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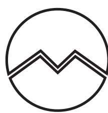

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The Effect of Miswak and Fluoride Toothpastes on Dental Plaque, A Comparative Clinical and Microbiological Study

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Abstract: Aim: The aim of this randomized, clinical trial was to compare the effect of miswak and fluoride toothpastes on the count of *S. mutans* and lactobacilli in dental plaque and also the effect of both toothpastes on plaque and saliva pH. **Methodology:** Children were randomly allocated into either the fluoride group (n = 20) or the miswak group (n = 20). The antimicrobial effect of both miswak and fluoridated toothpaste used in this study was determined using bacterial count. Two types of media were used for this purpose, Mitis Salivarius Agar Base for *S. mutans*, and tomato agar (ROGOSA AGAR) for Lactobacillus. Plaque and saliva pH were measured using a portable pH meter. Children were asked to brush their teeth twice daily for 2 weeks. **Results:** There was no statistically significant increase in the mean Plaque pH after using fluoridated toothpaste. While miswak group, showed a statistically significant increase in mean plaque pH after 2 weeks. Although the mean saliva pH values of both groups increased slightly yet it was statistically not significant. As regards fluoride group, there was a statistically significant reduction in mean $\log_{10}$ values of *S. mutans* CFU count after treatment. While miswak group, showed no statistically significant reduction in mean $\log_{10}$ values of *S. mutans* CFU count after treatment. Both fluoride and miswak group, showed a statistically significant reduction in mean $\log_{10}$ values of *Lactobacillus* CFU count after treatment. **Conclusions:** Both toothpastes have a good antimicrobial effect on caries producing bacteria. Miswak toothpaste raised plaque pH; while both toothpastes have no effect on saliva pH.

[Wafa K. M. Talha, Manal Elsaid, Ola M. Omarand Somaiya A. Eissa. **The Effect of Miswak and Fluoride Toothpastes on Dental Plaque, A Comparative Clinical and Microbiological Study.** *Nat Sci* 2013;11(9):1-7]. (ISSN: 1545-0740). http://www.sciencepub.net. 1

Key words: fluoride; miswak; toothpaste; plaque pH; saliva pH; *S. mutans*; lactobacilli

1. Introduction

Dental caries in children is a significant public dental health problem, affecting 60% to 90% of school children in industrialized countries (**WHO, 2003)**.It is not a self-limiting disease and without proper oral hygiene, it will progress until the tooth is destroyed ***(Fejerskov et al., 2003).*** It is a multifactorial disease in which, *streptococci mutans* and *lactobacilli in* dentalplaque, play an important role (***Featherstone, 2000; Selwitz et al., 2007***).

Tooth brushing and flossing removes dental plaque and antiseptic mouthwashes kills some of the bacteria that help in formation of plaque. Good oral hygiene is necessary for the healthy teeth, gum and fresh breath. Fluoride and plant extracts incorporated in toothpastes help to protect teeth by binding with enamel to make it stronger. It is of importance to look at the role plants play in oral hygiene as a number of them have medicinal properties (***Muhammad & Lawal, 2010).***

A study has shown that the concentrations of fluoride in biofilms are significantly increased after brushing with fluoridated toothpaste. The uptake of fluoride into the dental biofilm that was not removed by brushing is regarded as the main cariostatic effect of fluoride containing toothpastes (***Tenuta et al., 2009***).

Miswak *Salvadorapersica*is a small upright evergreen tree or shrub with white branches and aromatic roots, seldom more than 30 cm in diameter and three meters in height, has been used for centuries as oral hygiene tools (***Kassas & Zahran, 1965***).

Chemical analysis of *S. persica* demonstrated the presence of many components exhibiting antimicrobial effect of miswak aqueous extract (***Al-Bagieh et al., 1994)***. Some in *vitro* studies have shown that miswak extracts inhibited growth of various oral aerobic and anaerobeic bacteria, and *C. albicans* (***Al-Bagieh et al. 1994; Al.lafi & Ababneh, 1995***). Inhibition of in *vitro* plaque formation, growth and acid production of various cariogenic bacteria by miswak extracts have also been demonstrated (***Al Sadhan & Almas, 1999***). Miswak extract stimulate parotid saliva flow rate significantly (***Sofrata et al., 2007).***

In vitro studies showed that Miswak extract inhibited the growth of some dental plaque bacteria. And antibacterial effect of the herbal toothpaste was significantly greater than that of the placebo (*P* =0.002). Therefore due to antimicrobial effects of Miswak extract, its use in mouth rinses and toothpastes is highly recommended. ***(Hamid et al., 2007).***

An in *vivo* study aimed to document changes in plaque pH when an acidic challenge was followed by rinsing with miswak extract showed that classical plaque pH drop after 5% sucrose rinse. The maximum pH drop was recorded after 8 min with a mean minimum pH of 4.5. Rinsing with miswak extract or water at min 9 raised the plaque pH immediately. However, miswak rinse maintained the elevated plaque pH level, while water rinsing did not. The difference in plaque pH between water and miswak sessions was statistically significant. (***Sofrata et al., 2007).***

The aim of this study was to:

1. Compare the effect of miswak and fluoride toothpastes on the count of *S. mutans* and *lactobacilli* in dental plaque.
2. Compare the effect of both types of toothpastes on plaque and saliva pH.

2. Subjects and Methods

Patient selection:

Forty children of both sexes attending the Department of Pediatric Dentistry, Faculty of Oral and Dental Medicine, Cairo University were included in this study. Informed consent from the parents was obtained before inclusion of children in the study. Selected children fulfilled the following criteria:

1. Ranging in age from 7 - 9 years.
2. Permanent first molars and upper central incisors erupted.
3. Apparently healthy with no history of systemic diseases.
4. None of them were under antimicrobial agents since one month of sampling.
5. Number of carious primary teeth ranging between 4 and 8 and no caries in the permanent dentition. (DMF=0 def between 4 - 8)
6. Oral rehabilitation was done.

2. Materials:

- (Dabur MISWAK) herbal toothpaste formulated with pure extract of Miswak.
- (Signal)fluoridated toothpaste containing sodiummonofluorophosphosphate (1450ppm fluoride)..MitisSalivarius Agar Base9(HIMEDIA laboratories Pvt. Ltd. 23, Vadhani Ind. Est., LBS Marge, Mumbai-400 086, India.) Final pH (at 25ºC) 7.0 ± 0.2.
- ROGOSA AGAR (OXOID Ltd., Basingstoke, Hampshire, England) (500g makes 6litres) pH5.4 ± 0.2 at 25^0c.
- (HI 8424 Portable pH/mV/Temperature Meter).

Methods:

All patients were instructed to brush their teeth twice daily, refrain from any other oral hygiene procedures, and continue their routine dietary habits, throughout the duration of the study. They were also instructed to use the assigned toothpaste for 2weeks and refrain from brushing the night before their dental visit in order to allow for plaque accumulation as mentioned in previous studies **(*Edger & Geddes, 1986*).** The patients were examined at the first visit as baseline record and after 2 weeks for comparison.

Pooled plaque samples were collected from buccal surface of clinically sound upper incisors using sterile toothpicks for bacterial count. Plaque samples were placed in the screw capped vial containing 1ml of brain heart infusion broth (BHI).Plaque samples were inoculated on both the agar media. Another plaque sample was collected from buccal surface of clinically sound upper first molarsin a container containing 10 ml normal saline using sterile tooth picks for plaque pH measurement.

Saliva collection:

Children were seated comfortably on the dental chair. Unstimulated (resting) saliva was collected by expectorating in a sterile disposable container over 10 minutes (at base line before the use of toothpaste and after 2weeks).

For standardization of the saliva collection technique the children did not eat or drink (except water) 1 to 2hours before collection and did not perform any physical exercise before collection **(*Sudha et al., 2007*).**

The antimicrobial effect of both miswak and fluoride toothpaste used in this study was determined using bacterial count. Two types of media were used for this purpose, Mitis Salivarius Agar Base for *S. mutans*, and tomato agar (ROGOSA AGAR) for *Lactobacillus*.

Counting Bacteria by Dilution and Plating:

Colonies of *S. mutans* appear with morphologic characteristics 0.5 mm raised convex undulated colonies of light blue color with rough margins, granular frosted glass appearance (***Jabbarifaret al., 2005).***

Colonies of *Lactobacillus*characterized bySmall greyish-white, flat or raised, smooth, rough or intermediate (***Janetet al.,* 2003).**

Plates were examined and the numbers of bacterial colonies were counted.

Mean was counted from duplicate for each sample.

Real bacterial number=50ml (20 micron) x dilution factor x count /ml.

Measurement of plaque and saliva pH by sampling method:

A portable pH meter was used for measurements.

(HI 8424 Portable pH/mV/Temperature Meter).

Statistical analysis:

Numerical data were presented as mean and standard deviation (SD) values. A logarithmic transformation ($\log_{10}$ transformation) of each CFU count was performed to normalize the data before statistical evaluation because of the high range of bacterial counts. Student's t-test was used to compare between the two groups. Paired t-test was used to study the changes after treatment within each group. Percent change data showed non-normal (non-parametric) distribution, so Mann-Whitney *U* test was used to compare between the two groups. This test is the non-parametric alternative to Student's t-test.

3. Results

The mean age of children in group I (fluoride toothpaste) was (7.8±0.8), and group II (miswak toothpaste) was (7.8±0.9). (*P*-value=0.967).

Plaque pH

Table (1), shows the mean plaque pH of both groups before and after treatment: the mean pH value at baseline (before treatment) was (4.3±0.4) in fluoride group and (4.6±0.6) in miswak group. The mean pH value after treatment was (4.4±0.4) and (4.8±0.5) respectively.

As regards fluoride group, there was no statistically significant increase in the mean pH after treatment. While miswak group, showed a statistically significant increase in mean pH after treatment.

As regards the percent change, no statistically significant difference was noted between both groups. Table (2), figure (1)

Saliva pH

Table (3), shows the mean saliva pH of both groups before and after treatment, the mean pH value at baseline (before treatment) was (7±0.7) in fluoride group and (7.1±0.4) in miswak group. The mean pH value after treatment was (7.2±0.6) and (7.2±0.5) respectively.

Both fluoride and miswak group, showed no statistically significant increase in the mean pH after treatment.

Although the mean saliva pH values of both groups increased slightly yet it was statistically not significant, Table (4).

As regards the percent change, no statistically significant difference was noted between both groups, Figure (2).

Table (1): The mean values of plaque pH before and after treatment in both groups

Period \ Group	Fluoridated toothpaste		Miswak toothpaste	
	Mean	SD	Mean	±SD
Before treatment	4.3	0.4	4.6	±0.6
After treatment	4.4	0.4	4.8	±0.5
P-value	0.063		0.033*	

*: *Significant at $P \leq 0.05$*

Table (2): The mean values and percent change of plaque pH in both groups

Period \ Group	Fluoridated toothpaste		Miswak toothpaste		*P*-value
	Mean	±SD	Mean	±SD	
Before treatment	4.3	±0.4	4.6	±0.6	0.041*
After treatment	4.4	±0.4	4.8	±0.5	0.018*
Percent increase	3.7	±5.7	4.6	±8.5	0.375

*: *Significant at $P \leq 0.05$*

Table (3): The mean values in saliva pH before and after treatment in both groups.

Period \ Group	Fluoridated toothpaste		Miswak toothpaste	
	Mean	±SD	Mean	±SD
Before treatment	7	±0.7	7.1	±0.4
After treatment	7.2	±0.6	7.2	±0.5
P-value	0.304		0.056	

*: *Significant at $P \leq 0.05$*

Table (4): The mean values and percent change of saliva pH in both groups.

Period \ Group	Fluoridated toothpaste		Miswak toothpaste		*P*-value
	Mean	±SD	Mean	±SD	
Before treatment	7	±0.7	7.1	±0.4	0.688
After treatment	7.2	±0.6	7.2	±0.5	0.834
Percent increase	2.4	±5	1.3	±3.1	0.942

*: *Significant at $P \le 0.05$*

***Streptococcus mutans*count**

Table (5), shows the mean $\log_{10}$ SM count of both groups before and after treatment, the mean $\log_{10}$ SM count at baseline (before treatment) was (6±1.3) in fluoride group and (6.4±1) in miswak group. The mean $\log_{10}$ SM count after treatment was (5.6±1.3) and (6.1±1.2) respectively.

As regards fluoride group, there was a statistically significant reduction in mean $\log_{10}$ values of *S. mutans* CFU count after treatment. While miswak group, showed no statistically significant reduction in mean $\log_{10}$ values of *S. mutans* CFU count after treatment.

As regards the percent change, no statistically significant difference was noted between both groups. Table (6), and figure (3).

***Lactobacilli* count**

Table (7) shows the mean $\log_{10}$ LB count of both groups before and after treatment, the mean $\log_{10}$ LB count at baseline (before treatment) was (2.4±0.4) in fluoride group and (2.5±0.5) in miswak group. The mean $\log_{10}$ LB count after treatment was (2.2±0.5) and (2.3±0.4) respectively.

Both fluoride and miswak group, showed a statistically significant reduction in mean $\log_{10}$ values of *Lactobacilli* CFU count after treatment.

As regards the percent change, no statistically significant difference was noted between both groups. Table (8), Figure(4).

Table (5): The mean $\log_{10}$ values of *S. mutans*CFU count in both groups

Period \ Group	Fluoridated toothpaste		Miswak toothpaste	
	Mean	±SD	Mean	±SD
Before treatment	6	±1.3	6.4	±1
After treatment	5.6	±1.3	6.1	±1.2
P-value	0.026*		0.301	

*: *Significant at $P \le 0.05$*

Table (6): The mean log10 values of *S. mutans* CFU count and percent change in both groups

Period \ Group	Fluoridated toothpaste		Miswak toothpaste		*P*-value
	Mean $\log_{10}$	±SD	Mean $\log_{10}$	±SD	
Before treatment	6	±1.3	6.4	±1	0.259
After treatment	5.6	±1.3	6.1	±1.2	0.198
percent reduction	4.8	±3.2	2.8	±1.8	0.884

*: *Significant at $P \le 0.05$*

Table (7): The mean $\log_{10}$ values of *Lactobacilli* CFU count in both groups

Period \ Group	Fluoridated toothpaste		Miswak toothpaste	
	Mean	±SD	Mean	±SD
Before treatment	2.4	±0.4	2.5	±0.5
After treatment	2.2	±0.5	2.3	±0.4
P-value	<0.001*		0.002*	

*: *Significant at $P \le 0.05$*

Table (8): The mean $\log_{10}$ values and percent change of *Lactobacilli* CFU count in both groups

Period \ Group	Fluoridated toothpaste		Miswak toothpaste		*P*-value
	Mean $\log_{10}$	±SD	Mean $\log_{10}$	±SD	
Before treatment	2.4	±0.4	2.5	±0.5	0.513
After treatment	2.2	±0.5	2.3	±0.4	0.466
percent reduction	39.8	±20.9	35.4	±21.7	0.532

*: *Significant at $P \le 0.05$*

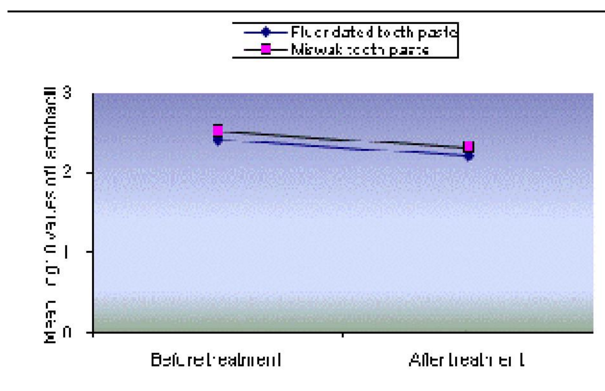

Figure (4): Changes after treatment in $\log_{10}$ values of *Lactobacilli* CFU count in both groups

4. Discussion

Children were selected from the Department of Pediatric Dentistry, Faculty of Oral and Dental Medicine, Cairo University to ensure comparable socioeconomic and feeding habits of all participants. Selected children ranged in age from 7-9 years as they can rinse and spit decreasing the risk of swallowing toothpaste.

Plaque samples were collected as they do not require sophisticated equipment, collected in a simple method and are efficiently used on a large number of subjects (***Sofrata, 2010).***

In miswak group the results were in agreement with **(*Sofrataet al., 2007),*** who demonstrated that rinsing with Miswak extract had a neutralizing effect on plaque pH after a previous sucrose exposure. The data indicated that rinsing with Miswak extract raised the plaque pH for a more prolonged time as compared to water rinsing.

Similarly another study showed that rinsing with Miswak extract resulted in protracted elevation of plaque pH (>6.0). The difference in plaque pH between Miswak extract and water rinse was statistically significant at 30 minutes ($p < 0.01$) (***Sofrata, 2010).***The elevated plaque pH with Miswak rinse could be due to a buffering capacity of the Miswak extract, salivary stimulation due to Miswak taste, and/or antibacterial activity against acid-producing bacteria. Also (**Edgar & O'Mullane, 1996**) explained that Miswak stimulated salivary secretions as it has a relatively strong taste, thus washing out and diluting acids.

Although the mean saliva pH values of Miswak and fluoride groups increased slightly yet it was not statistically significant. As regards the percent change, no statistically significant difference was noted between both groups.

On the contrary, ***(Dąbrowska, 2005)*** found that fluoride contained in oral hygiene preparations cause increase salivary pH. Differences in results between studies can be due to lack of compliance with the use of the assigned dentifrice which can occur in home-use studies.

Pooled plaque samples were used to determine levels of *S. mutans* because it is likely to be the most reliable method in children due to higher odds ratio between caries and *S. mutans* in plaque samples compared to saliva (***Sanchez-Pertez & Costa-Gio, 2001***). Plaque samples were collected from buccal surface of upper incisors as they have high concentration of plaque by sterile toothpick in accordance to ***Wennerholm et al., 1995***.

Decrease in the mean bacterial count has-been observed in both groups, toothpastes have reduced *S. mutans* and *Lactobacilli* count when used over a period of 2weeks. This substantiates the antibacterial properties of both toothpastes.

Similar results were noted in previous studies ***(Paiet al., 2004;Jabbarifar et al., 2005)*** where fluoride products containing500ppm and 1000ppm of fluoride, showed significant reductions of up to 50% after 21 days.

Similarly several studies demonstrated the antibacterial effect of miswak on *S. mutans* and found that *S. persica*has an antibacterial effect on different types of bacteria, including *mutansstreptococci* ***(Al-Lafi & Ababneh, 1995; Almas et al., 1997; Almas, 1999; Almas & Al-Bagieh, 1999).*** Another study demonstrated an immediate antibacterial effect of Miswak with significant reduction of *S. mutans*and non-significant reduction in *Lactobacilli* when comparing Miswak sticks with toothbrush ***(Almas& Al-Zeid, 2004).***

Contradicting our results ***(Petersson et al., 1991)*** foundno difference in levels of *S. mutans* or lactobacilli between subjects using or not using different fluoridated toothpastes. Also ***(Faiez, 1995)*** found that toothpaste containing Miswak extract was significantly more effective compared to fluoridated toothpaste,and attributed the anticaries effect of miswak to its fluoride content.

On the contrary testing the antibacterial effect of fresh Miswak pieces embedded in inoculated agar plates indicated that Miswak had very strong antibacterial activity. The inhibitory effect of miswak was most pronounced on *P. gingivalis, A. actinomycetemcomitans*, and *H. influenzae*, less on *S. mutans* and least on *Lactobacillus acidophilus,* which indicated that Miswak extract was more effective against Gram negative than Gram-positive species ***(Sofrata, 2010).***

Furthermore, the results from these studies cannot be directly compared as the Miswak sources and the concentrations predations are different, also method of extract preparation. However, the chemical compositions of *S. persica*roots and the exact amounts of each component are contradictory (***Ezmirly & Seif-Elnasr, 1981; Abdel- Wahab et al., 1990; Bader et al., 2002).***

Conclusion

From the present study the following conclusions can be drawn:

1. Both toothpastes have a good antimicrobial effect on caries producing bacteria, thus can be used in children as a regular home care preventive aid in combating dental caries.
2. Miswak toothpaste raised plaque pH; suggesting a positive role in the reduction and prevention of dental caries.
3. Both toothpastes have no effect on saliva pH.

Recommendations

1. Long term studies are needed to quantify the efficacy of miswak toothpaste among children.
2. Further researches with larger sample sizes are needed to evaluate the benefits of Miswak toothpaste use for children.
3. More researches are needed to study the combined effect of Miswak and fluoride in different oral hygiene products.
4. Further studies are needed to evaluate the effect of Miswak toothpaste on preschool children of high caries risk.

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Review of Fauna of Dehang-Debang Biosphere Reserve, Arunachal Pradesh (India)

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Abstract: The paper deals with the compilation of faunal diversity of Dehang-Debang Biosphere Reserve (DDBR) records from past works so as to create a comprehensive database of the faunal diversity of the BR at one place. Due to its rugged terrain and inaccessible landscape, the area has not been discovered so well from the biological diversity point of view, although its faunal diversity has been studied and reported by few workers. The areas in and around the Dehang Debang Biosphere Reserve contributes nearly 70% of the bird species of Arunachal Pradesh. The records reveal presence of 133 species of butterfly belonging to 8 families and 81 genera (as invertebrate record). While the vertebrate fauna is represented by 180 species of mammals, 492 species of birds,106 species of reptile, 43 amphibian species and 93 species of fish and yet to explore more.

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Key Words: Dehang Debang Biosphere Reserve, DDBR, Fauna, Arunachal Pradesh, Biodiversity.

1. Introduction

The Eastern Himalaya being the transition zone between Indian Indo-Malayan and Indian Indo-Chinese biogeographical regions is referred as "*Biogeographical gateway*" and it has created one of the biologically rich areas on the earth and recognized as Global Biodiversity Hotspots. Arunachal Pradesh is located between 26°28'and 29°30'North latitudes and 91°30' and 96°30' East longitude which covers an area of 83743sq.Km. The state is an abode for a wide range of floral and faunal wealth. It is one of the largest states of the Indian Himalayan Region (IHR). It is nature's repository of medicinal plants and has an assemblage of 5 hornbill species and is one of the topmost birding areas in the world and has the second highest Important Bird Area (IBAs) in the northeast India (IBCN, 2004; Islam *et al*, 2004) and has rich avifauna with over 760 bird species and 10 species of Pheasants (SFRI, 2008). The state has nearly 213 species of fish and 55 species of Amphibians, more than 55 species of Snakes (Maheswaran, 2012) and as many as 71 species of chiropterans.

The Dehang-Debang Biosphere Reserve (DDBR) is one of the biologically very rich and largely undisturbed protected areas in Arunachal Pradesh. The DDBR was notified on 02.09.1998, it spreads over an area of 5111.5 km^2, with buffer zone of 1016.7 km^2. It represents one of the most diverse wildlife assemblages hosting one of the Eastern Himalayan Biodiversity Hotspot. The Reserve is an abode for wide variety of floral and faunal species. The two protected areas, Mouling National Park (483 Km^2) and Debang Wildlife Sanctuary are located fully and partially within the Biosphere Reserve respectively. There are 12 major tribal communities inhabiting in and around DDBR whose main occupation is agriculture (mainly Jhum cultivation), cattle rearing and hunting and they depend directly on the forest resources for their daily needs. The topography of the Biosphere is characterized by steep to very steep and rugged terrain typical high mountainous, well drained with numerous rivers and gorges. Due to inaccessibility, the reserve though bestowed with rich faunal species, is poorly studied and much of its parts remains unexplored.

2. Methodology

The information available in the form of published literature paper, articles, reports, management plan and state faunal records from Zoological survey of India were collected and compiled. The different sources explored includes research papers, books, reports etc., for example Chaudhury (2003, 2004, 2008 & 2010), Sen and Mukhopadhyay (1999), Singh (1994), Athreya (2006), WWF (2006), Ghosh and Ringu (2002), Naoroji et al. (2005) etc. The data extracted from various sources was also processed and rectified with the help of online resources (such as www.iucnredlist.org) to prepare a detailed list of fauna with the systematic position, common name, scientific name, global distribution and status as per IUCN redlist of threatened animals.

Table 1. Faunal diversity with global threat status of DDBR.

Zoological Classification	Fauna	Family	Genera	Species	Global Status						
					CE	EN	NT	VU	LC	DD	NA
Vertebrate	Mammals	32	108	180	0	6	11	18	133	8	4
	Birds	71	237	492	2	4	15	16	455	0	0
	Reptiles	14	61	106	0	2	3	5	20	3	73
	Amphibian	7	26	43	0	0	1	1	27	4	10
	Fish	23	57	93	0	1	9	3	61	3	16
Invertebrate	Butterfly	8	81	133	0	0	0	0	0	0	133
Total		155	570	1047	2	13	39	43	696	18	236

TH-Threatened, CE-Critically Endangered, EN-Endangered, NT-Near Threatened, VU-Vulnerable, LC-Least Concern, DD-Data Deficient, NA-Not Assessed.

3. Results and Discussion

A total number of 1047 faunal species have been found reported in different sources belonging to (in and around) the Dehang Debang Biosphere Reserve, belonging to 570 genera and 155 families (Table 1). Table 1 is also representing faunal group wise number of species falling in different global conservation status as per IUCN redlist. Table 1 above clearly depicts that birds or avifauna is the largest group among all faunal groups reported in and around DDBR. The bird fauna contributes 47% with 492 species among other faunal groups followed by mammals (17%) and butterflies (13%) etc. (Figure 1). Amphibian is the lowest populated faunal group among others. Talking about the mammals, a total of 180 mammalian species are reported in and around DDBR which comprises of the order Chiroptera with the maximum 52 (29%) species followed by the Order Rodentia with 47 (26%) species, Carnivora of 39 (21%) species order Cetartiodactyla exhibits 16 (9%) species, order Eulipotyphla with 11(6%) species, order Primates contributes 6 (3%) species of which 5 are globally threatened, order Lagomorpha with 5 (3%) species and order Scandentia with 2 (1%) species, while the least of 1 species is contributed by the order Insectivora and Pholitoda.

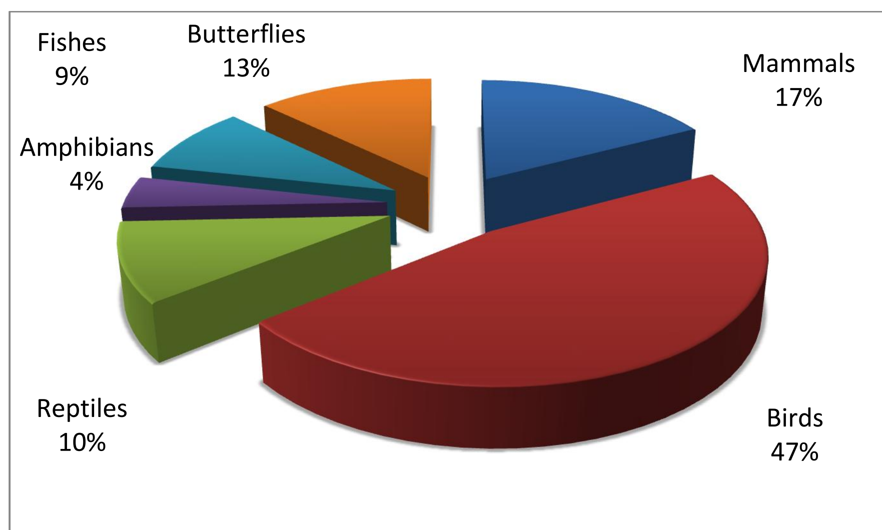

Figure 1. Percent species contribution of different faunal groups in DDBR.

Avifauna which is the largest faunal group reported in and around DDBR comprises 492 species of 18 order, 71 families and 237 genera. The order Passeriformes is dominant with total 287 (58.3%) species belonging to 34 families followed by the order Falconiformes with 34 (6.9%) species, Charadriiformes with 28 (5.6%) species, order Galliformes 25 (5.8%) species with 8 globally threatened species, Anseriformes 23 (4.7%) species, order Piciformes 19 (3.8%) species, Ciconiiformes

and Columbiformes 13 (2.6%) species each, order Coraciiformes 11 (2.2%) species, order Strigiformes and order Gruiformes 8 (1.6%) species each, order Cuculiformes, order Pelecaniformes and Apodiformes with 5 species each, order Caprimuliformes 3 species and the least species contributed by the order Podicipediformes and Trogoniformes (2 each) and Psittasiformes with only 1 species. Some 23 (4.6%) species of Migratory birds were also reported from the same area. It is important to highlight that out of 21 restricted range species in the Eastern Himalayan Endemic Bird Areas, 16 (76%) species namely *Arborophila mandellii, Garrulax virgatus, Spelaeornis caudatus, heterophasia pulchella, Tragopan blythii, Lophophorus sclateri, Stachyris oglei, sphenocichla humei, Actinodura waldeni, Yuhina bakeri, Alcippe ludlowi, Heterophasia picaoides, Phyllocospus cantator, Spelaeornis troglodytes, Tickellia hodgsoni and Harpactes wardii,* are reported to be found in DDBR.

Among the 106 species of reptilian fauna found in and around DDBR, order Squamata dominates the reptilian fauna with 97 (91%) species whereas, order Testudines represents only 9 (8.4%) species of which 8 species are threatened globally. The Threatened species includes *Batagur dhongoka, Cuora mouhotii, Cuora amboinensis*, Cyclemys dentate, *Melanochelys tricarinata, Nilssonia hurum, Ophiophagus Hannah, Pangshura smithii, Pangshura sylhetensis* and *Python molurus.*

The 43 species of Amphibian fauna is contributed by family Rhacophoridae contributing the maximum species of 15 (34.8%) species, followed by family Ranidae with 11 (25%) species, family Dicroglossidae with 7 (16%) species,family Megophryidae with 5 (11.6%) species, Bufonidae with 3 (7%) species, the family Microhylidae and Palobatidae contributes only 1 species each only. The species *Theloderma moloch* and *Rhacophorus reinwardtii* are the two threatened species that were reported among the amphibian fauna from DDBR. Among the Butterflies, the dominant family being Nymphalidea with 28 genera and 41 species followed by Papilionidae and Satyridae with 9 and 12 genera and 22 species each, Lycanidae comprising of 14 genera and 20 species and Peridae with 10 genera and 16 species.

While the 93 species of fish fauna comprises of 7 orders of which the order Cypriniformes represented by 53 (57%) species, followed by order Siluriformes with 22 (24%) species, Perciformes with 12 (12%) species, order Symbranchiformes with 3 species and least species by the order Osteoglossiformes, Clupeiformes and Beloniformes contributes only 1 species. There are 13 globally threatened fauna that were recorded from the BR, these includes *Aborichthys kempi, Bagarius bagarius, Botia rostrata, Cyprinion semiplotum, Labeo pangusia, Neolissochilus hexagonolepis, Ompok bimaculatus, Ompok pabda, Ompok pabo, Schizothorax richardsonii, Tor putitora, Tor tor* and *Wallago attu.* Most of the fish species representing the fish fauna of DDBR were recorded from Mouling National park as per the available sources.

4. Conclusion

As per the available database it is clearly reflected that DDBR is indeed a treasure of faunal wealth and certainly there is a lot more to be further explored. In general the biotic components face a lot of threats globally but as far as Dehang-Debang Biosphere Reserve is concern, due to its inaccessible terrain and topography the place is somewhat safe from many forms of the threats. However, in contrast, many studies have revealed that a significant number of faunal species found in and around DDBR are facing local threats because of various reasons. Among these species, many are listed under different IUCN threat categories and also listed under schedule I-IV of Wildlife Protection Act (WPA) 1972 (Table 2). As presented in Table 2, there are 36 species belonging to different faunal groups which are being harnessed for medicinal, cultural and different other uses (Chakravorty et al., 2011; Chakravorty et al., 2011; Solanki & Chutia, 2004; Chinlampiaga et al, 2013 etc.). Consequently, these species are under stress locally. Hornbills and galliformes are most threatened species. Some of the faunal species those are known to be Least Concern (LC) as per the Global status in the IUCN redlist are being hunted for various ritualistic and medicinal values. For example, Squirrels are hunted for marriage related rituals (Table 2), if hunting of this species continuous there are likely chances that squirrels may come under vulnerable (VU) or endangered (EN) status in near future. In Arunachal Pradesh, there is the seasonality of hunting and hunting is intensive during jhum cultivation and harvesting of the crops (Chutia and Solanki, 2013), although the role of Jhum agriculture can again be a matter of separate debate. However, it is important to understand that the presented facts and figures are alarming and clearly showing the need of conservation efforts.

Table 2. Conservation status of faunal species found in and around DDBR and their local uses.

S. No.	SCIENTIFIC NAME	COMMON NAME	IUCN STATUS (IUCN 3.1 RED DATA	STATUS AS PER WPA 1972 AND CITES	USES OF FUANAL RESOURCES IN ARUNACHAL PRADESH
1.	Neofelis nebulosa Griffith, 1821	Clouded Leopard	Vulnerable	WPA,1972 Sch – I	Hunted for meat,skin and Bone marrow to massage body pain (Chakravorty *et al*., 2011)
2.	Panthera pardus Linnaeus, 1758	Leopard	NT	WPA,1972 Sch – I/CITES appen - I	Hunted for meat and medicine for Malaria,typhoid and rheumatic pain. (Solanki & Chutia, 2004)
3.	Panthera tigris Linnaeus, 1758)	Tiger	EN	WPA,1972 Sch – I/CITES appen - I	Hunted for meat,skin and dried bones as paste used for curing rheumatic and body pain. (Solanki & Chutia, 2004)
4.	Pardofelis marmorata Martin, 1837	Marbled Cat	VU	WPA,1972 Sch – I	Hunted for meat and skin. (Solanki & Chutia, 2004)
5.	Ursus thibetanus G. [Baron] Cuvier, 1823	Himalayan Black Bear	VU	WPA,1972 Sch – I	Hunted for meat, teeth and gall bladder (medicine for malaria, typhoid and T.B.), dried skin as armour (known as *Khuk* by Monpas) and headgear & shoulder belt (Nyshi), (Solanki & Chutia, 2004)
6.	Felis chaus Schreber, 1777	Jungle cat	LC	WPA,1972 Sch – II/CITES - II	Skin and fur for aesthetic use by males during special occasion. (Solanki & Chutia, 2004)
7	Vulpes bengalensis Shaw, 1800)	bengal fox	LC	WPA,1972 Sch – II/CITES – III	Hunted for food. Meat boiled or roasted is used for treatment of TB and bones in fertility. (Chakravorty *et al*, 2011)
8.	Canis aureus Linnaeus, 1758	golden jackal	LC	WPA,1972 Sch – III/ CITES – III	Hunted for meat and bones to treat skin disease. (Solanki & Chutia, 2004)
9.	Bos gaurus C.H. Smith, 1827	Indian Bison	VU	WPA,1972 Sch – I/CITES appen – I	As wild meat (Solanki & Chutia, 2004)
10.	Budorcas taxicolor Hodgson, 1850	Takin	VU	WPA,1972,Sch – I/ CITES appen - II	One of the endemic species is hunted for meat,skull and skin display at household. (Solanki & Chutia, 2004)
11	Moschus fuscus Li, 1981	Black musk deer	EN	CITES append - I	Musk pod exported illegally from the local by outsider (Solanki & Chutia, 2004)
12	Moschus moschiferus Linnaeus, 1758	Siberian Musk Deer	VU	CITES appen - II	Hunted for meat and musk which is used for therapeutic purpose for malaria and diarrhea (Solanki & Chutia, 2004)
13	Naemorhedus goral Hardwicke, 1825	Himalayan Goral	NT	WPA,1972,Sch – III/ CITES - I	Skin to partly covered hand fan (Solanki & Chutia, 2004)
14.	Rusa unicolor Kerr, 1792	Sambar	VU	WPA,1972,Sch – III	Dried skin as coat (known as *Pakcha)* to protect from severe climate. Crush horn with salt for bursting boils (Chakravorty *et al*, 2011)
15.	Sphaerias blanfordi Thomas, 1891	Blandford's fruit bat	LC	-----------	Hunted for meat,skin used for skin diseases and bones as taboo item. (Chinlampiaga et al., 2013)
16.	Manis pentadactyla Linnaeus, 1758	Chinese pangolin	EN	WPA,1972,Sch – I/CITES append - II	Nails used to pierce boils (Chakravorty *et al*, 2011)
17.	Macaca assamensis M'Clelland, 1840	Assamese Macaque	NT	WPA,1972,Sch – II/CITES append - II	Hunted for meat as it has good medicinal properties and used to treat disease like small pox,malaria etc. (Solanki & Chutia, 2004)
18.	Trachypithecus pileatus Blyth, 1843	Capped langur	VU	WPA, 1972,Sch – I/CITES append - I	Meat as food and as ethno-medicine and for socio-cultural practices (Solanki & Chutia, 2004)

19.	Macaca mulatta Zimmermann, 1780	Rhesus Monkey	LC	WPA, 1972,Sch – I/CITES append - II	Meat for treating diseases like malaria, cholera. Skull & finger or palms are hung to door to calm evil spirit (Solanki & Chutia, 2004)
20.	Macaca munzala Madhusudan & Mishra, 2005	Arunachal Macaque	VU	CITES append -II	Hunted for meat,sport and medicine for sick livestocks as well,juveniles as pet. (Solanki & Chutia, 2004)
21.	Ratufa bicolor Sparrman, 1778	Malayan Giant Squirrel	NT	WPA,1972,Sch – II	Hunted for meat and used dowry item. (Chinlampiaga et al, 2013)
22.	Hystrix brachyura Linnaeus, 1758	Himalayan Crestless Porcupine	LC	WPA, 1972,Sch – II	Boiled gall bladder,stomach and intestine are used to cure Diahoea, gastritis and TB. (Chakravorty *et al*, 2011)
23.	Dremomys lokriah Hodgson, 1836	Orange bellied Himalayan Squirrel	LC	WPA, 1972,Sch – IV	As gift to bride's family during marriage ceremony and other social ceremonies and as medicine also. (Chinlampiaga et al, 2013)
24	Callosciurus pygerythrus, I. Geoffroy Hilaire, 1832	Irrawaddy squirrel	LC	WPA, 1972,Sch – II	Hunted for meat and used dowry item (Chinlampiaga et al, 2013)
25.	Hylopetes alboniger (Hodgson, 1836)	Particolored Flying Squirrel	LC	WPA, 1972,Sch – II	Hunted for meat and used dowry item (Chinlampiaga et al, 2013)
26.	Belomys pearsonii Gray, 1842	hairy footed flying squirrel	DD	WPA, 1972,Sch – II	Hunted for meat and used dowry item (Chinlampiaga et al, 2013)
27.	Tamiops macclellandi Horsfield, 1840	Himalayan striped Squirrel	LC	WPA, 1972,Sch – IV	Hunted for meat and used dowry item (Chinlampiaga et al, 2013)
28.	Aceros nipalensis Hodgson, 1829	Rufous necked hornbill	VU	WPA, 1972,Sch- I /CITES append – I/II	Mostly hunted species, beak, decorate traditional headgear (*Nyshi* tribe), feathers to adorn headdresses (*Wancho* & *Nocte* tribe), fat for body massage. (Chakravorty *et al*., 2011)
29.	Tragopan blythii Jerdon, 1870	Blyths tragopan	VU	WPA,1972 Sch – I/CITES append- I	Tail feather as hand fan. (Aiyadurai, 2012).
30.	Lophophorus sclateri Jerdon, 1870	Sclater's monal	VU	WPA,1972 Sch - I	Feather used as hand fan by chanting priest, wing feather worn around neck by women (Aiyadurai, 2012)
31.	Gracula religiosa Linnaeus, 1758	Hill myna	LC	--------------	Hunted for meat as energy enhancement. (Chinlampiaga et al, 2013)
32.	Python molurus Linnaeus, 1758	Indian Python	NT	WPA,1972 Sch – I/CITES, append- I	Body fat massage for joint pain (Chakravorty *et al*., 2011)
33.	Naja kaouthia Lesson, 1831	Monocled Cobra	LC	CITES, appendix – I	Cooked meat used in disease like cold (Chakravorty, *et al*., 2011)
34.	Bagarius bagarius Hamilton, 1822	NA	NT	-------------	Smoked dried bones/Fins are used for burns and stomach ache. (Chakravorty, *et al*, 2011)
35.	Sus scrofa Linnaeus, 1758	Wild Boar	LC	WPA, 1972,Sch – III	Meat in food,bones in fertility and skull in hunting art. (Chinlampiaga et al, 2013)
36.	Varanus bengalensis.	Monitor lizard	LC	WPA, 1972,Sch – III	Flesh boiled and taken as preventive measure for cough and fever. (Chakravorty *et al*, 2011)

TH-Threatened, CE-Critically Endangered, EN-Endangered, NT-Near Threatened, VU-Vulnerable, LC-Least Concern, DD-Data Deficient, NA-Not Assessed.

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7/5/2013

Serum Copper Concentration in type-1 diabetes mellitus by Atomic Absorption Spectroscopy

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Abstract: Type 1 diabetes mellitus (DM) is an endocrinological disease associated with hyperglycemia characterized by both insulin resistance and defective insulin secretion. It is associated with the alteration of trace elements like copper which may be a contributing factor in the progression of Diabetes Mellitus and its complications. The aim of the present study was to estimate serum copper, in patients with type 1 DM and compare it with controls .This study was conducted in 100 subjects, out of which 50 were type 1 diabetes mellitus patients and 50 were healthy subjects (controls). Serum copper was determined by Atomic Absorption Spectroscopy using air-acetylene flame (AAS, Model Varian A-20). Serum Copper concentrations was significantly higher in patients as compared with the controls and It could be concluded that there is an association between oxidative stress and metabolic control in diabetic patients.

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Keywords: Type-1 diabetes mellitus, copper, serum, Atomic absorption spectrometer

1. Introduction

Diabetes Mellitus is characterized by metabolic disorders related to high blood glucose levels. This hyperglycemia leads to various vascular complications like coronary artery disease, (1) neuropathy, retinopathy and nephropathy. In type 1 DM prevalence gradually increase from onset of disease while in type 2 DM it can be observed in both newly diagnosed and established (2) diabetes .Diabetic nephropathy is the most important cause of (3) death in type 1 diabetes patients compares to type 2 DM. In general (non-diabetic) population, hypertension is found to be the major responsible factor of microalbuminuria. Individuals with essential hypertension who develop microalbuminuria have (4) higher incidence of biochemical disturbances. Trace metals are important for optimum human metabolic function, and serve a variety of functions including catalytic, structural and regulatory activities in which, they interact with enzymes, prohormones, presecretory granules and biological membranes.(5) There is accumulating evidence that the metabolism of several trace elements are altered in type-1 diabetes mellitus (DM) and that these nutrients might have specific roles in the pathogenesis and progression of this disease.(6) It is reported that plasma copper levels has been found to be elevated in type-1 DM patients, while the urinary excretion of copper has been found to be affected by DM.(6,7) has been reported in diabetic patients This study evaluated the serum concentration of copper in diabetic patients and controls.

2. Material and Methods

The metal copper in the serum was determined by using Atomic Absorption Spectrometry (AAS) (Model, A-20 Varian). The concentration of the Copper was determined by air-acetylene flame. The standards from 1 to 5 ppm were run on the spectrometer and the calibration curves were obtained prior to running the samples for the determination of copper in the blood serum of controls and the patients. Blood samples were collected from fifty healthy controls in fasting conditions and a similar condition was maintained while taking blood samples of patients. Each blood sample was centrifuged at 5000 rpm for 20 min. The supernatant of blood serum was used for the analysis of metals copper using Atomic Absorption Spectrometer inserting appropriate hollow cathode lamp in it. All standards used were of analytical grade.

Chemicals and reagents

Sulphosalicylic acid was obtained from Merck, Darmstadt, Germany and Copper chloride to prepare standards were purchased from Sigma Chemical Company. All chemicals were of analytical grade.

Stock Solutions and working Metal standards

Commercial trace metal (**Cu**) atomic absorption standard solution (1000 μg/mL, Sigma Co.) was used. Working standards were prepared from the stock standard solution by diluting with deionized water with addition of few drops of corresponding concentrated acid solution.

Statistical analysis was carried out with Statistical Package for Social Sciences (SPSS) version 16 All data are expressed as mean ± standard error of mean (SEM).

3. RESULTS

Serum copper concentration in controls and in patients determined to be 70.01±1.30 ppm and 75.04 ±0.62 respectively. Serum copper was found statistically highly significant in patients as compared with the controls.

All values are expressed as mean SEM.

Table 1. Serum Copper concentration in patients and controls.

Variables	Controls	Patients
Copper	70.01±1.30	75.04 ±0.62

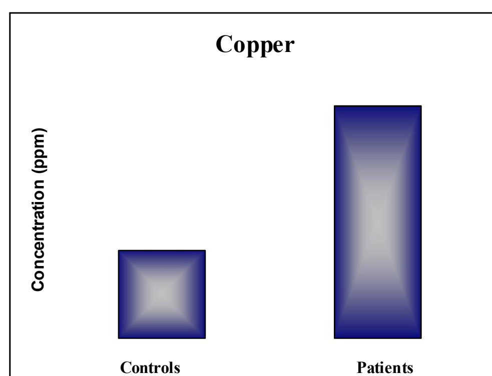

Fig: 1 shows Copper mean concentration in patients as compared to the controls.

4. Discussion

Trace elements have important physiological effects when present at concentrations other than those associated with classical toxicity or with extreme deficiency. (5) Many studies have reported significant though variable alteration in trace element concentration in type-1 and type-2 DM patients. (6, 7, 8, 9) In a recent study, we documented that serum copper concentration was comparable between patients with normal control subjects. Copper, an essential trace element, plays an important role in cytochrome oxidase function in the mitochondria. Copper deficiency results in swelling and subsequent disruption of the mitochondria of metabolically active tissues like hepatocytes and pancreatic acinar cells. (5) In our study, copper levels were comparable between type-1 DM patients and non-diabetic controls. Glycemic control and presence of microalbuminuria did not affect the serum copper levels. Earlier, we have documented elevated plasma copper levels in type-2 DM patients (10). Conflicting results have been reported regarding the copper levels in type-1 DM, both elevated as well as decreased plasma copper concentrations had been reported. (11, 12).

The redox chemistry of Cu makes this both a powerful enzyme catalyst and a dangerous reactant that generates hydroxyl radical. Although virtually all cells from microbes to mammals must acquire Cu to drive important biochemical reactions, the potential toxicity of Cu demands an exquisite level of vectorial transport and homeostatic control (13). Abnormal copper metabolism can lead to several chronic pathogenesis, such as diabetes or diabetic complications (14). Serum Cu level in the diabetic group did not show statistical significant difference compared to the nondiabetic which is not consistent with findings of Kazi *et al*. (15). Our study indicates possible increase in copper mediated generation of ROS leading to increased consumption of available antioxidants in the body. These findings further confirm that alteration of serum copper levels is due to antioxidant imbalance in diabetic patients. From this study, it could be concluded that there is an association between oxidative stress and metabolic control in diabetic patients.

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6/28/2013

Efficacy of Fractionated CO_2 Laser and Pulsed Dye Laser in Treatment of Keloids and Hypertrophic Scars

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Introduction: Keloids and hypertrophic scars are skin proliferative disorders with altered dermal matrix deposition. Although laser improves scars, a universal treatment protocol regarding laser type, dosage and number of sessions has not yet been established; in addition Laser indications and efficacy have not been fully defined. We studied the impact of laser therapies on keloids and hypertrophic scars. **Methods:** This study aimed to assess keloids and hypertrophic scars response to 6 sessions of combined pulsed dye laser and fractionated CO_2 laser (4 sessions of pulsed dye laser and 2 sessions of fractionated CO_2 laser). Pulsed Dye laser parameters were fluence: 6- 6.5 joules/cm^2, spot size: 10 millimeter and pulse duration: 0.5 millisecond. Fractionated CO_2 laser parameters were fluence: 15 Watts, spacing: 500 micrometer and dwell time: 0.7-1 millisecond. Scar textural pliability, pigmentation and vascularity were subjectively scored according to the modified Vancouver Scar Scale comparing readings before laser sessions and after the 2nd, 4th, 6th laser sessions and after follow up for 6 months. Collected data was analyzed with appropriate statistical tests. Hemoglobin, melanin and contour indices were objectively calculated. **Results:** Combined pulsed dye laser and fractionated CO_2 laser ameliorated (**with highly statistically significance**) modified Vancouver Scar Scale from 7.00±0.60 to 2.00±1.35 in keloids and from 7.13±0.99 to 1.63±0.74 in hypertrophic scars. Hemoglobin, melanin and contour indices showed amelioration with combined pulsed dye laser and fractionated CO_2 laser. **Conclusion:** When pulsed dye laser is associated with fractionated CO_2 laser in the treatment of keloids and hypertrophic scars, scar amelioration is achievable.

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Keywords: Fractionated CO_2 laser, Hypertrophic scars, Keloids, Microscopic treatment zones, modified Vancouver Scar Scale, Pulsed dye laser

1. Introduction

Cutaneous dermal injury eventuates in the inevitable formation of scar, which may be cosmetically acceptable or unacceptable. The reparative process involves inflammation, granulation tissue formation, and matrix remodeling resulting in variable degree of fibrosis. In some cases, exuberant fibrosis may produce disfiguring keloids and hypertrophic scars **(Clark, 1993).**

Keloids and hypertrophic scars have been notoriously difficult to eradicate with traditional treatments, including surgical excision, corticosteroids and continuous laser destruction, yielding either unsatisfactory results or high lesional recurrence rates **(Alster and Handrick., 2000)**

Over the past decade, advances in pulsed laser technology have enabled successful treatment of these lesions, giving millions of patients a new therapeutic option. Pulsed dye laser improves the textural quality, the appearance of scar erythema, also it affects the scar pliability, hypertrophy and symptoms of patient discomfort **(Bouzari et al., 2007).**

Fractionated CO_2 laser may represent the most popular ablative fractionated laser type. The recent development of ablative fractionated CO_2 technology represents a significant advance for scars treatment. Ablative fractionated laser in general are blamed to induce hyper pigmentation and prolong healing time when used for treatment of scars in the neck, chest and hands (**Tierny and Hanke, 2009**). However, **Stebbins and Hanke in 2011** demonstrated that with appropriately conservative settings, ablative fractionated CO_2 laser can safely be used in off-face areas. This work aimed to study the effect of pulsed dye laser + fractionated CO_2 laser in treatment of keloids and hypertrophic scars.

2. Subjects and methods

Patient Population and setting

A total of 20 patients with keloids and hypertrophic scars underwent laser sessions, during the study period. Patients were seen, treated and followed up in Dermatology department at Salus Medical Center, Florence, Italy and National Institute of Laser Enhanced Sciences, Cairo University, Egypt. The scar

duration ranged from 2 to 11 years. Demographic data collected include age, sex, location and scar duration. Outcome data include incidence of complications and number of procedures.

Study design

We conducted a prospective, before-after, study of all keloids and hypertrophic scar patients who underwent laser treatment of keloids and hypertrophic scars during a 24-month period. In this model, both keloids and hypertrophic scars were assessed before and after treatment, using modified Vancouver Scar Scale (**Table 1**) and a specialized camera (Cutaneous Multi Spectral Analyzator) that detects hemoglobin, melanin and contour. All patients with keloids and hypertrophic scars were treated, with no patients serving as negative, untreated, internal controls. Rather, post treatment patients were compared to pretreatment patients, to measure the effect of the intervention

.

Table 1: Modified Vancouver Scar Scale (Bowes et al, 2002).

Parameter	Rating	Description
Vascularity	0, normal	Color closely resembles the color over the rest of one's body.
	1, pink	A slight increase in local blood supply.
	2, red	A significant increase in local blood supply.
	3, purple	Excessive local blood supply.
Pigmentation	0	Hypopigmentation.
	1, normal	Color closely resembles the color over the rest of one's body.
	2,	Hyperpigmentation.
Pliability	0, normal	Normal pliability.
	1, supple	Flexible with minimal resistance.
	2, yielding	Giving way to pressure; offering moderate resistance.
	3, firm	Solid, inflexible unit, not easily moved, resistant to manual pressure.
	4, banding	Rope like tissue, does not limit the range of motion.
	5, contracture	Permanent shortening of scar, limit the range of motion.

Main Outcome Measures

For this study, we measured functional outcomes, as determined by a scar scale and a specialized camera. For subjective, provider-rated outcomes we used the modified Vancouver Scar Scale which is an easy-to-use method that provides measurement in 3 distinct categories: vascularity, pigmentation and pliability. For objective, patient-reported outcomes, we used Cutaneous MultiSpectral Analyzator that detects hemoglobin, melanin and contour indices. Range of scores for modified Vancouver Scar Scale varied from 0 to 10 with higher score associated with more morbidity.

Data Collection

Both the **modified Vancouver Scar Scale** and the Cutaneous MultiSpectral Analyzator were assessed immediately before the 1st session, after the 6th session and after 6 months follow up after 6th session. **Modified Vancouver Scar Scale** was determined by 2 blinded observers at the time of assessment, regarding the previous **scale** and type of therapy. Hemoglobin, melanin and contour indices were assessed using the Cutaneous Multi Spectral Analyzat or. Data regarding, laser type, laser setting, complications and follow up were recorded and housed in a separate, secure database.

Surgical Technique

All procedures were performed in a hospital based ambulatory surgery center. Patients underwent laser treatment with topical anesthesia only. Specific operative technique, including type of laser and range of settings, has been documented elsewhere, but overall treatment algorithm includes the following modalities:

1. Vascular specific, 595-nm wavelength, pulsed dye laser photothermolysis to reduce hyperemia and improve pliability of the scar (**Dermobeam 2000, DEKA, Calenzano, Italy**). Typical settings include a fluence of 6-6.5 j/cm^2, spot size of 10 mm, pulse duration of 0.5 ms and 1 pass.
2. Ablative, fractionated, 10,600 nm wavelength CO_2 laser resurfacing to correct abnormal texture, thickness, and stiffness of keloids and hypertrophic scars (**SmartXide DOT; DEKA, Calenzano, Italy**). Typical settings include energy per session measuring 15 Ws. Spacing was adjusted at 500 µm and dwell time at 0.7-1 ms. Only 1 pass is performed with the hand piece.

Laser treatment of keloids and hypertrophic scars begins no sooner than 2 years after injury and wound closure and continues every 4 to 6 weeks for 6 sessions (4 sessions with pulsed dye laser and 2 sessions with fractionated CO_2 laser). Wound care includes topical antibiotic ointment (fusidic acid) application twice daily for 7 days and return to work or school within 3 to 5 days after the procedure. Patients do not routinely receive preoperative antibiotic or antiviral prophylaxis, unless they are carriers of methicillin-resistant Staphylococcus aureus or have frequent outbreaks of oral herpes simplex. Post operative analgesia is accomplished with non steroidal anti inflammatory agents.

Statistical analysis

Data were analyzed using SPSS software package version 16.0 (SPSS/Windows Version 16.0, SPSS Inc., Chicago, IL, USA). Student T test, with statistical significance assigned to P values less than 0.05.

3. Results

Patient Demographics

From January 2010 to January 2012 we treated 20 patients with keloids and hypertrophic scars. The duration of keloids ranged from 2 to 9 years (4.62±2.32). The duration of hypertrophic scars ranged from 2 to 11 years (5.06±2.61). The sites involved in the keloids group patients, in order of frequency, were: the shoulder in 5 (41.6%), the neck in 3 (25%), the pre-sternal area in 3 (25%), the hand in 1 (8.4%). The sites involved in the hypertrophic scars group patients were: the forearm in 3 (37.5%), the neck in 2 (25%), the face in 2 (25%), the infra mammary area in 1 (12.5%). Mean age of keloids was 36.25±10.27 and mean age of hypertrophic scars was 41.25±7.88.

Outcome Measures

Over the course of the study modified Vancouver Scar Scale in keloids and hypertrophic scars decreased from 7.00±0.60 to 2.00±1.35 and from 7.13±0.99 to 1.63±0.74 respectively (**Table 2 and 3**). By analyzing the data of Cutaneous MultiSpectral Analyzator camera, the **hemoglobin** index of both keloids and hypertrophic scars reported a highly significant decrease from 47.58±15.93 to 7.61±4.47 and from 48.37±14.24 to 7.75±9.54 ($p<0.01$) respectively. **Melanin** index showed a highly significant decrease in both keloids and hypertrophic scars from 9.50±10 to 5.69±6.07 and from 14.81±8.82 to 8.45±5.03 ($p<0.05$) respectively. **Contour** showed a highly significant improvement in both keloids and hypertrophic scars (**Figure 1 and 2**).

Table 2: Modified Vancouver Scar Scale in keloid patients treated with combined pulsed dye laser and fractionated CO_2 laser**.**

	1 Before the 1st laser session	2 After the 2nd laser session	3 After the 4th laser session	4 After the 6th laser session	5 After 6 months follow up	*P*-value 1&5
Vascularity mean±SD range	2.50±0.52	1.83±0.39	1.00±0.43	0.42±0.51	0.42±0.51	0.000
Pigmentation mean±SD range	1.42±0.51	1.42±0.51	1.25±0.62	0.92±0.67	0.92±0.67	0.007
Pliability mean±SD range	3.08±0.51	2.25±0.75	1.50±0.52	0.67±0.78	0.67±0.78	0.000
Total score mean±SD range	7.00±0.60	5.50±0.80	3.75±0.87	2.00±1.35	2.00±1.35	0.000

Significant *P* value (<0.05) Highly significant *P* value (<0.01)

Table 3: Modified Vancouver Scar Scale in hypertrophic scar patients treated with combined pulsed dye laser and fractionated CO_2 laser**.**

	1 Before the 1st laser session	2 After the 2nd laser session	3 After the 4th laser session	4 After the 6th laser session	5 After 6 month follow up	*P*-value 1&5
Vascularity mean±SD range	2.50±0.53	1.63±0.58	1±0	0.25±0.46	0.25±0.46	0.000
Pigmentation mean±SD range	1.63±0.52	1.50±0.53	1.50±0.53	1.13±0.64	1.13±0.64	0.033
Pliability mean±SD range	3.13±0.64	2.25±0.46	1.37±0.52	0.25±0.46	0.25±0.46	0.000
Total score mean±SD range	7.13±0.99	5.38±0.74	3.88±0.64	1.63±0.74	1.63±0.74	0.000

Significant *P* value (<0.05 Highly significant *P* value **(<0.01)**

Complications

Pulsed dye laser treatments were well tolerated. Subjects described the procedure as near painless. Immediately after treatment all subjects were noted to have purplish discoloration of the scar. No complications were noted during the course of the study. On the other hand, fractionated CO_2 laser was accompanied by post operative erythema which subsided from 3 to 6 days. Shedding of the scabs caused by fractionated CO_2 laser was seen during the 1st week after treatment and patients were reassured that this was a normal sign. We may attribute the absence of complications after the use of pulsed dye laser laser to the fair skin types of patients studied in this work (Fitzpatrick type II and III). Adequate pre and post treatment preparations of patients in the present study with sunscreens, topical retinoids, cold fomentations, and local antibiotics share in the fortunate absence of complications after both pulsed dye and fractionated CO_2 laser sessions. Histo-pathological specimens could not be harvested from patients in this study because they refused any surgical insult for fear of scar deterioration.

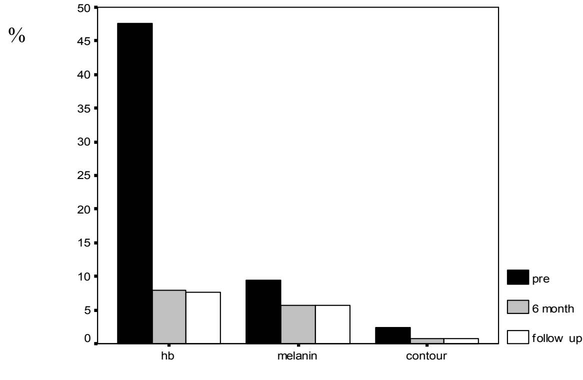


Figure 1: Hemoglobin (hb), melanin and contour of keloids pre, post 6 sessions of combined pulsed dye laser and fractionated CO_2 laser and after follow up of 6 months.

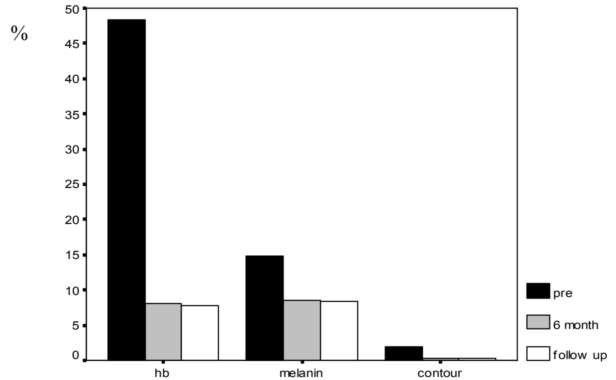


Figure 2: Hemoglobin (hb), melanin and contour of hypertrophic scars pre, post 6 sessions of combined pulsed dye laser and fractionated CO_2 laser and after follow up of 6 months.

Improvements of scar characters were evident in keloids and hypertrophic scars patients to variable levels. The recorded character improvement of the 2 different scar types varied according to the used laser modality (**Figures 3-6**).

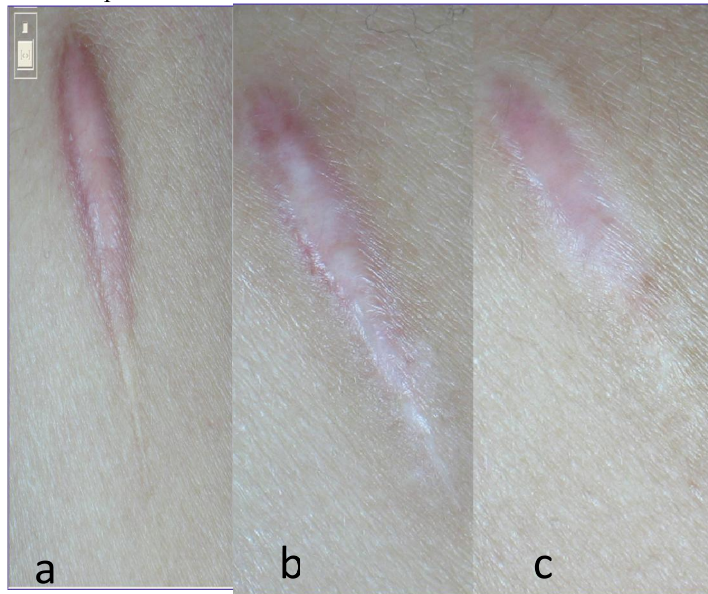


Figure 3: Left shoulder keloid before (a), after (b) 6 sessions of combined pulsed dye laser and fractionated CO_2 laser and after follow up of 6 months (c).

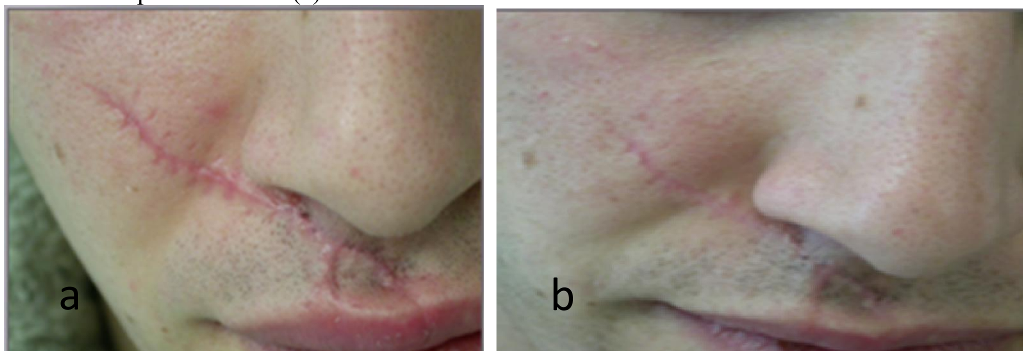


Figure 4: Right cheek and upper lip hypertrophic scar before (a) and after (b) 6 sessions of fractionated CO_2 laser.

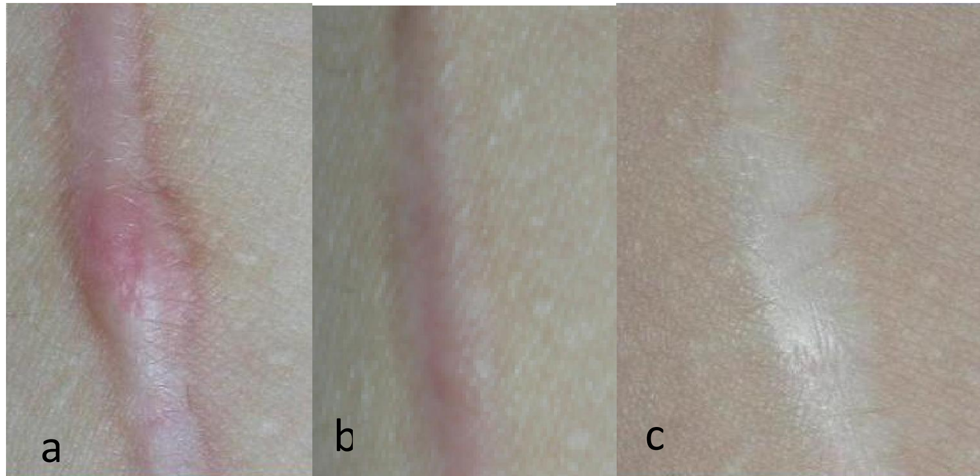
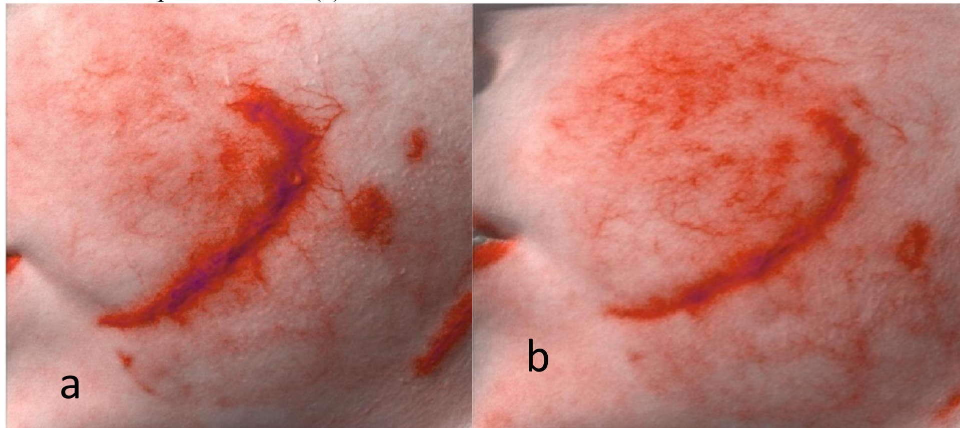


Figure 5: Right arm hypertrophic scar before (a), after (b) 6 sessions of combined pulsed dye laser and fractionated CO_2 laser and after follow up of 6 months (c).



(Figure 6) Cutaneous MultiSpectral Analyzator image for left cheek hypertrophic scar before (a) and after (b) combined pulsed dye and fractionated CO_2 laser treatment showing a decrease in the hemoglobin amount.

3. Discussion

In this prospective, before-after cohort study assessing the effect of laser therapy on keloids and hypertrophic scars, we provide strong evidence that treatment with pulsed dye laser and fractionated CO_2 laser improves the objective and the subjective components of these scars, based on both the modified Vancouver Scar Scale and the Cutaneous MultiSpectral Analyzator. Within the 24 months of this study period, the modified Vancouver Scar Scale decreased significantly in both keloids and hypertrophic scars from 7.00±0.60 to 2.00±1.35 and from 7.13±0.99 to 1.63±0.74 respectively. Hemoglobin, melanin and contour indices decreased with a highly significant improvement. These results were achieved after 6 laser sessions (4 pulsed dye laser and 2 fractionated CO_2 laser sessions). We concluded that laser can be safely combined to treat keloids and hypertrophic scars and achieve nearby results of invasive surgical interference. Laser may not only complement traditional modalities of compression garments, silicone sheeting, deep massage and moisturizing agents, but these emerging technologies may also disrupt current algorithms and reset our expectations of what we can achieve, in restoring form of patients with keloids and hypertrophic scars.

In addition to improving the characteristics of keloids and hypertrophic scars, we note that laser treatments result in substantial gain in the physical components of keloids and hypertrophic scars. The improvement in keloids and hypertrophic scars continue with consecutive sessions. For this reason we recommend several sessions and guide our overall treatment plan based on the response of the patient to these laser treatments. Not only does laser therapy improve the physical aspects of keloids and hypertrophic scar, this approach may also eliminate the need for or decrease the magnitude of reconstructive surgery.

The study results showed that combined pulsed dye laser and fractionated CO_2 laser decreased total modified Vancouver Scar Scale in the keloids patients and hypertrophic scars patients from 7.00±0.60 to

2.00±1.35 and from 7.13±0.99 to 1.63 ±0.74 respectively. **Martin and Collawn in 2013** combined both 7-session laser (pulsed dye laser and fractionated CO_2 laser) with triamcinolone acetonide (TAC) intra-lesional injections and reported a lighter purple color and a flatter appearance of treated keloids. In a study by **Hultman et al, 2013** combined pulsed dye laser (3 to 5 sessions) and fractionated CO_2 laser (1 to 3 sessions) in treatment of burn hypertrophic scars. The interval between sessions was 6 weeks. They wrote that pulsed dye laser is useful for reducing the size and hyperemia and the fractionated CO_2 laser is useful for correcting the abnormal texture, thickness, and stiffness. Improvement in vascularity, pigmentation and pliability, was recorded. Changes in the burn hypertrophic scars were measured by the Vancouver Scar Scale that decreased from 11.3 to 5.5 after the last session. It does worth to mention here that despite the lower pulsed dye laser energy (6-6.5 vs 6-11 j/cm^2) and the shorter pulse duration (0.5 vs 1.5 ms) delivered in the present study, the results were comparable.

Fractionated CO_2 laser seems to be an encouraging approach in treatment of keloids. The CO_2 laser decreases fibroblasts proliferation, increases basic fibroblast growth factor (bFGF) production (that reduces collagen synthesis) and inhibits transforming growth factor beta 1 (TGF-β1) secretion (that increases collagen synthesis) (**Scrimali et al., 2012**). Fractionated resurfacing may improve Hypertrophic scars through vaporization or coagulation of microscopic dermal columns and this in turn stimulates collagen production and remodeling **(Hultman et al., 2012)**.

Pulsed dye laser affects blood vessels of keloids and hypertrophic scars through the concept of selective photothermolysis, in which the light energy emitted from pulsed dye laser, is absorbed by hemoglobin, generating heat and leading to coagulation necrosis **(Liu et al., 2012).** Vascular changes were also noted in pulsed dye laser irradiated tissue beginning with occlusion of the papillary vascular plexus and evolving to longitudinal rearrangement of blood vessels and cross-filling between vessels of adjacent territories (**Lack and Rachel, 2004**). **Manuskiatti et al in 2001** reported a decrease in erythema after treating erythematous keloids segments with pulsed dye laser in laser parameters close to those used in the present study (5-7 j/cm^2 fluence, 0.45 msec pulse duration and 5 mm spot size). Vascularity improvement came significant in their results (p=0.03) after 5-6 sessions pulsed dye laser and came highly significant in the results of the present study (0.000) after 4 sessions Pulsed dye laser and 2 sessions fractionated CO_2 laser. **Chan et al., (2004)** showed a decrease in vascularity after treatment of linear erythematous hypertrophic scars with pulsed dye laser. Fractionated laser may cause microscopic thermal damage to the small blood vessel walls of the dermal vasculature under the effect of photothermolysis (**Glaich et al., 2007**). It does worth to mention here that we could not find published work justifiable for comparison with our study recording keloids vascularity changes after its irradiation with fractionated CO_2 laser.

In the present study after combined pulsed dye laser with fractionated CO_2 laser, improved hypertrophic scars colors: 4 red and 2 purple colored hypertrophic scars changed to normal and 2 purple hypertrophic scars changed to pink.

Pulsed dye laser 585nm targets melanin (**Zelickson et al., 1999**). **Groover and Alster (2000)** used pulsed dye laser 585 nm with fluence between 5-5.5 j/cm^2 with 10 mm spot size for 4 sessions. Improvement of hyper pigmentation was noticed. In the present study using pulsed dye laser and fractionated CO_2 laser 6 out of 10 hyperpigmented keloids and hypertrophic scars turned to normal color. The remaining 4 scars partially responded to the combined laser treatment showing new areas of normal color.

Alster and Handrick., (2000) reported that pulsed dye laser makes dermal collagen finer, more fibrillar, and less dense. They also claimed that ischemia from micro vascular destruction caused by laser, releases collagenase, leading to collagenolysis. They noted that dermal heat produced from blood vessels irradiated by laser can stimulate the collagen synthesis. **Manuskiatti et al., (2007)** used 595-nm pulsed dye laser at a fluence of 7 j/cm^2 and pulse duration of 0.45 ms in treatment of keloids. Patients were treated every 4 weeks for a total of 3 treatments. Improved scar softening and elasticity of segments were noticed. With the same 595-nm pulsed dye laser and very similar parameters for 4 laser sessions of pulsed dye laser and 2 sessions of fractionated CO_2 laser, the results in the present study showed significantly decreased keloids pliability from 3.08±0.52 to 0.67±0.78 and improved its contour from 2.38±0.63 to 0.71±0.29. **Alster and Handrick (2000)** noticed improved pliability and texture of hypertrophic scars treated with 585-nm pulsed dye laser with energy fluencies ranging from 5.5 to 7.5 j/cm^2 and 7-mm or 10 mm spot size. In the present study combining 595-nm pulsed dye laser with fractionated CO_2 laser showed a highly significant decrease in hypertrophic scars pliability from 3.12±0.64 to 0.25±0.46 and improved its contour from 2.01±0.68 to 0.30±0.10 (according to modified Vancouver Scar Scale).

Conclusion:

Combined pulsed dye laser and fractionated CO_2 laser is a new effective modality for treatment of both keloids and hypertrophic scars. In hypertrophic scars the combined fractionated CO_2 laser and pulsed dye laser is very good as we target both hemoglobin and collagen.

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6/25/2013

A survey of Traditional medicinal plants of Uttar Pradesh (India) - Used in treatment of infectious diseases

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Abstract: The present study was to explore the traditional plant knowledge of Uttar Pradesh, India used in infectious disease. Ethnobotanical information was obtained through open informal interviews. The informants were residents, belonging to families living in the study area since generations, and involved at least partially in agriculture. A total of 184 botanical taxa were recorded along with local name, family, habit and parts used. These 184 plants species which provide the crude drugs pertain to 151 genera and 74 families. These plants used to cure 12 infectious ailments. The most widely sought after plant parts in the preparation of remedies in the area are the leaves (17.74%) and bark (14.51%). Plants used by local people were compared with previous ethnobotanical literature, concerning the neighboring areas of Uttar Pradesh. Our results highlight the role of the traditional use of plants in the maintenance of health and the prevention of infectious diseases.
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Keywords: Ethnobotany, Traditional plant, Infectious diseases, Uttar Pradesh, India.

1. Introduction

Traditional medicine is now recognized worldwide as a healthcare resource. The World Health Organization has pointed out the traditional medicine is an important contributor to its health goals (www.wpro.who.int/health). Today, according to the WHO, 80% of the world's population depends on traditional medicine for their primary health care needs. Infectious diseases are the second leading cause of death worldwide (World Health Report, 2002). Infectious disease is a clinically evident disease resulting from the presence of pathogenic microbial agents, including pathogenic viruses, pathogenic bacteria, fungi, protozoa, multicellular parasites, and aberrant proteins known as prions. These pathogens are able to cause disease in animals and or plants. Infectious pathologies are usually qualified as contagious diseases due to their potential of transmission from one person or species to another Infectious disease. Transmission of an infectious disease may occur through one or more of diverse pathways including physical contact with infected individuals. These infecting agents may also be transmitted through liquids, food, body fluids, contaminated objects, airborne inhalation, or through vector-borne spread (MacGraw-Hill Encyclopedia of Science and Technology 2005). The term infectivity describes the ability of an organism to enter, survive and multiply in the host, while the infectiousness of a disease indicates the comparative ease with which the disease is transmitted to other hosts. An infection however, is not synonymous with an infectious disease, as an infection may not cause important clinical symptoms or impair host function. In the current scenario, the integration of local indigenous knowledge for sustainable management and conservation of natural resources is receiving more and more recognition (Posey, 1989). Moreover, an increased emphasis is being placed on possible economic benefits especially of the medicinal use of tropical forest products instead of pure timber harvesting (Reddy et al., 2008). The present study is to focus on the current status of knowledge of medicinal plants of Uttar Pradesh which are used in infectious disease.

2. Material and Methods

Uttar Pradesh (U.P.) is located between 23º 52' N and 31º 28' N lat and 77º 3' and 84º 39' E long. The altitude varies between 50 -500 m above mean sea level. The state is bounded by Nepal on the north, Uttarakhand on the north-east, Himachal Pradesh on the north-west, Haryana on the west, Rajasthan on the south-west, Madhya Pradesh on the south and south-west and Bihar on the east. The climate of the state is tropical monsoonal with annual rainfall varying between 600-2000 mm. The average maximum and minimum temperatures are 48ºC and 2ºC, respectively. There are three distinct seasons: winter from October to February, summer from March to mid-June, and rainy from June to September.

The field work was carried out from September 2009 to March 2010, by gathering ethnobotanical information regarding the use of wild

plants and, in addition, of some cultivated plants. Data were obtained mainly through open informal interviews. All informants were residents, belonging to families living in the study area since generations, and involved, at least partially, in agriculture. Quite often the interviews occurred in the square of village, in the fields, or in the houses of informants. Plant samples were generally collected in the fields upon the indications provided by the informants. Informants were requested to indicate vernacular names, folk uses, parts used, preparation procedures and associations with other plants. Only the plants indicated by at least two informants were considered.

Samples of medicinal plants were collected for scientific identification and herbarium preparation following standard procedures (Jain & Rao, 1977) Citation of author names was given according to Brummitt and Powell (1992). The voucher specimens of plants were deposited in the herbarium of National Botanical Research Institute, Lucknow (LWG). The nomenclature adopted was according to (Uniyal et al., 1994; Uniyal et al., 1997, 1999; Khanna et al., 1999).

3. Results

Through this study, 184 medicinal plant species belonging to 151 genera in 74 families were collected. All the medicinal plants have been written down with their traditional prescriptions. Most ingredients of the prescriptions are plants, of which the local name and the botanical Latin name were written down; some ingredients are things like liquid wax, tallow and muskiness. All the plants have been listed in alphabetical order with the prescriptions (Table 1).

The most cited families of medicinal plants were Caesalpiniaceae, Euphorbiaceae (24.32% each), Fabaceae (21.62%), Malvaceae, Scrophulariaceae, Mimosaceae (8.10% each) and Bignoniaceae, Anacardiaceae, Convolvulaceae and Moraceae (6.75% each) (Fig. 1).

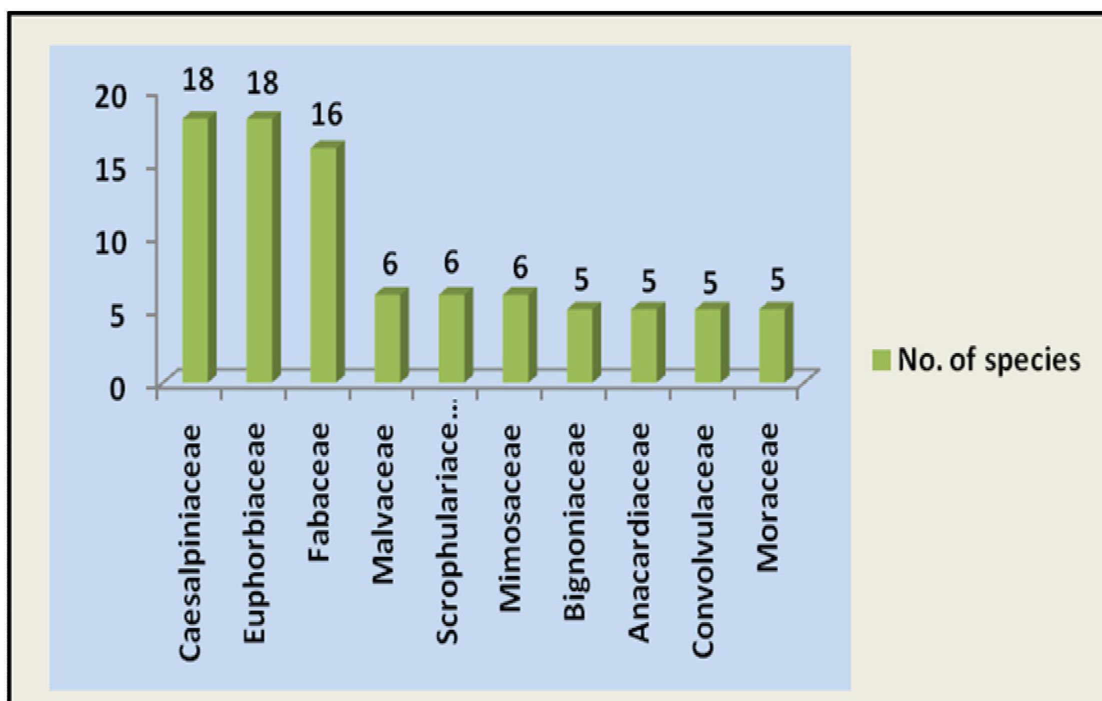


Figure 1. Dominant families of medicinal plants

The results of growth form analysis of medicinal plants showed that herbs made up the highest proportion being represented with (72 spp., 39.13%), followed by trees (61 spp., 33.15%), shrubs (27 spp., 14.67%), climbers (7 spp., 3.84%), twiners (6 spp., 3.26%), undershrubs (3 spp., 1.63%) and lianas (2 spp., 1.08%) (Fig. 2).

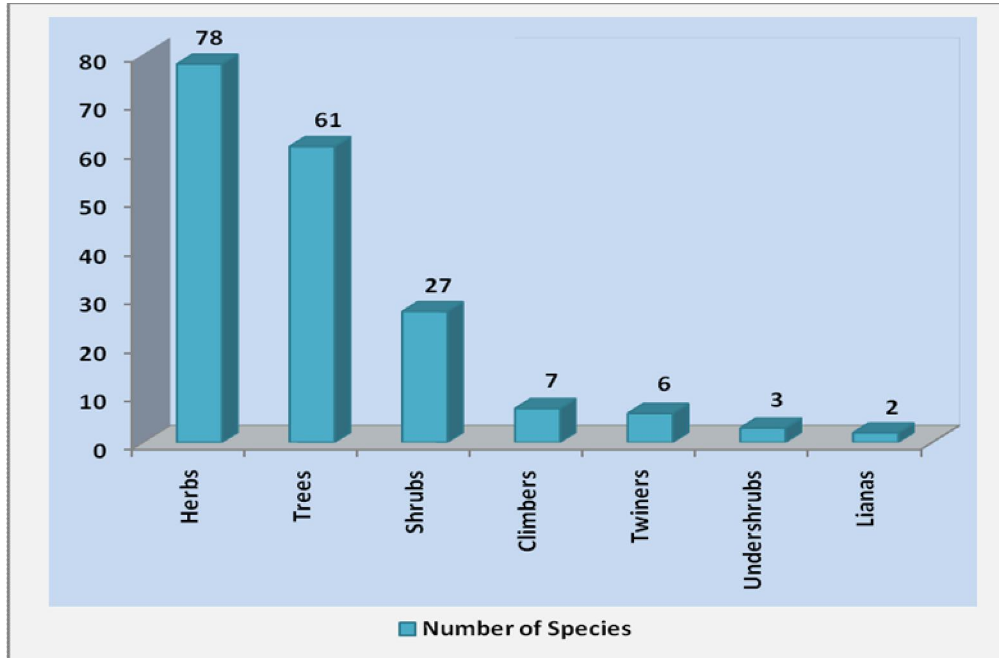


Figure 2. Habit wise distribution of ethnobotanical species

One hundred seventy (93.47%) of the medicinal species are Dicotyledons, twelve are Monocotyledons (6.52%), one is fern and gymnosperm (1.5% each). Different preparations and applications of medicinal plants are shown in Fig. 3. The methods reported for internal use were paste, decoction, powder, juice, oral, extract, infusion, oil and ash. The methods documented for external use included paste, decoction, powder, juice, extract, infusion, oil, ash and direct application of fresh or boiled plant material. Overall, paste was the most cited preparation (31%) followed by decoction (24%), powder (15%), juice (9%), extract, oral (8% each), infusion, oil (2%) and ash (1%).

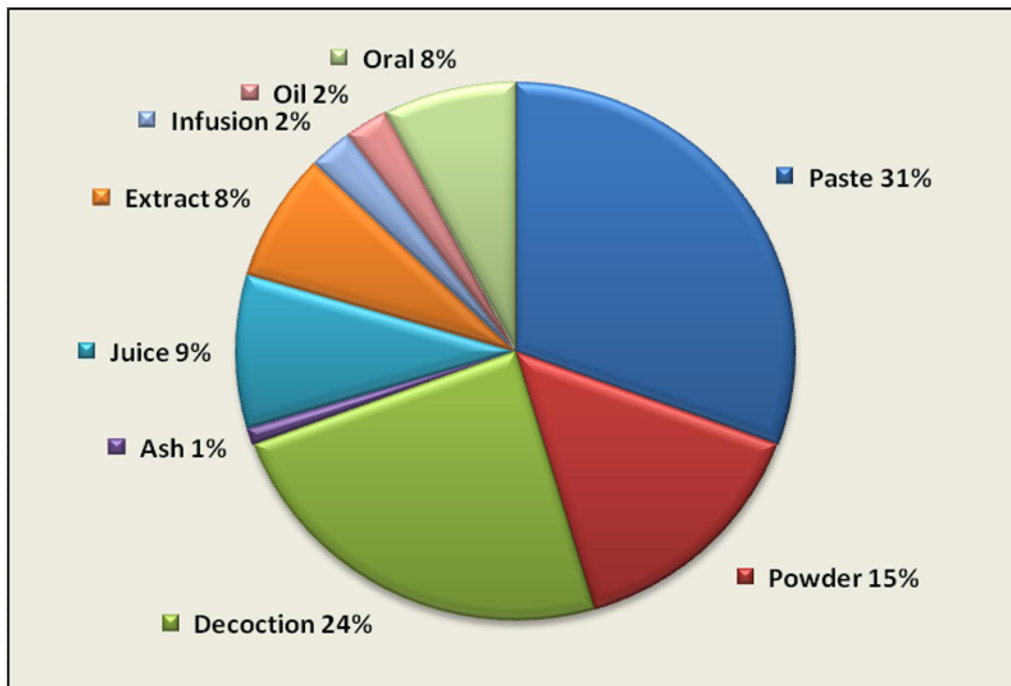


Figure 3. Different preparation methods

The plant parts/product used for medicinal purposes are shown in the Fig. 4, and the and the leaves (17.74%) may be concluded as the most frequently used part followed by bark (14.51%), roots, whole plant (12.90% each), seeds (9.67%), latex (8.06%), rhizome (6.45%), flowers (4.83%), gum, resin (3.22% each) and wood, branch, root bark (1.61% each).

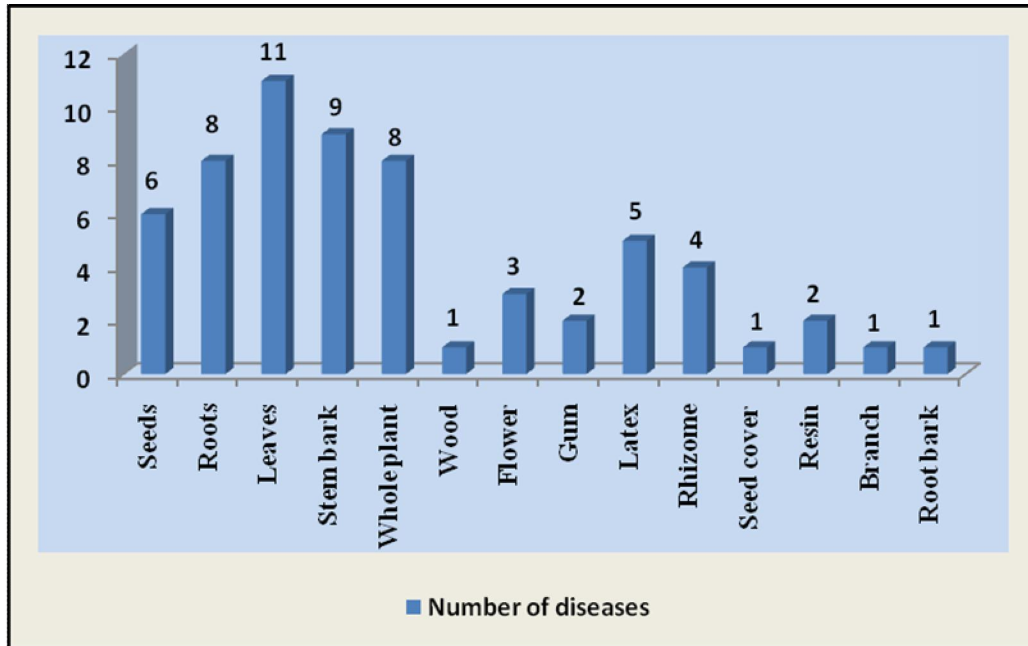


Figure 4. Plant parts used as medicine

The 184 traditional medicinal plants may cure 12 kinds of infectious diseases, among which the dysentery (28.07%) is the most common illness treated followed by Diarrhoea (21.92%), Skin disease (17.69%), Jaundice (9.23%), Ringworm (6.15%), Tuberculosis, Leprosy (4.61% each), Eczema (2.69%), Syphilis, Itches (1.92% each), Cholera (0.76%), Dandruff (0.38%) (Fig. 5).

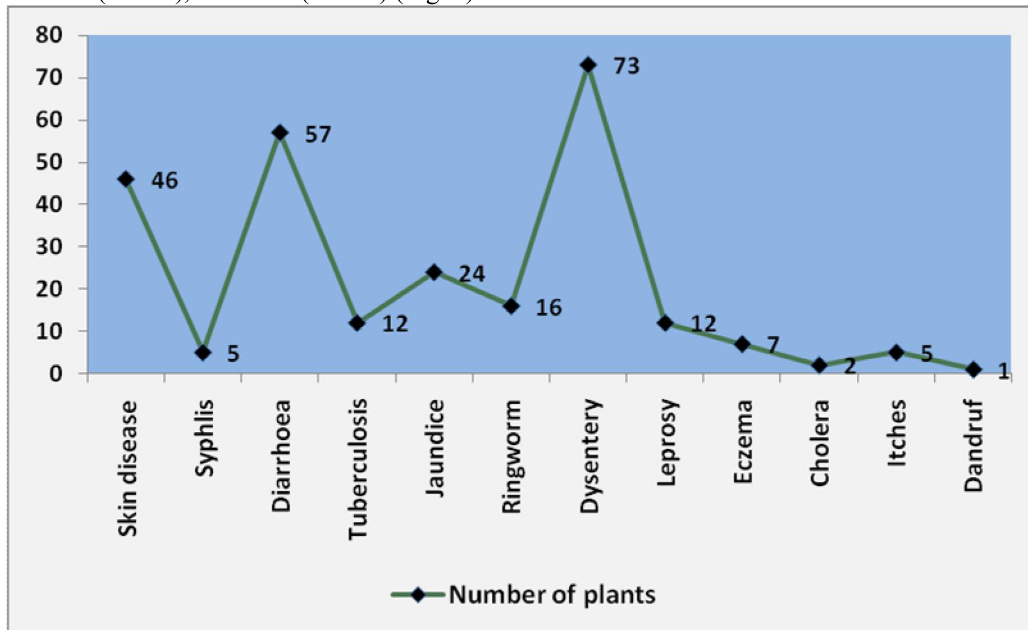
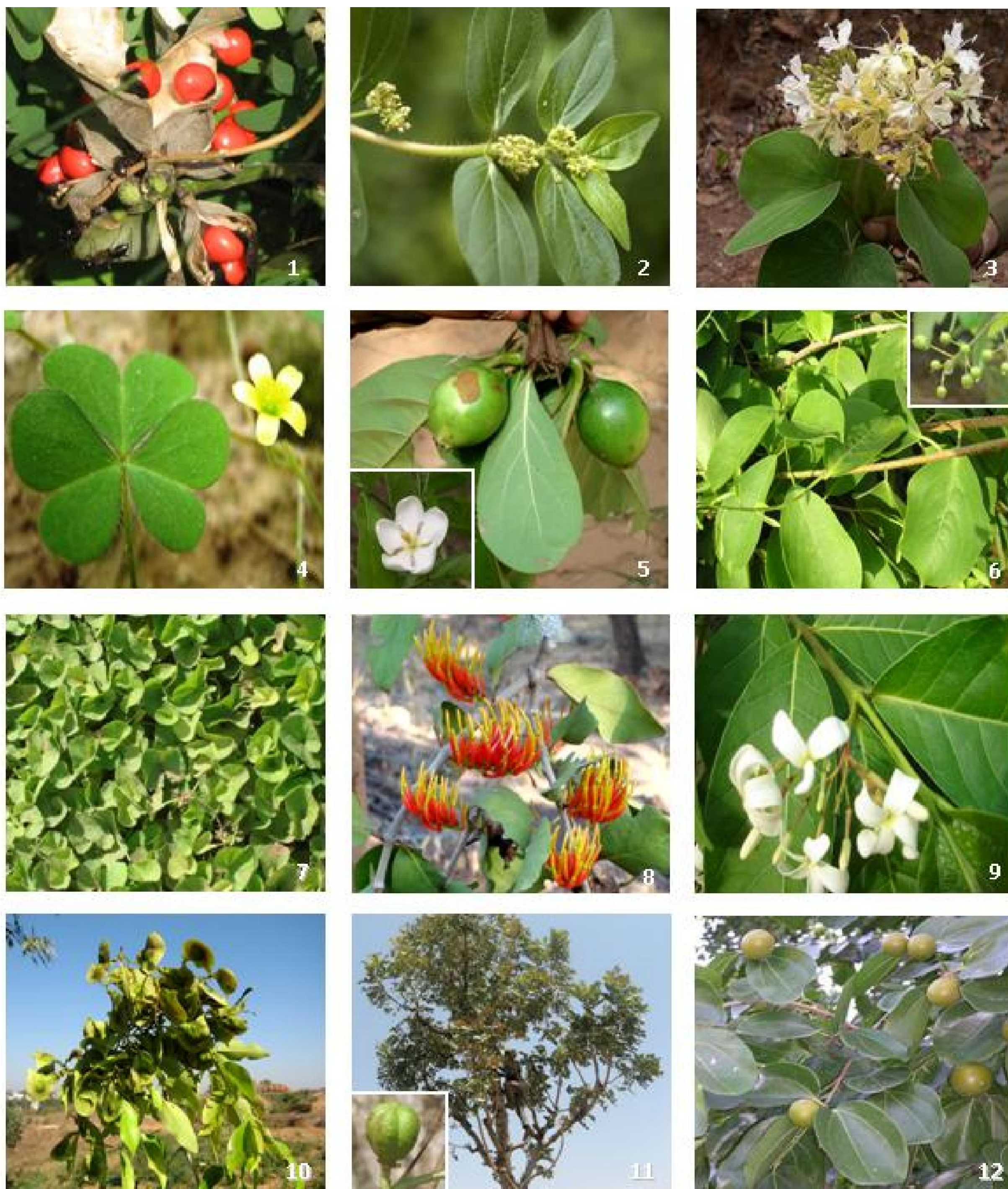


Figure 5. Diseases/symptoms cured by the 184 plants

Appendix Figures



Appendix Figures: **Fig. 1.** *Abrus precatorius* L.; **Fig. 2.** *Euphorbia hirta* L.; **Fig. 3.** *Bauhinia vahlii* Wight & Arn.; **Fig. 4.** *Oxalis corniculata* L.; **Fig. 5.** *Catunaregam uliginosa* (Retz.) Sivarajan; **Fig. 6.** *Celastrus paniculatus* Willd.; **Fig. 7.** *Centella asiatica* (L.) Urban; **Fig. 8.** *Dendrophthoe falcata* (L.f.) Etting.; **Fig. 9.** *Holarrhena pubescens* (Buch.-Ham.) Wall. ex G.Don; **Fig. 10.** *Pterocarpus marsupium* Roxb.; **Fig. 11.** *Soymida febrifuga* (Roxb.) A.Juss.; **Fig. 12.** *Strychnos nux-vomica* L.

The local traditional medicinal knowledge and managing experiences which are practiced, accumulated and passed down from generation to generation may play a significant role in the sustainable use and development of plants resources. Nevertheless, along with the disappearing of biodiversity and negative effects of mainstream culture, the traditional/folk medicinal knowledge of many ethnic groups is facing the danger of losing. Furthermore, the losing of the traditional medicinal knowledge and culture which is the same as the disappearing of biodiversity is not a reversible process. Therefore, it is imperative to carry out the systematic investigation and research on the local traditional medicinal knowledge of Uttar Pradesh.

Table 1. Medicinal plants of Uttar Pradesh used in treatment of infectious diseases

Species Name	Field voucher no.	Local Name	Family	Habit	Parts used	Uses
Abrus precatorius L.	262601	Ghumchi, Ratti	Fabaceae	Climber	Seed and root	Seeds paste in skin diseases; roots paste in dandruff and extract in syphilis.
Abutilon indicum (L.) Sweet	262602	Kakai	Malvaceae	Shrub	Leaf and root	Powdered leaves in diarrhoea. Powdered roots in tuberculosis.
Acacia catechu (L.f.) Willd.	262603	Khair	Mimosaceae	Tree	Bark	Bark paste in skin diseases.
Acacia leucophloea (Roxb.) Willd.	262604	Rewenja, Rayunch	Mimosaceae	Tree	Bark and root	Bark decoction in diarrhoea. Powdered roots in jaundice.
Acacia nilotica (L.) Willd. ex Delile subsp. *indica* (Benth.) Brenan	262605	Babool	Mimosaceae	Tree	Leaf	Pounded leaves in jaundice.
Acalypha indica L.	262606	Khokali	Euphorbiaceae	Herb	Leaf	Leaves paste in ringworm.
Acanthospermum hispidum DC.	262607	-	Asteraceae	Herb	Leaf	Leaves paste in skin diseases.
Achyranthes aspera L.	262608	Chirchiri, Latjira	Amaranthaceae	Herb	Whole plant and root	Plant ash in jaundice. Powdered roots in dysentery.
Adhatoda zeylanica Medik.	262609	Adusa	Acanthaceae	Shrub	Leaf	Leaves decoction in tuberculosis and paste in ringworm.
Agave americana L.	262610	Patangwar	Agavaceae	Herb	Leaf	Pulp of leaves in skin diseases.
Albizia lebbeck (L.) Benth.	262611	Siris	Mimosaceae	Tree	Leaf	Leaves juice in curing white spots on skin.
Albizzia odoratissima (Willd.) Benth.	262612	-	Mimosaceae	Tree	Bark	Bark paste in leprosy, and other skin diseases.
Ammannia baccifera L.	262613	-	Lythraceae	Herb	Leaf	Leaves paste in skin diseases.
Anagallis arvensis L.	262614	Krishna neel	Primulaceae	Herb	Whole plant	Plants paste in leprosy.
Apluda mutica L.	262615	Sutara	Poaceae	Herb	Whole plant	Plants juice in dysentery.
Ardisia solanacea Roxb.	262616	-	Myrsinaceae	Shrub	Root	Roots decoction in diarrhoea.
Argemone mexicana L.	262617	Bharbhanda, Kateli	Papaveraceae	Herb	Whole plant and root	Plants juice in jaundice and skin diseases. Roots extract in itching.
Argyreia nervosa (Burm. f.) Bojer	262618	Samandar-ka-pat	Convolvulaceae	Climber	Leaf	Leaves paste in skin diseases.
Asparagus racemosus Willd.	262619	Satawar	Liliaceae	Herb	Root	Powdered roots in jaundice.
Azadirachta indica A.Juss.	262620	Neem	Meliaceae	Tree	Leaf and seed	Leaves decoction in skin diseases. Seeds oil in leprosy.
Bacopa monnieri (L.)	262621	Jalneem,	Scrophulariaceae	Herb	Whole	Plants decoction in

Wettst.		Brahmi			plant and seed	epilepsy. Seeds paste in skin disease.
Balanites aegyptiaca (L.) Delile	262622	Ingua, Hingot	Simaroubaceae	Small tree	Fruit	Fruits pulp in tuberculosis.
Bambusa arundinacea (Retz.) Willd.	262623	Bans	Poaceae	Tree	Wood	Wood oil in eczema.
Bauhinia acuminata L.	262624	Sivamalli	Caesalpiniaceae	Shrub	Bark	Bark decoction in leprosy.
Bauhinia racemosa Lam.	262625	Kachnal	Fabaceae	Small tree	Bark	Pounded bark powder in dysentery and diarrhoea.
Bauhinia tomentosa L.	262626	Kachnar	Caesalpiniaceae	Small tree	Leaf and flower	Dried leaves and flowers in dysentery.
Bauhinia vahlii Wight & Arn.	262627	Mohlain	Caesalpiniaceae	Liana	Bark and root	Bark decoction in dysentery and cholera. Roots extract in syphilis.
Bauhinia variegata L.	262628	Kachnar	Caesalpiniaceae	Small tree	Bark	Bark decoction in dysentery, leprosy, syphilis and other skin diseases.
Boerhavia diffusa L.	262629	Santan, Santh, Gadapurena, Punarnava	Nyctaginaceae	Herb	Leaf	Leaves decoction in jaundice. Cooked leaves in jaundice.
Bombax ceiba L.	262630	-	Bombacaceae	Tree	Fruit	Fruits in dysentery.
Bridelia stipularis (L.) Blume	262631	Khaji	Euphorbiaceae	Small tree	Leaf	Leaves decoction in jaundice.
Buchanania lanzan Spreng.	262632	Chirongi	Anacardiaceae	Tree	Leaf, gum, and seed	Leaves paste in skin diseases. Gum in diarrhoea. Seeds paste in skin diseases.
Buddleja asiatica Lour.	262633	Nayari	Loganiaceae	Shrub	Leaf	Leaves paste in eczema.
Bulbostylis barbata (Rottb.) C.B.Clarke	262634	-	Cyperaceae	Herb	Whole plant	Plant paste in dysentery.
Butea monosperma (Lam.) Taub.	262635	Chihula, Palas	Fabaceae	Small tree	Leaf, flower, bark and gum	Leaves paste in blood dysentery and ringworms. Flowers decoction in skin diseases. Bark decoction in dysentery. Gum in diarrhoea and dysentery.
Butea parviflora Roxb.	262636	Vyavhar	Fabaceae	Liana	Flower	Flowers decoction in skin disease.
Cajanus cajan (L.) Millsp.	262637	Arhar, Rahar	Fabaceae	Shrub	Leaf and root	Leaves paste in cholera and jaundice. Powdered leaves and roots in dysentery.
Cajanus scarabaeoides (L.) du Petit-Thouars,	262638	Bankulthi	Fabaceae	Shrub	Whole plant	Plants decoction in dysentery.
Calotropis gigantea (L.) R.Br.	262639	Akaua	Asclepiadaceae	Shrub	Root	Powdered root bark in jaundice.
Calotropis procera (Aiton) R.Br.	262640	Madar	Asclepiadaceae	Shrub	Leaf and latex	Leaves paste on skin diseases. Latex in itching, eczema, ringworm, and leprosy.
Capparis spinosa L.	262641	Baferu	Capparaceae	Shrub	Root	Roots extract in jaundice.
Capsella bursa-pastoris (L.) Medik.	262642	-	Brassicaceae	Herb	Leaf	Leaves juice in dysentery.
Carica papaya L.	262643	Papita	Caricaceae	Tree	Latex	Latex in ringworm and jaundice.
Casearia tomentosa Roxb.	262644	Chilla	Samydaceae	Small tree	Bark	Bark juice in ringworm.
Cassia absus L.	262645	Chaksu	Caesalpiniaceae	Herb	Seed	Seeds paste in skin diseases.

Cassia fistula L.	262646	Amaltas	Caesalpiniaceae	Small tree	Leaf, Fruit and seed	Leaves paste in skin diseases. Fruits in dysentery. Powdered seeds in jaundice.
Cassia obtusifolia L.	262647	Panevar	Caesalpiniaceae	Herb	Root	Roots paste in ringworm.
Cassia occidentalis L.	262648	Chakwad	Caesalpiniaceae	Undershrub	Leaf	Leaves paste in skin disease.
Cassia tora L.	262649	Chakunda	Caesalpiniaceae	Undershrub	Whole plant and seed	Plants paste in skin diseases. Seeds paste on itches, eczema and ringworm.
Cassine glauca (Rottb.) Kuntze	262650	Mamar	Celastraceae	Tree	Root	Root bark extract in dysentery.
Casuarina equisetifolia L.	262651	Jhau, Jangli saru	Casuarinaceae	Tree	Bark	Bark decoction in diarrhoea and dysentery.
Catunaregam uliginosa (Retz.) Sivarajan	262652	Pindalu	Rubiaceae	Small tree	Fruit	Fruits in dysentery.
Celastrus paniculatus Willd.	262653	Unjan	Celastraceae	Liana	Fruit	Fruits paste in dysentery and diarrhoea.
Celosia argentea L.	262654	Safed murga ka phul	Amaranthaceae	Herb	Seed	Powdered seeds in diarrhoea and dysentery.
Centella asiatica (L.) Urban	262655	Brahmi-buti	Apiaceae	Herb	Whole plant	Plants decoction in skin diseases and leprosy.
Chloroxylon swietenia DC.	262656	Bharhul, Bharuee	Meliaceae	Tree	Bark	Bark decoction in jaundice.
Cissampelos pareira L.	262657	Batulia	Menispermaceae	Climber	Root	Roots extract in dysentery.
Citrus medica L.	262658	Neembu	Rutaceae	Shrub	Fruit	Fruits juice in dysentery.
Clerodendrum cordatum D.Don	262659	Bhat	Verbenaceae	Shrub	Bark	Powdered bark in skin diseases.
Clitoria ternatea L.	262660	Gokari, Aparajita	Fabaceae	Climber	Root	Powdered roots in dysentery.
Cocculus hirsutus (L.) Diels	262661	Charenti	Menispermaceae	Herb	Whole plant and Leaf	Plants decoction in dysentery. Leaves paste in eczema.
Commelina diffusa Burm.f.	262662	-	Commelinaceae	Herb	Whole plant	Plants paste on itches.
Corchorus aestuans L.	262663	Chench	Tiliaceae	Herb	Whole plant	Plants decoction in diarrhoea.
Corchorus capsularis L.	262664	Kharenti	Tiliaceae	Herb	Leaf	Leaves decoction in dysentery.
Coriandrum sativum L.	262665	Dhania	Apiaceae	Herb	Fruit	Powdered fruits in dysentery.
Croton bonplandianus Baill.	262666	-	Euphorbiaceae	Herb	Leaf	Leaves decoction in diarrhoea.
Cuminum cyminum L.	262667	Jeera	Apiaceae	Herb	Seed	Powdered seeds in diarrhoea.
Curculigo orchioides Gaertn.	262668	Kali-musali	Hypoxidaceae	Herb	Whole plant and root	Plants paste in jaundice and diarrhoea. Roots paste on itching and other skin diseases.
Curcuma longa L.	262669	Haldi	Zingiberaceae	Herb	Rhizome	Powdered rhizomes in skin diseases.
Cynodon dactylon (L.) Pers.	262670	Dubghas	Poaceae	Herb	Whole plant	Plants paste in leprosy.
Dendrophthoe falcata (L.f.) Etting.	262670	Banda	Loranthaceae	Shrub	Whole plant	Plants extract in tuberculosis.
Desmodium triflorum (L.) DC.	262672	Throughout years	Fabaceae	Herb	Leaf	Leaves paste in diarrhoea and dysentery.
Diospyros exsculpta	262673	Tendu or	Ebenaceae	Shrub	Fruit	Fruits pulp in dysentery and

Buch-Ham.		Kundi				diarrhoea.
Diospyros malabarica (Ders.) Kostel	262674	Tendu	Ebenaceae	Tree	Bark	Powdered bark in dysentery.
Emblica officinalis Gaertn.	262675	-	Euphorbiaceae	Tree	Bark	Bark decoction in diarrhoea, dysentery and cholera.
Euphorbia hirta L.	262676	Dudhi	Euphorbiaceae	Herb	Whole plant	Plants juice in dysentery.
Euphorbia thymifolia L.	262677	Lal dudhi	Euphorbiaceae	Herb	Whole plant	Plants decoction in blood dysentery.
Feronia limonia L.	262678	Kaith	Rutaceae	Tree	Fruit	Fruits in diarrhoea and dysentery.
Ficus benghalensis L.	262679	Bargad	Moraceae	Tree	Bark	Bark infusion in diarrhoea and dysentery.
Ficus glomerata Roxb.	262680	Gular	Moraceae	Tree	Fruit	Fruits infusion in diarrhoea and dysentery.
Ficus heterophylla L.f.	262681	Traymana	Moraceae	Shrub	Leaf	Leaves juice in dysentery.
Ficus racemosa L.	262682	Gular	Moraceae	Tree	Latex and fruit	Latex in dysentery. Fruits in diarrhoea.
Flacourtia indica (Burm.f.) Merr.	262683	Rakatsonk, Kateyya	Flacourtiaceae	Small tree	Bark and fruit	Bark decoction in dysentery and eczema. Fruits juice in jaundice.
Flacourtia jangomas (Lour.) Raeusch.	262684	Paniyala	Flacourtiaceae	Small tree	Leaf	Leaves paste in diarrhoea.
Hamelia patens Jacq.	262685	-	Rubiaceae		Fruit	Berries-syrup in dysentery.
Hedychium coronarium Buch.-Ham.	262686	Seerh	Zingiberaceae	Shrub	Rhizome	Powdered rhizomes in diarrhoea.
Helicteres isora L.	262687	Murerua or Rasbhari or Aithan	Sterculiaceae	Shrub	Fruit, bark and seed	Fruits paste in dysentery. Bark in diarrhoea and dysentery. Seeds paste in dysentery.
Hemidesmus indicus (L.) R.Br.	262688	Cherdudhia	Asclepiadaceae	Herb	Leaf and root	Leaves infusion in diarrhoea. Roots extract in eczema and ringworm infection.
Hibiscus rosa-sinensis L.	262689	Gurhal	Malvaceae	Shrub	Flower	Flowers extract in tuberculosis.
Holarrhena pubescens (Buch.-Ham.) Wall. ex G.Don	262690	Chirol	Apocynaceae	Shrub	Bark	Bark decoction in dysentery; paste in diarrhoea.
Holoptelea integrifolia (Roxb.) Planch.	262691	Chilbil	Ulmaceae	Tree	Leaf	Leaves paste in ringworm.
Hyptis suaveolens (L.) Poit.	262692	Bantulsi	Lamiaceae	Herb	Seed	Seeds oil in skin diseases.
Indigofera astragalina DC.	262693	Hairy Indigo	Fabaceae	Herb	Leaf	Leaves decoction in diarrhoea.
Indigofera linnaei Ali	262694	Latahai	Fabaceae	Herb	Leaf	Leaves decoction in diarrhoea.
Ipomoea eriocarpa R.Br.	262695	Nakhari	Convolvulaceae	Twiner	Whole plant	Plants paste in leprosy.
Ipomoea nil (L.) Roth	262696	Paturia/ Kala dana	Convolvulaceae	Climber	Seed	Pounded seeds in diarrhoea.
Jacaranda mimosaefolia D.Don	262697	Nila gulmohar	Bignoniaceae	Tree	Leaf and bark	Leaves in syphilis.
Jasminum humile L.	262698	Pili - Chameli	Oleaceae	Shrub	Root	Roots paste in ringworm.
Jatropha curcas L.	262699	Rtanjot	Euphorbiaceae	Shrub	Bark	Powdered bark in dysentery

						and tuberculosis.
Jatropha gossypifolia L.	262700	Banren	Euphorbiaceae	Shrub	Leaf	Leaves paste in eczema and itches.
Kigelia africana (Lam.) Benth.	262701	Balamkhira	Bignoniaceae	Tree	Fruit	Fruits paste in skin diseases.
Kyllingia brevifolia Rottb.	262702	-	Cyperaceae	Herb	Leaf	Powdered leaves in diarrhoea.
Lablab purpureus (L.) Sweet	262703	Sem	Fabaceae	Climber	Leaf	Leaves paste on skin diseases.
Lannea coromandelica (Houtt.) Merr.	262704	Jingna	Anacardiaceae	Tree	Bark	Bark decoction in skin disease.
Lawsonia inermis L.	262705	Mehandi	Lythraceae	Shrub	Leaf	Leaves juice in dysentery.
Leonotis nepetifolia (L.) R.Br.	262706	Baraguma, Hejurchei	Lamiaceae	Herb	Flower	Flowers paste in skin infection.
Leucas cephalotes (Koenig ex Roth) Spreng.	262707	Gumma	Lamiaceae	Herb	Leaf	Leaves decoction in dysentery and diarrhoea.
Limonia acidissima L.	262708	Kaitha/Kait	Rutaceae	Tree	Fruit	Fruits pulp in diarrhoea and dysentery.
Lindenbergia muraria (Roxb. ex D.Don) Bruehl	262709	-	Scrophullariaceae	Herb	Leaf	Powdered leaves in tuberculosis.
Lindernia crustacea (L.) F.v.Muell.	262710	-	Scrophullariaceae	Herb	Whole plant	Plants in dysentery and ringworm.
Ludwigia adscendens (L.) Hara	262711	-	Onagraceae	Herb	Whole plant	Plants paste in skin diseases.
Ludwigia octovalvis (Jacq.) Raven	262712	-	Onagraceae	Herb	Whole Plant	Plants extract in diarrhoea.
Luffa acutangula (L.) Roxb.	262713	Satputia	Cucurbitaceae	Climber	Leaf	Leaves paste in leprosy.
Mallotus philippensis (Lam.) Muell. Arg.	262714	Rohini	Euphorbiaceae	Tree	Leaf, fruit and bark	Leaves paste in skin diseases. Powdered fruits in dysentery. Bark decoction in jaundice.
Malvastrum coromandelianum (L.) Garcke	262715	Bariara	Malvaceae	Herb	Seed	Seeds decoction in dysentery.
Mangifera indica L.	262716	Aam	Anacardiaceae	Tree	Bark and fruit	Fruits pulp in dysentery. Bark decoction in jaundice.
Martynia annua L.	262717	Biswat	Martyniaceae	Shrub	Seed	Seeds oil in eczema and other skin diseases.
Medicago polymorpha L.	262718	Jangli ghas	Fabaceae	Herb	Leaf	Leaves decoction in dysentery.
Melilotus indica (L.) All.	262719	Senji	Fabaceae	Herb	Seed	Seeds in diarrhoea.
Melochia corchorifolia L.	262720	Bilpat	Sterculiaceae	Undershrub	Leaf	Leaves decoction in dysentery.
Mimusops elengi L.	262721	Maulsiri	Sapotaceae	Tree	Bark	Bark decoction in dysentery.
Nelumbo nucifera Gaertn.	262722	Kamal	Nelumbonaceae	Herb	Rhizome	Powdered rhizomes in diarrhoea and dysentery.
Nymphaea nouchali Burm.f.	262723	Kamal	Nympheaceae	Herb	Rhizome	Powdered rhizomes in dysentery and diarrhoea.
Ochna pumila Buch.-Ham. ex D.Don	262724	Bhuikusum	Ochnaceae	Undershrub	Root	Roots decoction in dysentery and diarrhoea.
Operculina turpethum (L.) Manso	262725	Pipal pant	Convolvulaceae	Climber	Leaf	Leaves sap in itching and ringworm.
Oroxylum indicum (L.) Venten	262726	-	Bignoniaceae	Tree	Leaf, bark and	Leaves decoction in diarrhoea. Bark decoction in

					root	jaundice. Roots decoction in dysentery.
Ougeinia oogeinensis (Roxb.) Hochr.	262727	Sanan	Fabaceae	Tree	Bark	Bark decoction in dysentery and diarrhoea.
Oxalis corniculata L.	262728	Khatibuti, Khatamithi, Teepatiya	Oxalidaceae	Herb	Leaf	Leaves decoction in dysentery.
Peltophorum pterocarpum (DC.) Baker ex K.Heyne	262729	Copper pod	Caesalpiniaceae	Tree	Bark	Powdered bark in dysentery.
Phanera integrifolia (Roxb.) Benth.	262730	Mohlain	Caesalpiniaceae		Root	Roots extract in syphilis.
Phyllanthus virgatus Forst.f.	262731	Banaunri	Euphorbiaceae	Herb	Leaf	Leaves juice in dysentery.
Pistia stratiotes L.	262732	Pistia, Jalkumbhi	Araceae	Herb	Leaf	Leaves juice in skin diseases.
Plantago ovota Forssk.	262733	Isaphgol	Plantaginaceae	Herb	Seed cover	Husk in dysentery and diarrhoea.
Pongamia pinnata (L.) Pierre	262734	Karanj	Rhamnaceae	Tree	Seed	Powdered seeds in ringworm.
Premna latifolia Roxb.	262735	Bakar	Verbenaceae	Small tree	Bark	Bark extract in ringworm.
Prosopis cineraria (L.) Druce.	262736	Chaonka	Mimosaceae	Small tree	Leaf	Leaves paste in ringworm.
Psidium guajava L.	262737	Amrood	Myrtaceae	Small tree	Leaf	Leaves extract in cholera, diarrhoea and dysentery.
Pterocarpus marsupium Roxb.	262738	Vijasal	Fabaceae	Tree	Leaf	Leaves paste in skin diseases.
Quisqualis indica L.	262739	Malti	Combrataceae	Tree	Seed	Seeds in diarrhoea.
Raphanus sativus L.	262740	Mooli	Brassicaceae	Herb	Root	Roots juice in jaundice
Ricinus communis L.	262741	Rendi	Euphorbiaceae	Small tree	Leaf	Leaves decoction in jaundice.
Rivea hypocrateriformis (Desr.) Choisy	262742	Phang	Convolvulaceae	Climber	Leaf and seed	Leaves paste in tuberculosis. Powdered seeds in tuberculosis.
Saccharum officinarum L.	262743	Ikh	Poaceae	Herb	Whole plant	Plants juice in jaundice.
Sapindus trifoliatus L.	262744	Reetha	Sapindaceae	Tree	Fruit	Fruits paste in leprosy.
Saraca asoca (Roxb.) de Wilde	262745	Ashok	Caesalpiniaceae	Tree	Flower	Flowers infusion in blood dysentery.
Scoparia dulcis L.	262746	Sweet broom weed	Scrophullariaceae	Herb	Leaf	Powdered leaves in diarrhoea.
Selaginella bryopteris (L.) Bak.	262747	Kamraj	Selaginellaceae	Herb	Whole plant	Plants paste in dysentery.
Semecarpus anacardium L.f.	262748	Bhela	Anacardiaceae	Tree	Resin	Gum-resin in leprosy and skin diseases.
Shorea robusta Roxb. ex Gaertn.f.	262749	Sal, Sakhul	Dipterocarpaceae	Tree	Fruit and gum	Fruits paste in dysentery. Gum in dysentery.
Sida cordata (Burm.f.) Borss.	262750	Baharbuta	Malvaceae	Herb	Whole plant	Plants extract in dysentery.
Sida rhombifolia L.	262751	Sahadevi	Malvaceae	Herb	Whole plant	Plants powder in tuberculosis.
Solanum nigrum L.	262752	Makoi	Solanaceae	Herb	Whole plant and leaf	Plants juice in diarrhoea and decoction in jaundice. Leaves paste in skin diseases and decoction in jaundice.
Solanum surattense Burm.f.	262753	Kantakeri	Solanaceae	Herb	Flower	Flowers juice in diarrhoea.
Solanum viarum	262754	-	Solanaceae	Herb	Fruit	Fruits extract in jaundice.

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Sonchus wightianus DC.	262755	Gobhi	Asteraceae	Herb	Root	Powdered roots in tuberculosis.
Soymida febrifuga (Roxb.) A.Juss.	262756	-	Meliaceae	Tree	Bark	Bark decoction in diarrhoea.
Spathodea campanulata P.Beauv.	262757	Rugtoora	Bignoniaceae	Tree	Bark	Bark paste in skin diseases and dysentery.
Spergula arvensis L.	262758	-	Caryophyllaceae	Herb	Whole plant	Plants in tuberculosis.
Spondias pinnata (L.f.) Kurz	262759	Amra, Wild Mango	Anacardiaceae	Tree	Bark	Bark decoction in diarrhoea and dysentery.
Stephania japonica (Thunb.) Miers	262760	Vanatikatika	Menispermaceae	Herb	Root	Roots decoction in diarrhoea.
Streblus asper Lour.	262761	Sihoor	Moraceae	Tree	Bark	Bark decoction in diarrhoea and dysentery.
Strychnos nux-vomica L.	262762	Kuchila	Loganiaceae	Tree	Seed	Seeds paste in eczema and ringworm.
Syzygium cumini (L.) Skeels	262763	-	Myrtaceae	Tree	Bark	Bark juice in diarrhoea.
Syzygium heyneanum Wall. ex Duthie	262764	Jamati	Myrtaceae	Tree	Bark	Bark paste in dysentery.
Tecomaria capensis (Thunb.) Spach	262765	-	Bignoniaceae	Shrub	Leaf	Leaves decoction in diarrhoea.
Tephrosia purpurea (L.) Pers.	262766	Sarpokha	Fabaceae	Herb	Leaf	Leaves paste in skin diseases.
Terminalia arjuna (Roxb. ex DC.) Wight & Arn.	262767	Arjun	Combrataceae	Tree	Bark	Bark decoction in dysentery, jaundice and cholera.
Terminalia bellirica (Gaertn.) Roxb.	262768	Bahera	Combrataceae	Tree	Fruit	Powdered fruits in leprosy and diarrhoea.
Terminalia chebula Retz.	262769	Harra	Combrataceae	Tree	Fruit and seed	Fruits paste in dysentery and diarrhoea. Seeds powder in diarrhoea.
Thalictrum foliolosum DC.	262770	Supowa	Ranunculaceae	Herb	Root	Roots extract in dysentery and diarrhoea.
Thespesia populnea (L.) Soland. ex Corrêa	262771	Parsipu	Malvaceae	Small tree	Bark and seed	Seeds paste in skin troubles. Bark in dysentery.
Thuja orientalis L.	262772	Morpankhi	Cupressaceae	Small tree	Leaf	Leaves ash in dysentery.
Toona ciliata M.Roem.	262773	Tun, Mahaneem	Meliaceae	Tree	Bark	Bark paste in dysentery.
Trachyspermum ammi (L.) Sprague	262774	Ajwain	Apiaceae	Herb	Seed	Seeds paste in diarrhoea.
Urtica dioica L.	262775	Bichhughas	Urticaceae		Whole plant	Plants decoction in jaundice.
Verbascum chinense (L.) Sant.	262776	-	Scrophulariaceae	Herb	Leaf	Leaves juice in skin disorders.
Veronica anagallis-aquatica L.	262777	Titlokia	Scrophulariaceae	Herb	Root	Roots decoction in diarrhoea.
Vitis vinifera L.	262778	Angur	Vitaceae	Climber	Branch	Sap of young branches in skin infections.
Wahlenbergia marginata (Thunb.) DC.	262779	-	Campanulaceae	Herb	Whole plant	Plants paste in skin troubles.
Xanthium indicum Koenig	262780	Bichchu, Gokharu, Kaktoni	Asteraceae	Herb	Leaf and seed	Leaves juice in ringworms. Seeds oil in skin diseases.
Zingiber officinale Roscoe	262781	Adrak	Zingiberaceae	Herb	Rhizome	Powdered rhizomes in tuberculosis.
Ziziphus mauritiana	262782	Ber	Rhamnaceae	Small tree	Seed	Seeds decoction in

Lam.						diarrhoea.
Ziziphus oenoplia (L.) Mill.	262783	Jharberi	Rhamnaceae	Shrub	Fruit	Fruits paste in dysentery.
Ziziphus xylopyra Willd.	262784	Guthar	Rhamnaceae	Small tree	Root bark	Roots bark extract in dysentery.

4. Conclusions

The study is of great importance to preserve the knowledge of medicinal plants used by the tribal people and exploit the knowledge in treatment of various diseases. Moreover, further phytochemical and pharmacological studies of little noticed medicinal plants are an urgent need to understand the underlying mechanism of traditional treatment systems. On the other hand, these plants hold tremendous potentials for pharmaceutical products of commercial values. The search for new biologically active compounds from plants usually starts in the field and depends on the specific ethnic and folk information obtained from local practitioners. The conservation of the medicinal plants is an essential requirement for maintaining traditional medicine as a medicinal and cultural resource.

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The Influence of Raw Material Composition on the Quality of Sinter

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Abstract: In this study provides an overview of chemical and physical properties of commercial iron ores as well as their influence on sintering performance. The sinter structure and its characteristics mainly depend on the raw material chemistry, phase body, crack distribution and the sintering process parameters. The aim of the studies is to present a new approach to the characterization of complex macrostructures and microstructures, especially those found in effect of sinter quality and productivity. A number of the commercial iron ores were tested in an industrial sinter plant to study the effect of iron ore composition on the sintering properties. Sintering process was performed for each individual using iron ore as constant basicity, coke dust and flux. The sintering properties of blending ores, including productivity, tumbler index (TI), suitable moisture and coke rate were approximately equal except reduction degradation index (RDI) values, to the weighted means of the individual ores. An important feature of this system is the simultaneous use of X-Ray Diffraction and Scanning Electron Microscope (SEM-EDS) which enables to determine both macro and microstructure of a sinter with high accuracy.
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1. Introduction

Iron ore sinter is usually the major component of a blast furnace's iron bearing material. Most of impurities and running factors are combined to create molten silicates which called (iron or furnace blast) and floated over the molten iron, so they are separated from the molten iron. The Iron ore is carried then to steel furnace where the content of carbon decreases from 4% to 0.5% and the other impurities are removed. Solid iron slag is added in scrap iron and steel furnaces and iron slag (Zain et al., 2013). There are various types of iron ores traded in the international market. Many researches demonstrated that various types of iron ores affected the mineral structure of sinter and the sintering properties. In industrial sintering applications, the iron ores are blended and composition of the blending ore plays an important role in controlling the sintering properties. The quality properties of sinter are dependent on the mineral structure of sinter (Barnaba, 1985; El-Didamony et al, 2011). Therefore, it is required to understand the fundamental aspects of phase formation during sintering process in order to control and improve the characteristics of sinter. The reaction in sintering and consequently development of phases has been the subject of many studies (Alexander, 1981; Antunes, 1998). All these were basic studies conducted on sinter samples. It has been reported that the metallurgical properties of self-fluxing sinters, such as the mechanical strength, the degradability during reduction at around 1200°C, and the reducibility at high temperatures are greatly dependent on minerals compounds (Barnaba, 1985; Boyd & Ferron, 1995; Brock, 1983). Accordingly, an explanation of the fundamental aspects of the physical and chemical nature of the sintering processes, particularly the mineral formation processes that is required in order to control the qualities of self-fluxing sinters (Camci & Aydın, 2000).

Sinter product consists of various mineral phases produced by sintering of iron ore with fluxes and coke breeze. But yet comprehensive studies have been accomplished, many phenomena in the mineral formation processes have been left unsolved (Chaigneau, 1994; Egundebi, 1989). The problem has been studied through the sintering of very simple mixtures consisting of reagents, and several bits of interesting information have been obtained especially on the formation processes of the secondary or granular hematite, a product detrimental to the degradability (Erünsal, 2000; Fujimori, 1998). But, many complexities can be expected on the mineral formation processes that consist of different points within production process at iron steel plants (Jasienska & Durak, 1999). Because of their complex chemical structure and mineral components, much of solid wastes can be used to utilize in sinter blend (i.e. sludge, flue dust, slag, mill scale). Many investigations on the sinter structure and its qualities

have been done. A sufficient clarification has not obtained, however, made on the relationship between the sinter structure and its physical properties. A few research reports have recently been released in an attempt to clarify the relationship between the sinter structure and its physical properties through the quantitative study by image analysis (Hamilton, 1951; Hida et al., 1983).

Several authors were investigated sinter microstructure, which directly influences sinter properties, is largely governed by the type of ore being treated (Hino et al., 2003). And the sinter strength is also a function of original ore properties (Higuchi & Heerema, 2003). Hida et al, concluded that sinter productivity can be influenced by the type of the iron ore used.

In this study, the sinter tests were applied to study the relationship between the sintering mineralogical properties, of the blending and individual iron ores, and attempted to find the way to improve the sintering properties of the blending ore containing with high productivity. Moreover, the effects of different type of iron ores on sinter properties with the basicity of 1.7 were studied. The effect three different types of foreign iron ores and domestic iron ores usage in sinter making processes effects of some sinter properties were examined. For this purpose preparation of sinter mix was done by considering the sintering conditions at an industrial sinter plant. Experiments were carried out in three stages; preparations of the sinter raw mix, baking, followed by the treatment of the burned sinter by some standard tests. Iron ores, coke, limestone and dunite were supplied by an industrial sinter plant for the tests.

2. Materials and Methods

Initially; iron ores are crushed to a suitable size (< 8mm) which are stacking on the sinter blend according to their chemical analyses by a stacker. Some flux material, coke and return dust can also be added at proportion unit. Besides, collected dust and mill scale, flue dust and sludge of gas cleaning from steel making can be added to the iron ore blend at the mixing stage. At the start of the sintering operation, the iron ore blend is transferred from the beds to storage bunkers. The ore blend and the coke breeze are weighed on conveyer belts and loaded into a mixing drum (Figure 1). Here, they are blended completely and the mixture is moistening to enhance the agglomerated, which improve the permeability of the sinter bed.

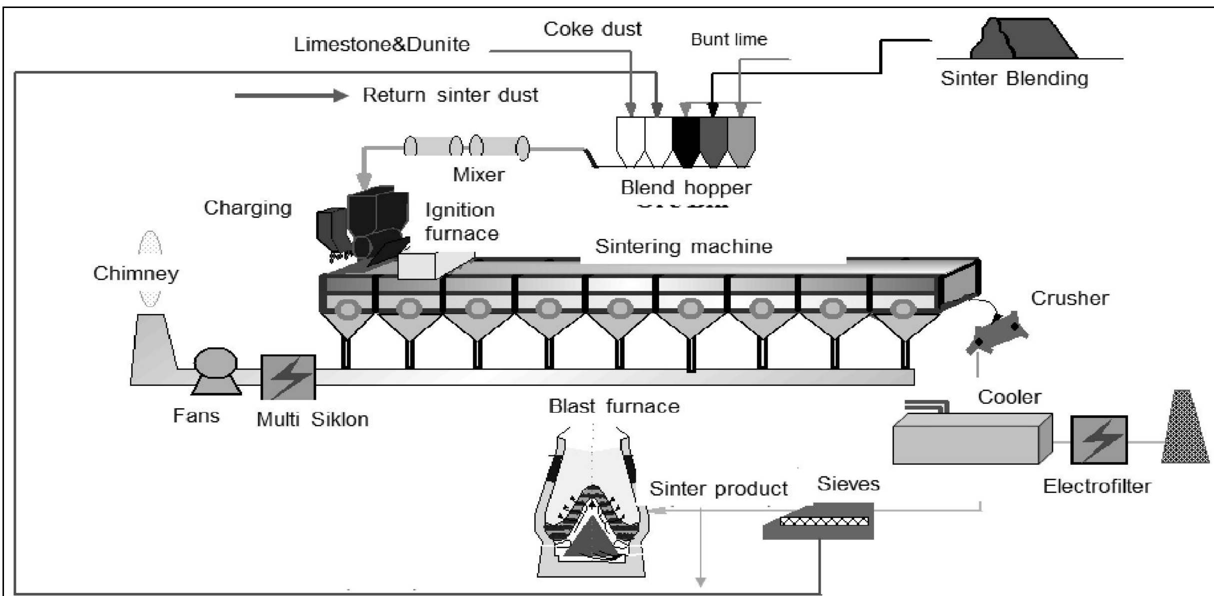


Figure 1. Iron Ore Sintering Process Flow Diagram

Raw materials: Iron ore X, iron ore Y, iron ore Z were domestic ore, while iron ore K, iron ore L and iron ore M were foreign ore fines mentioned in sinter test program. These iron ores were uniformly mixed respectively to reduce fluctuation of chemical composition and obtain representative sample. Chemical compositions of main iron mineral ores and sintered samples are given in table 1 and 2.

Fixed proportioning ratio among iron ore fines and relevant parameters applied in test program is listed as follows;

- Foreign iron ores (K, L and M): domestic iron ores (X, Y and Z) = 60: 40 (Table 3)

- X: Y: Z: = 10:20:10 (based on weight %)
- BF flue dust and iron bearing sludge account for 1.5 % and 2.4% of raw mix respectively. According to the test program, sintering test was carried out under condition of basicity (CaO/SiO_2) 1.7. Moisture in raw mix is fixed at 7.0%.

Table 1. Chemical and Mineral Composition of Main Minerals for Iron Ores

Raw materials	Chemical Composition (%)							Size Distribution (mass%)				Mineral Composition (%)
	Fe	*SiO_2*	*Al_2O_3*	*CaO*	*MgO*	*Zn*	*Ni*	*+6.35 mm*	*+1 mm*	*+0,15 mm*	*-0,15 mm*	
Ore K	63.86	3.73	0.89	0.11	0.04	0.002	0.001	14.3	34.8	22.7	28.2	*Hematite*
Ore L	62.25	3.65	1.12	0.03	0.05	0.006	0.002	17.3	26.7	24.4	31.6	*Hematite*
Ore M	61.88	3.18	2.09	0.02	0.03	0.005	0.001	23.5	20.9	18.2	37.4	*Hematite, Limonite*
OreX	55.68	6.27	0.59	1.30	0.78	0.002	0.002	15.5	35.8	23.3	25.4	*Hematite, Geothite*
Ore Y	53.38	6.47	1.41	1.29	0.86	0.038	0.003	13.5	33.9	23.4	29.2	*Hematite, Limonite*
Ore Z	58.72	6.78	1.64	0.48	1.48	0.016	0.180	14.4	27.2	19.8	38.6	*Hematite, Magnetite*
Limestone	0.36	1.30	0.27	53.45	1.24	0.000	0.000	1.2	42.7	33.4	22.7	
Dunite	3.39	38,42	1.17	4.68	36.5	0.04	0.240	2.2	34.2	36.5	27.1	
Coke dust	1.20	51.76	25.65	2.69	1.26	0.00	0.014	4.3	35.05	31.1	29.5	

Table 2. Chemical Analysis of Sinter Samples

Sample	*Fe*	*Al_2O_3*	*CaO*	*Alkaline $K_2O.Na_2O$*	*MgO*	*SiO_2*	*Ni*	*MnO*	*Zn*
Sinter 1	56.94	1.68	9.56	0.04	1.15	4.89	0.01	0.48	0.02
Sinter 2	53.72	1.25	11.63	0.15	1.64	6.78	0.03	0.64	0.03
Sinter 3	54.65	1.54	9.58	0.09	1.35	5.68	0.02	0.72	0.02
Sinter 4	53.07	0.92	8.04	0.26	1.52	9.95	0.05	0.93	0.03
Sinter 5	52.85	1.45	12.65	0.14	1.42	7.67	0.02	0.92	0.04

Table 3. The Sintering Properties of Blending Ores.

Sample No	Blending of iron ores (mass %)				Productivity		RDI(-3.15) (%)	Machine Productivity (t/ m^2.24h)
	Total domestic iron ore (X, Y, Z)	*K*	*L*	*M*	*Moisture (Mass %)*	*Coke Breeze (kg/t sinter)*		
Sinter 1	-	33.3	33.3	33.3	6.8	76.27	24.85	40.45
Sinter 2	40	60	-	-	5.5	78.02	32.58	32.68
Sinter 3	40	-	-	60	8.4	75.38	27.41	38.78
Sinter 4	100	-	-	-	8.9	79.95	36.02	29.89
Sinter 5	40	-	60	-	6.4	77.45	30.35	36.42

Table 3 shows the sintering properties varied with iron ore type. The productivity of the sinter ranged from 29.89 to 40.45 t/m^2.24h; the RDI ranged from 24.85 to 36.02 %; the proper moisture of raw mix, 5.5-8.9 mass % (Table 3). The sinters made from the low alumina commercial iron ores (S3, S1, S5) presented the lower coke rate (75.38-77.45 kg/t sinter) and required the lower moisture (6.4-8.4 mass %).

Later dry mixing of the blend, sufficient amount of water was added, and wet mixing was done to obtain adequate ball. Then, sinter mix was placed in the sinter strand in which all sintering experiments were done during with this study. The baking of the sinter mix was done in a plant scale sinter .The baked sinter, called the sinter cake was then subjected to tumbling and abrasion test in sequence. Test results were explained depending on sinter qualities such as sinter strength, return fine balance, sinter mineralogy. Tumbler index test was made to final product using 1/5 ISO standard method. Tumbler drum is of 1000 mm in diameter and 100 mm in width with rotation speed of 25 rpm (Int. Org and Stand., 2007). One batch of 11,3 kg sinter with grain size of 10-50 mm was put into tumbler drum rotating at 200 rpm and screen analysis was applied. The weight percentage of +6.35 mm fraction was taken as tumbler index as the weight percentage of -0.5 mm was taken as abrasion index (Figure 2). The final size was minus 100 microns and 100 gr of the sample was taken for chemical analysis (Table 2).

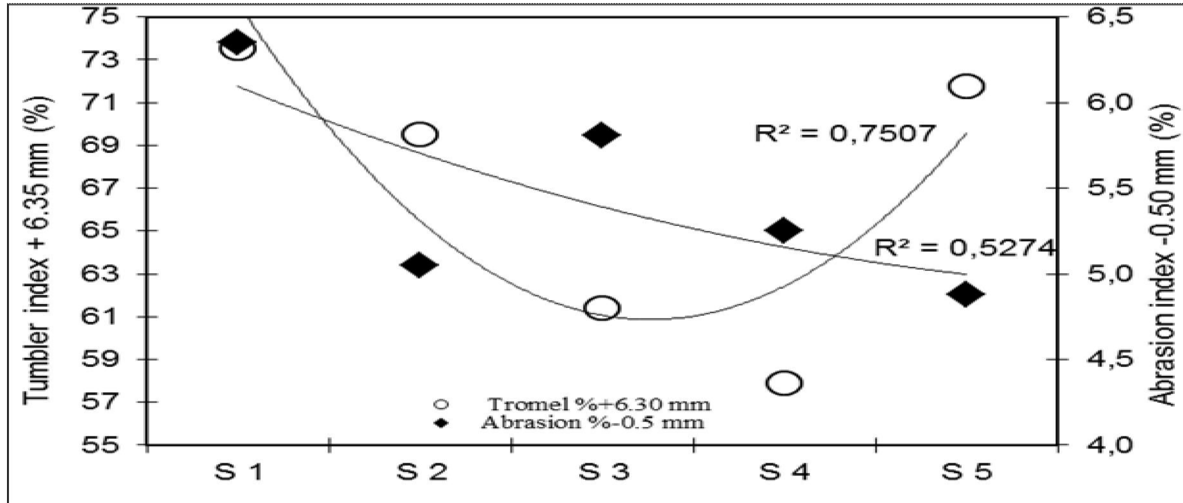


Figure 2. Changes of Tromel and Abrasion Index Depend on Iron Ore Types.

3. Results

3.1. Macrostructure Analyses of the Sinter Samples

The sintered samples were collected from an industrial sintering plant. Various defects existing on the surface and inside of the sintered ore made easy to form crack propagates under the action of external forces and ambient media, which would finally lead to the breaking and cracking of the sintered ore. The distribution of crack formation in various sintered ore structures are shown in figure 3. The lengths of the indentation and cracks were measured by a microscope of 30 times magnification. The aim of the present work is to provide some reference rules for the macrostructure designing of the sintered ore so as to meet the requirement of BF production. Results of examinations, it is observed that calcium ferrite is a major factor influencing crack resistance of sintered ore. The finer the grain size of calcium ferrite, the greater is the crack resistance of sintered ore.

Porosity is an important parameter that has to be measured to comment on strength and reduction properties of a blast furnace burden. The production of sinter was required an optimum pore size and the strength for used in Blast Furnace (Kawaguchi & Usui, 2005).The results of the distribution of crack lengths in various sintered ore structures are shown in figure 4. It is believed that the strength, reducibility and size distribution of sinter particles and the yield from a sinter strand are determined by the inherent strength of the bonding phases present and the structure of the pores (Hsieh & Whiteman, 1993).

Additionally, different pore sizes seem to have different effects on sinter strength, reducibility and other properties; the high temperature reducing property is controlled by the micro pore sinter structure, while the sinter strength is determined by the macro pore sinter structure (Tsukihashi, Kimura, & Yazawa, 2003). It is therefore important to optimize the pore structure in sinter to improve its reducibility, while maintaining the cold strength, reduction degradation and load softening properties.

Moreover, a number of other researchers suggested that the cracks resulting from a volumetric change accompanying the phase transformation of crystalline hematite to magnetite is mainly responsible for the reduction degradation of sinter (Jasienska & Durak, 1999; Kawaguchi & Usui, 2005).

Recent results suggested that it is possible to further improve the permeability of a sinter blend, and therefore control the pore size distribution of the sinter product, by adjusting the particle sizes of both limestone and coke breeze simultaneously (Kawaguchi & Usui, 2005). S1 and S3 are a sample of the homogeneous a size distribution (average 149.8μm and 141.9μm). Pore size distribution required by the blast furnace is observed in the sample. S2 sample indicates the distribution of heterogeneous porosity (average 161.3μm.) (Figures 4).

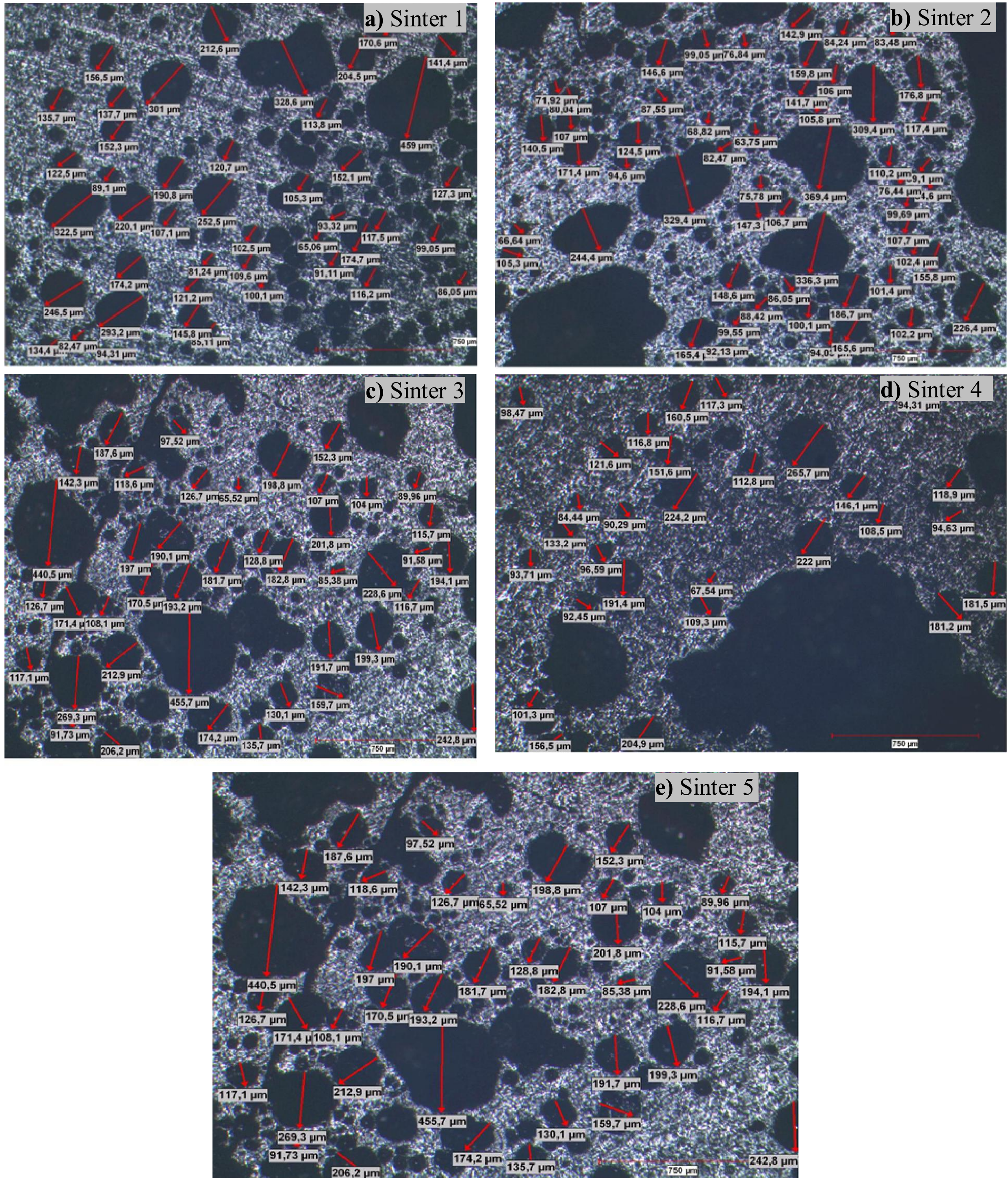


Figure 3. The Crack Formation of Sintered Granules with Different Iron Ores. a)Sinter1, b)Sinter2, c)Sinter3, d)Sinter4, e)Sinter5

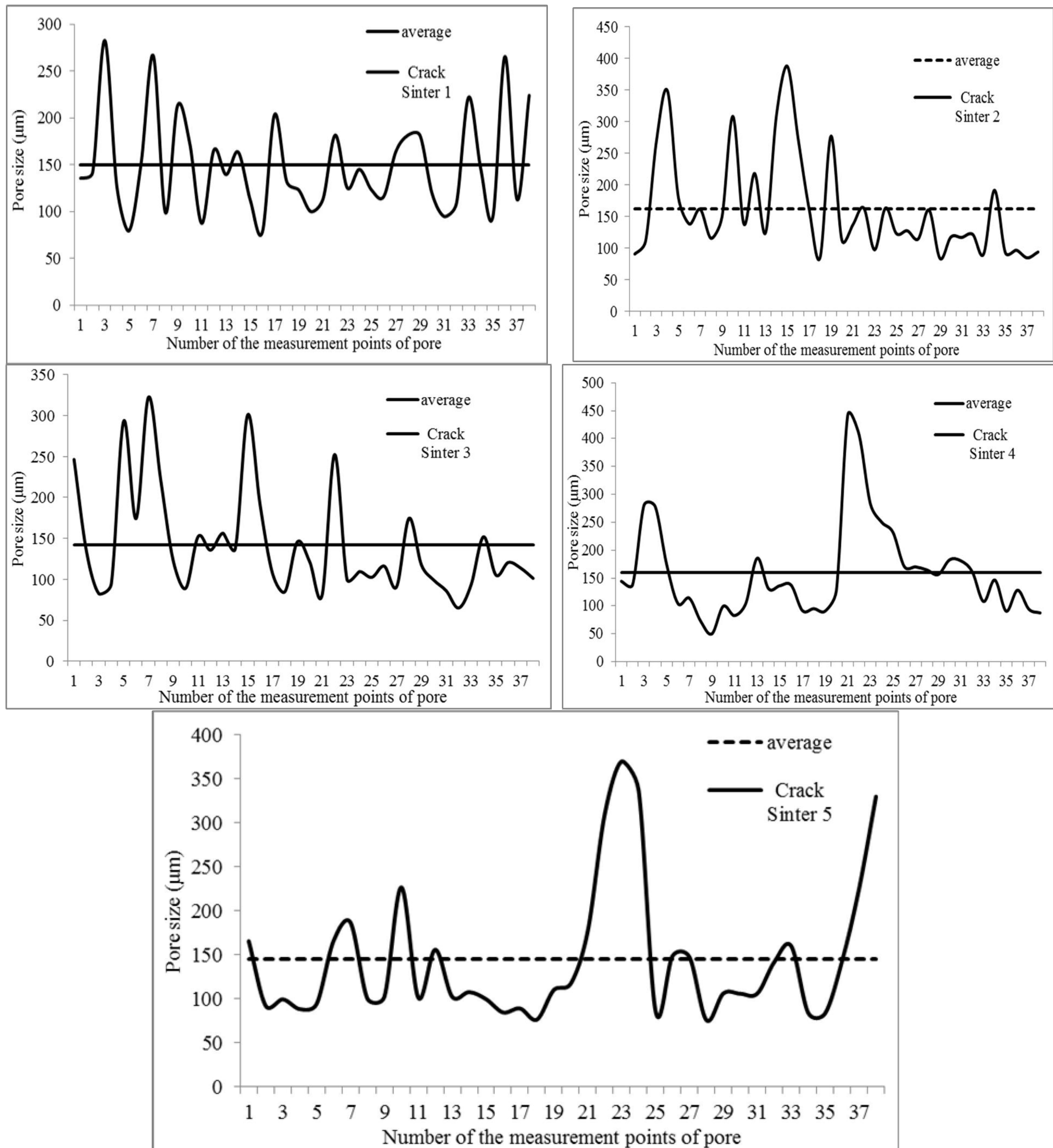


Figure 4. Crack Distribution in Various Sintered Ore Structures

3.2. Microstructure Analyses of the Sinter Samples

Sinter products of all experiments were collected for microstructural examination. After sintering, the specimens were mounted in epoxy resin and then vacuum impregnated. The sinter grains of ~1 to 3 mm size were mounted in epoxy resin and vacuum impregnated. When a large ore particle was present, the specimens were polished to expose a plane section vertical to the top surface on a diameter of the specimen so that a cross section of the ore particle could be seen. These sections were polished by using silicon carbide paper to 1000 grit employing ethanol as a lubricant, and finally to 0.3 micron by using diamond paste. The specimens were examined using a light microscope in reflection.

The mineralogical content and microscopic texture of sinter was analyzed using optical

microscope. The microcosmic textures of sinter are demonstrated on the micrographics a, b, c, d and e.

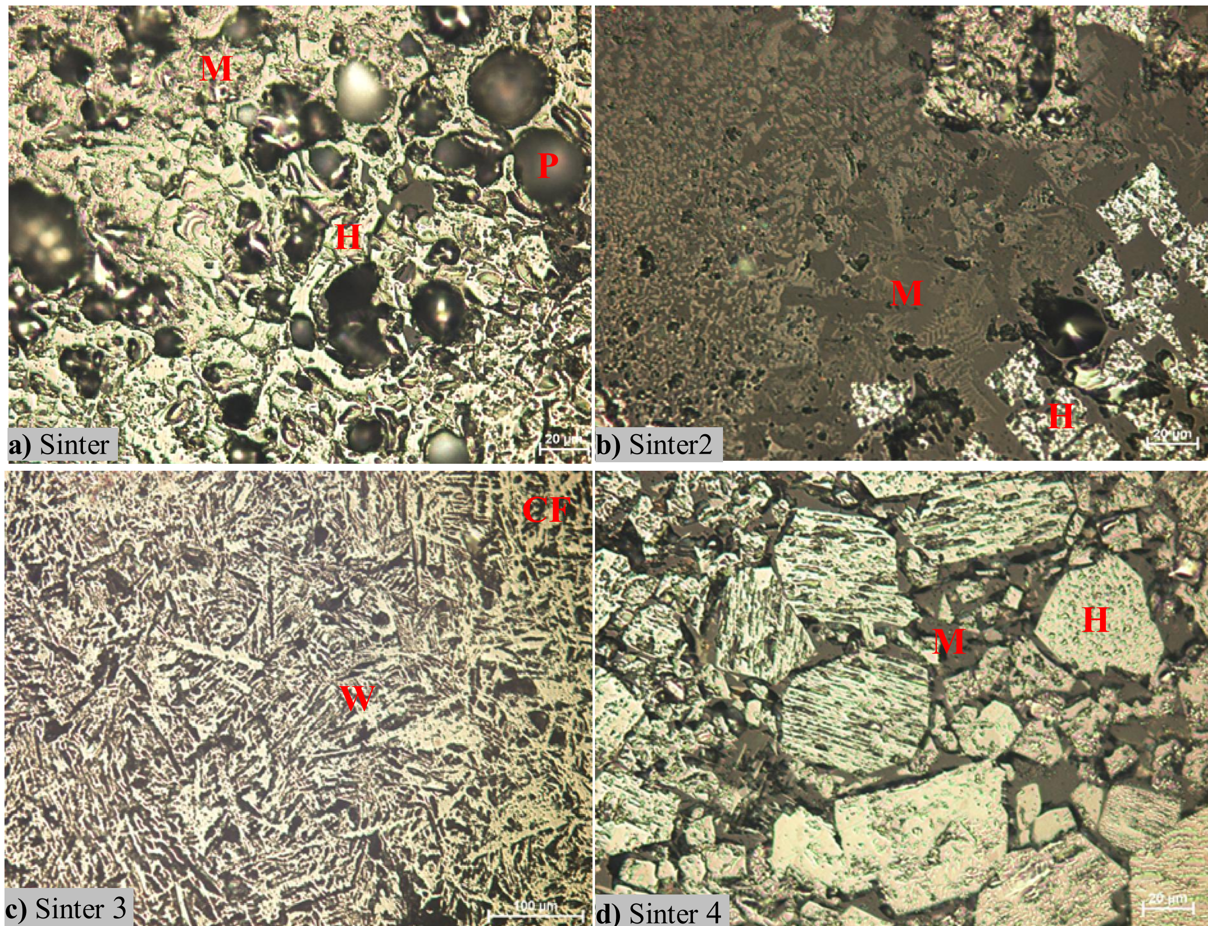
Figure 5a observes typical appearance of sinter structure for iron ore M mix, at 1250°C sinter consists of fine texture of needle like calcium ferrites and hematite, porosity is irregular and disrupted; at 1320°C hematite and magnetite crystals precipitate from slag; calcium ferrites are less abundant but are forming plates. There are a few intergrowths of fine magnetite crystal grains and hematite.

In the event of iron ore L mix (Figure 5b) the same general trends are observed. In addition, typical figures are obtained at low temperature: some kinds of quasi particles are formed; large amounts of needle like calcium ferrites are present at their periphery. The glass phases between hematite together with hematite form eutectic texture. Along edges of some hematite crystal grains, there is oxidation occurrence.

In the case of sinter, made from domestic ore mix to contain high gangue ores (Figure 5c), the same general trends apply. However, the reactions involving slag are promoted, due to the amount of gangue. The amount of calcium ferrites is rather high. At high temperatures, large plates are formed. Their chemistry is quite complex, with remarkable substitutions, involving silica, alumina, lime an even magnesium oxide.

In the event of sinter made a foreign iron ore mix (Figure 5d) consists mainly of molten texture formed by magnetite and calcium ferrite. A few needle shaped or branch shaped calcium ferrite and magnetite form interlacing textures, which occur along caverns holes. Magnetite amount is increased and also intergrowth of magnetite and hematite can be observed. Oxidation occurs at the edges or along caverns holes. At some places in the glass phase, separation out of magnetite fine crystal grains is investigated.

In point of iron ore K mix (Figure 5e) consists mainly of molten texture formed by magnetite and calcium ferrite or second birth hematite that are liquid phase filled by calcium ferrite. The crystal grains of some fine grain magnetite and hematite form interlacing texture, and the crystal grains of some magnetite are filled with calcium silicate. Few gangues are visible. There are a few intergrowths of fine magnetite crystal grains and hematite.



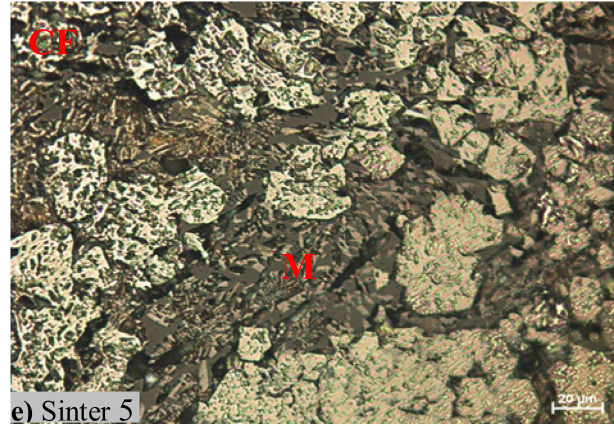


Figure 5. Microstructures of Sinter Samples. a) Sinter1, b) Sinter2, c) Sinter3, d) Sinter4, e) Sinter5 (H:Hematite, M:Magnetite, P: Pore, CF: Calcium Ferrite, W: Widmanstaaten (x500)

SEM analysis was performed in all of the samples, but only Sinter2 and Sinter4 were results shown here. SEM analysis was examined by D.E.U. Metallurgy and Materials Eng. Brand in the laboratories of the Department Jeol JSM-6060 model scanning electron microscope. As seen in figure 6 that demonstrates comparison of SEM images of sinter surfaces. There are a few interlacing existences of crystal grains of magnetite, hematite and a few coarse grain porous hematite (Figure 6a). Thus, high capacity of water absorption of S2 was caused by readily intrusion of water thorough open pores from the ore surface. Granulation in high moisture content is necessary to enhance granulation ability. It was clearly shown that each ore had individual surface morphology. In particular, S2 sample consists mainly of molten texture formed by magnetite and calcium ferrite (Figure 6b).

Calcium ferrite melt was found to form within residual ores of Sinter4 (Figure 6b). There is intergrowth of magnetite and hematite. Oxidation is seen at edges or along caverns and holes. At some places between coarse grain hematite, there is glass phase and much separation out of magnetite fine crystal grains (Figure 6b).

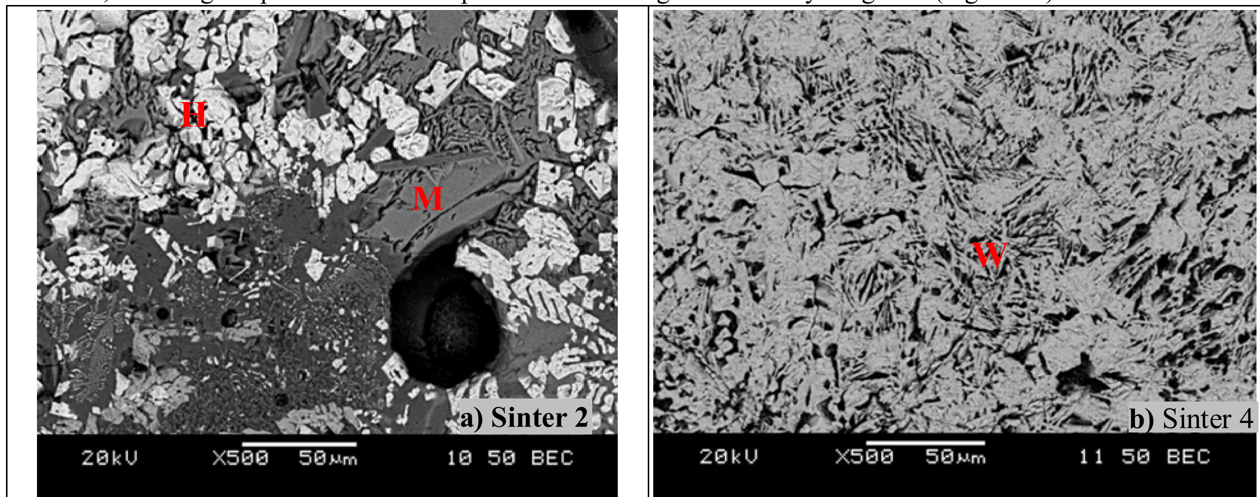


Figure 6. SEM Images of Sinter Surfaces. a) S2, b) S4. (H:Hematite, M:Magnetite, W:Widmanstaaten (X500)

3.3. X-Ray diffraction studies

Sinter samples were examined by Rigaku D/Max-2200 model X-ray diffraction and results was given in figure 7. The aim of investigating the sinter samples under a microscopy was to find out the morphologies of phases. As a result of the mineralogical research, the samles were found to be rich in hematite (Fe_2O_3), magnetite (Fe_3O_4) and wustite (FeO), calcium ferrite ($CaO.Fe_2O_3$ or $CaFe_2O_4$), SFCA (silico-ferrites of calcium and aluminium), wollastonite ($CaSiO_2$), and various CaO-FeO-SiO_2 solid solutions like ferro-monticellite ($CaFeSiO_4$). Also Mn_3O_4 (Manganese Oxide), Ca_2SiO_4 (dicalcium silicate) $K_2S_2O_4$ (Potassium Sulfate) Na_3PO_4 (Sodium Phosphate) and amorphous glassy phase were observed. The sinters were very heterogeneous, due to the limited diffusion possible during the short period at melt conditions and variations at the peak temperature reached. Secondary hematite known as skeletal rhombohedra hematite is

the major cause of poor reduction degradation resistance of sinter (Jasienska & Durak, 1999 & Larrca, 1992). This is based on frequent observations of cracks around the narrow neck regions of such hematite.

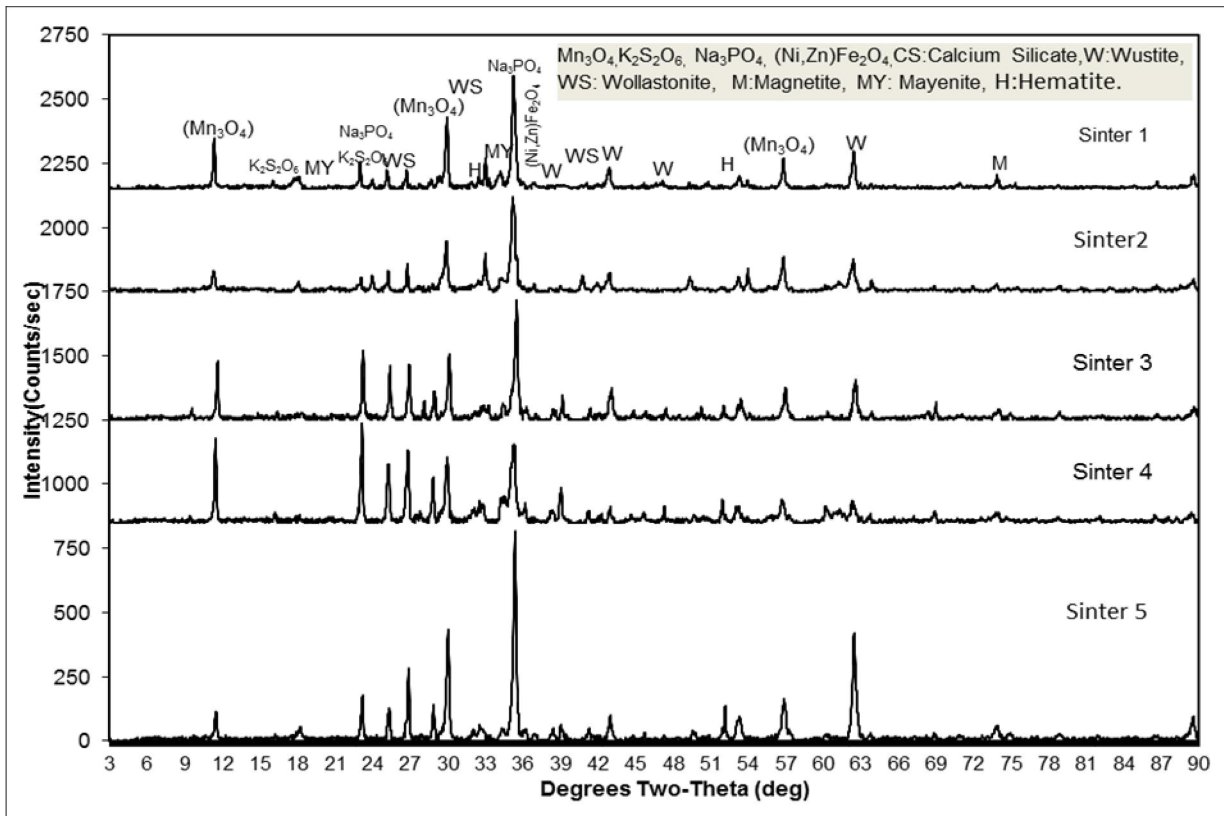


Figure 7. X-Ray Diffraction Studies of Sinter Samples

4. Discussion

Current literatures have not clearly established the relationship between the sintering properties of blending and individual iron ores. The small particles of blending ore were coated on the large particles to form the quasi particles in the granulation of industrial sintering.

The studies indicated that a little change raw material composition of sinter blends can have a significant adverse impact on the strength and reduction degradation characteristics of final sinter leading to deterioration in gas permeability in the upper part of the blast furnace (Lu, Holmes & Manuel, 2007). The trends of total hematite, reoxidized hematite, calcium ferrite and tumble strength which might affect the RDI were identical for all iron ores, but the trend of porosity varied with iron ore type (Kawaguchi & Usui, 2005; Meyer, 1980). In this study, Sinter3 consisted of with the lowest coke rate showed the largest increase in porosity; Sinter1 was less marked. For Sinter4 comprises with a high coke rate, the porosity was lowest and the porosity was reduced slightly as the alumina content increased. Probably because a high porosity in sinter promoted the transformation of hematite to magnetite during reduction, the RDI would seem to be related to the change in porosity in the same ore sinters that had a variation in alumina. Thus, the slope of the trend in RDI varied with iron ore type (Morrissey, 1953).

Prior work indicated that the high ratio of the pisolitic ore deteriorated the productivity of sinter (Nosovitskii & Zhilin, 1980; Pammer et al., 1980). In the experiments X and Y iron ore of this study, the pisolitic ore also showed the lowest relatively productivity index and increasing the ratio of the pisolitic ore decreased the productivity of sinter. Decreasing the SiO_2 content to raise the basicity and decreasing the MgO content may improve the sintering properties the productivity and RDI. This improvement may be related to the phenomena of which the increase in CaO or basicity and the decrease in MgO are able to decrease the porosity in sintering.

If the sinters have a low Al_2O_3 content (1.0–1.6%), it can be beneficial. The most harmful effect

of alumina is to worsen the sinter's RDI, which increases as the alumina content rises. Industrial experience with the blast furnace shows that a sinter within a 10–10.5 % CaO content range an increase of 0.1% in the alumina content raises the RDI by 2 points (Inazumi, Nagano & Kojima, 1970). Sinter with good strength and reducibility, which is able to maintain these properties as long as possible in the blast furnace that is desirable.

The tumbler index of sinter is dependent on critical flaws in the sinter and the propagation of these flaws through a sinter particle. Cold mechanical strength is directly related with the tendency to form fines during transportation and handling from the sinter machine to the blast furnace throat. The best sinters, from the point of view of strength, were achieved with mixtures Sinter1 and Sinter3, with a tumbler index of more than 70%.

Calcium ferrite is the main bonding phase in sinter. It increases with an increase of basicity (Sasaki & Wan, 2001). Generally, the high content of calcium ferrite favors the tumbler strength of sinter, but probably not for the RDI. The hematite includes the unreacted and the secondary hematite and most of the secondary hematite is reoxidized hematite. With an increase in basicity, the reoxidized hematite decreased in sinter (Figures 5a&e). An increase in magnesia can slow the formation of reoxidized hematite during the cooling stage of sintering. The secondary hematite is the most disadvantage phase to the RDI of sinter.

During the cooling stage, the calcium ferrite has previously been supposed to form the solidification of the melt (Scarlett et al., 2004; Sevinç & Topkaya, 2001). However, in this study it has been clearly shown that a large amount of calcium ferrite also may be generated by the reaction of magnetite with silicate melt and oxygen (Figures 6a&b) The analysed ferrites are SFCA type (silico ferrites of calcium and aluminium) and form by solid liquid reaction between the hematite and the Fe_2O_3 CaO melt, with the subsequent assimilation of SiO_2 and Al_2O_3 in this melt. The chemical formula of SFCA can be written as $5CaO.2SiO_2.9(Fe,Al)_2O_3$. These ferrites are beneficial for the sinter structure because they improve its strength and reducibility.

These approaches will contribute to understand more deeply the actual mechanisms of sintering, allowing thus the production of a sinter meeting closely the requirements for efficient blast furnace operation.

5. Conclusions

Different ore mixtures have been tested in an industrial plant using various operating parameters to establish the best sinter manufacturing conditions. The structure and composition of a series of sinter samples has been studied by electron microscopy, SEM and XRD. The presence of hematite, secondary hematite, primary magnetite, secondary magnetite, and ferrites has been detected as majority phases, along with a smaller amount of gangue. There is sufficient porosity, with micropores in many cases, to favour the reducibility of the sinter. The structure is always highly heterogeneous with the phases considerably mixed up. The results obtained are summarized as follows:

- In general the structure of the sinters includes the presence of ferrites with beneficial properties for sinter strength and reducibility. The optimum structure, formed by a hematite nucleus surrounded by an acicular ferrite lattice, has been detected. This structure is favoured when working with a higher basicity.
- The size of iron ore also affected the sintering properties. An increase in the size of iron ore promoted the productivity of sinter, but may reduce the tumbler strength slightly and save a little coke.
- The MgO content of the sinters is between 1.2–1.7%. The addition of MgO to the ore mixtures used to manufacture the sinters improves the RDI, because MgO stabilizes magnetite and thus decreases the hematite content, causing a lower stress in the sinter during the hematite to magnetite reduction in the blast furnace.
- The sinters present a low FeO content (<8%), which favours their reducibility. When the chemical composition of an ore mixture is fixed, FeO can provide an indication of sintering conditions, in particular the coke rate. It has been found that increase in the FeO content in the sinter lowers (improves) the RDI index. However, when the FeO content increases, reducibility decreases. It is important to find an optimum FeO content in order to improve the RDI without altering other sinter properties.
- Because of various chemical compositions and heterogeneous particle size distributions from raw materials are blending in industrial iron ore sintering. Thus, a microscopic point of view and the reactions of sintering are heterogeneous. Therefore, done experiments which can only closely simulate the typical microstructure of industrial sinter.
- The information reported in the present work, on the basis of the results obtained on the composition of ore mixtures, will be useful to the operators of the industrial sinter plant to allow improve the sinter manufacturing.

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Morphology and anatomy of egg and oncomiracidium of the monogenean gill parasite *Diplectanum aequanus*

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Abstract: Morphology and anatomy of the egg and free swimming oncomiracidia of *Diplectanum aequanus* infesting the gills of *Dicentrarchus labrax* were studies. Freshly laid egg of *Diplectanum aequanus* is yellowish brown in color, operculate, more or less tetrahedral with rounded protuberances at each corner and is provided with non-adhesive single appendage. The findings of the present study indicate that the appendage of the egg may ensure to a suitable anchoring and prevent the egg drift away from areas inhabited by potential host. Embryonation period ranged from 6 to 7 days at (20±2) °C with prominent developmental features is the formation of eyespots, which appeared on day 4 at 20±2°C. Hatching take place at the sixth day after deposition. Hatching process continued for about 10 to 15 minutes in the morning, one to two hours after sunrise. Oncomiracidial behaviour patterns were recorded. The free swimming oncomiracidium possesses four zones of ciliated cells (one anterior; two lateral and one posterior); four pigmented eyes; a prominent pharynx and a groups of gland cells on each side of the pharynx. The opisthaptor of each oncomiracidium has two pairs of well-developed hamuli in the centre of the opisthaptor in addition to usually present of 14 peripherally marginal hooklets. The distribution of the ciliated cells and possible functions of the pigmented eyes; lateral gland cells and haptoral sclerites are discussed.

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Key words: Diplectanides, *Dicentrarchus labrax, Diplectanum aequanus*, monogenean life cycle.

INTRODUCTION

Dicentrarchus labrax (Sea bass) is the most important commercial fish species in Egypt and it is commonly used in aquaculture **(Holder, 2003)**. Sea bass is an economically important cultured fish species in the Mediterranean coastal waters. The market demand is great and as a result, the price for fresh *Dicentrarchus labrax* has increased markedly over the past decade due to the desirable aroma and quality attributes of this fish; consequently, its farming is deemed to be a profitable business. Thus, many fish farms on the Mediterranean coasts have gradually expanded for the last decade **(Manera and Dezfuli, 2003; Johnson et al., 2004 and Caruso et al., 2005)**.

Nevertheless the infections with parasite have assumed an increasingly role among the factors limiting aquaculture productivity, particularly those with direct life cycles, such as Monogeneans **(Buchmann and Lindenstrøm 2002; Fioravanti et al., 2006 and Hayward et al., 2007)**. They are often responsible for economic losses for the activities of fish farming and have acquired an increasing importance as pathogens in marine fish **(Poulin and Rohde, 1997).**

A monogenean parasite, *Diplectanum aequans* is a common parasite of *D. labrax* and has a wide geographic distribution **(Dezfuli et al., 2007** and **Whittington and Chisholm, 2008).** The simple life cycle has sufficient evolutionary plasticity to enable monogeneans to adapt to fishes living in a range of marine and freshwater habitats **(Kearn, 1986)**. Much has been learned about the life cycles of ectoparasitic monogenean in the context of their serving as pathogens of fishes in aquaculture **(Whittington and Chisholm, 2008)**, details of their life histories on associated fishes are understudied. For example, it is generally unknown what transpires i) in the water column as the oncomiracidium seeks its fish host, ii) on the newly infected host as the post-oncomiracidium seeks an optimal microhabitat, and iii) within the optimal microhabitat as the post-oncomiracidium develops to an adult. Therefore, the present study is plane to give some light on life cycle of *Diplectanum aequans*. Moreover, this study may give some useful informations which help fisheries during control strategies parasite infections of the life cycle of *Diplectanum aequans* in order to design prophylactic and therapeutic measures against the parasite in fish cultures.

MATERIAL AND METHODES:

Specimens of the marine water fish *Dicentrachus* labrax were collected from the Mediterranean coast at Ras El-Bar at Damietta Province, in Egypt. Fishes were dissected to be searched for the monogenean parasites. The living mature monogenean parasite Diplectanum aequanus were carefully removed from excised gills and placed in separate dishes containing marine water where they were allowed to lay eggs at different

temperatures (14°C±2 and 20°C). Freshly laid eggs were studied morphologically using light microscopy. Groups of eggs were transferred to small dishes containing filtered marine water and incubated at room temperatures (20±2°C) under natural light. Eggs were observed daily to examine the free swimming oncomiracidium. The behaviour and anatomy of the oncomiracidium were observed using stereo-microscopy. The anatomy of the free swimming oncomiracidia was studied. All measurements are given in µm.

RESULTS:

1) The Egg:-

Freshly laid eggs of *Diplectanum aequanus* are yellowish brown in color, operculate, more or less tetrahedral with rounded protuberances at each corner (Fig. 1). The egg measures 73.6µm by long and measured 105.6µm by width. The filament (appendage) measures about 25.6 µm by long and arises from one pole.

The operculum has not been observed in the freshly laid eggs but begins to observe at the late stages of egg development, on the corner of tetrahedral egg. Numerous vitellaria fill most of the internal space of the egg (Fig. 2).

Embryonation to hatching takes (6-7) days at (20±2) °C. One of the prominent developmental features is the formation of eyespots, which appeared on day 4 at 20±2°C.

2) Hatching and the hatching time:-

Hatching take place at the sixth day of incubation at temperature 20±2°C under natural light. A few minutes before hatching, contraction and relaxation of oncomiracidia body started and the secretion from the median head gland cells was discharged towards the suture of the operculum. As a result of the rotation of oncomiracidia head towards the operculum and the continuous secretion produced by the median head glands, two small lateral splits were observed through the operculum suture.

Consequently, the operculum is opened suddenly and most of oncomiracidia liberated from the egg by its anterior and first end the fluid-filled sac was swelled during the emergence of the oncomiracidia and finally was burst. The whole process of hatching continued for about 10 to 15 minutes. In most cases, hatching occurs in the morning, one to two hours after sunrise.

3) Behavior of the oncomiracidia:-

Swimming pattern of the oncomiracidia was mainly of two types: vertical swimming in the water column and horizontal swimming close to the bottom. After emerges from the eggshell, it swimmed in the mid water near the side of the dish for a short time, then moved to the upper surface of the water in a wavy movement. After vertical swimming, some oncomiracidia continued swimming in spiral path or showed a horizontal and vertical whirling movement to the bottom of the dish and crept on the bottom with its anterior half of the body for a short time, then swimmed to the middle of the dish again.

4) The oncomiracidia:-

Free swimming oncomiracidia of *Diplectanum aequanus* are fusiform in shape and slightly compressed dorso-ventrally (Figs. 3 and 4). The body measurements of 10 well flattened, living specimens are 112 (100-130) µm in length and 48 (30-60) µm in width.

Each oncomiracidia has 59 ciliated cells (Figs. 3 and 4) arranged in four zones; one anterior, two median and one posterior. The anterior zone posseasss 27 ciliated cells, 10 of them are dorsal while 17 cells are ventro-lateral. Each of two median zones is ventro-laterally located and consists of 10 ciliated while the posterior zone possesses 12 ciliatd cells, 10 of them are dorsal and 2 are ventral.

The head of each oncomiracidium contains four pigmented eyes (Figs. 3 and 4). Each eye consists of a pigmented cell which appears as a cup containing numerous brown to black cylindrical bodies and a single crystalline-like lens (Fig. 3). The lenses of the anterior eyes are directed posterolaterally whereas the lenses of the posterior eyes are directed antero-laterally. The posterior eyes appear to be in close contact with each other while the anterior eyes are widely separated (Fig. 4). The muscular pharynx was observed posterior to the eyes but mouth and rest of gut was not observed (Figs. 3 and 4).

Gland-cells and their ducts were observed in each lateral region of the head just posterior to the pharynx (Fig. 4).

At the posterior end of the oncomiracidium an adhesive opisthaptor was observed in the form of a ventrally-directed disc shape and measured (32µm) long and (38.4µm) wide. The opisthaptor is equipped with seven pairs of marginal hooklets (I_VII). Each hooklet has a domus. The points of the fourteen marginal hooklets are ventrally located and distributed around the edge of the circular haptor; there are no centrally situated hooklets. At the center of the haptor, there are two pairs of well-developed median hamuli. Each hamulus consists of two roots and a shaft with recurved pointed hooked end. The hamuli appear as slender primordial (Figs. 3 and 4).

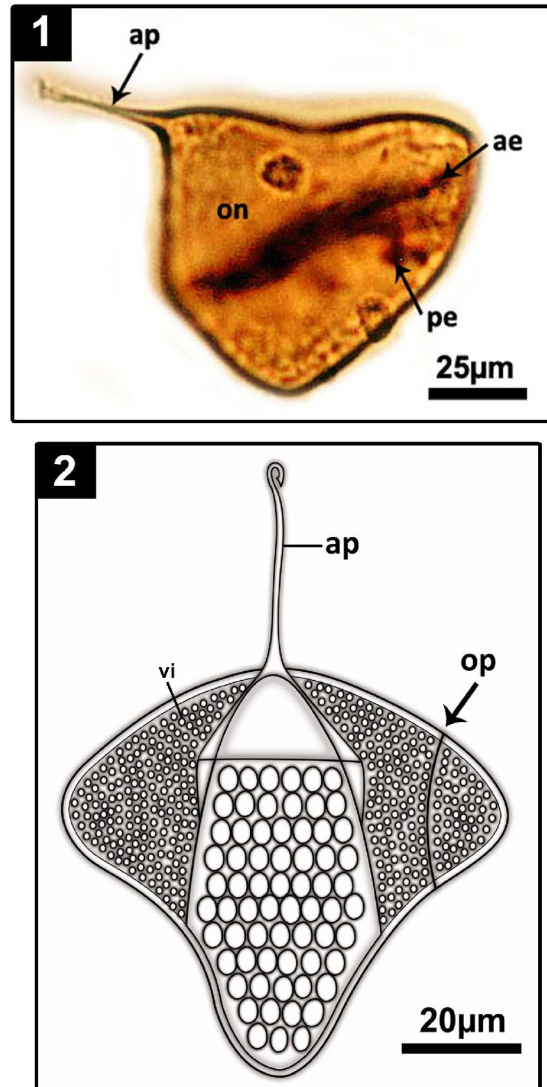
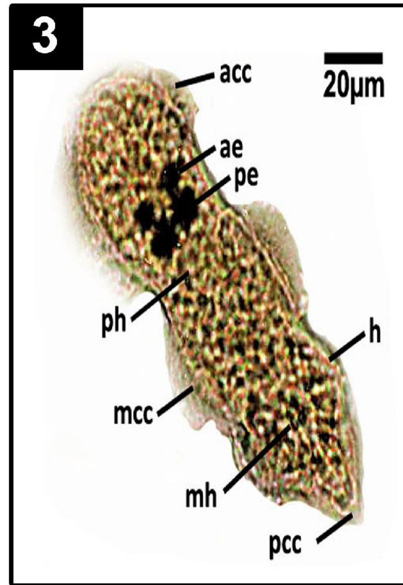


Figure (1):- Light microscope photograph of the well-developed tetrahedral egg of *Diplectanum aequanus* containing oncomiracidium (on). *Note* appendage (ap) arise from one pole and anterior eye (ae); posterior eye (pe).

Figure (2):- Schematic drawing showing the well-developed tetrahedral egg of *Diplectanum aequanus* with operculum (op) and appendage (ap) and vitellaria (vi) fill most of the internal space.



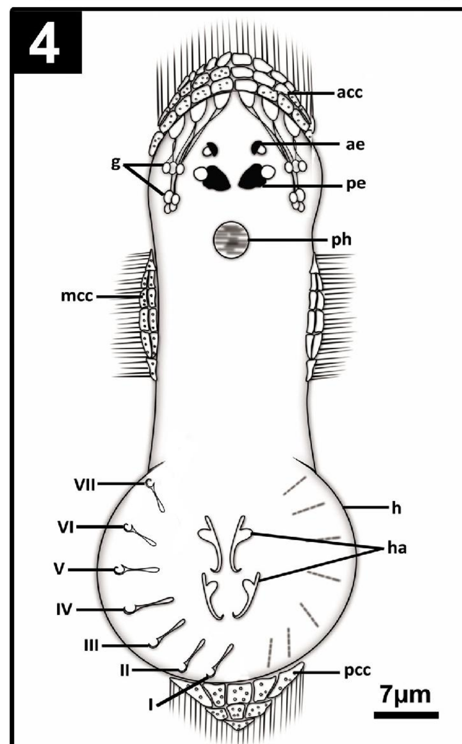


Figure (3):- Light microscope photograph showing the oncomiracidium of *Diplectanum aequanus* with ciliary zones (acc, anterior ciliated cells; mcc, median ciliated cells; pcc, posterior ciliated cells) and two pairs of pigmented eyes (ae, anterior eye; pe, posterior eye). ph, pharynx; ha, median hamulus; h, opisthaptor.

Figure (4):- Schematic drawing showing the anatomy of the oncomiracidium of *Diplectanum aequanus*. acc, anterior ciliated cells; ae, anterior eye; g, gland cell; h, opisthaptor; ha, median hamulus; mcc, median ciliated cells; pcc, posterior ciliated cells; pe, posterior eye; ph, pharynx;; I-VII, seven pairs of marginal hooklets.

DISCUSSION

The egg morphology of *Diplectanum aequanus* in the present studyshowed that great similarities of the eggs of *Diplectanum aequanus* that originally described as triangular and flat by **Oliver (1969);** and is more or less tetrahedral with prominent protuberances. Each egg has an operculum at one pole and an elongate flexible filament from another pole. **Kearn (1986)** mentioned that eggs of several species of monogeneans are roughly tetrahedral in shape due to the shape of the ootype chamber in which they are made. Entanglement happens to attach them to the host gills.

Oliver (1969) referred to that rounded appearance of ridges and corners of well-developed eggs of *Diplectanum aequanus* and to the fact that the egg resumes its tetrahedral shape after hatching, observation that is consistent with an increase in fluid pressure within the egg prior to hatching. The possible functions of the egg appendages are not known. However, **Kearn (1986)** has reviewed the possible functions of long egg appendages in monogeneanes. He proposed that the long, slender appendages of some mongenean eggs may promote the lifting of eggs off the bottom by water turbulence, in the same way that young spiders are carried aloft by the interaction of air currents with long threads of silk produce by the spiders and may also increase frictional resistance to sinking.

The appendage of the egg is better for ensuring a suitable anchoring as the egg drifts **(Kearn *et al.,* 1992)** the findings of the present study indicate that the appendage of the egg of *Diplectanum aequanus* could be the anchoring function. This is an advantage because if the egg failed to become anchored, it may be drift away from areas inhabited by potential host.

However, **Kearn (1975)** suggested that egg appendage of *Entobdella solea* serves as anchoring site which assists in egg hatching. Moreover, Egg appendages of *Kuhnia scombri* and capsalid monogeneans were suggested by **Kearn (1986)** to increase the surface area of the eggs and serve to reduce sinking rates. Also, he suggested that the egg appendage of *Diplozoon paradoxum* may serve to attach egg to the substrate if suitable projections, such as plant material or hydroid colonies in the sea, were available to snare the appendages of the egg drifting in the current; or may promote the lifting of eggs off the bottom by water turbulence or may also increase frictional resistance of skinning. However, egg appendages of *Diplozoon lucscae* resembling grappling hooks, may be adaptations to reduce the chances of the eggs being swept away from areas inhabited by the bottom-dwelling host **(Whittington and Kearn, 1988)**. In contrast, **Cone (1979a)** reported that short appendages of egg of *Urocleidus adspectus* have no role in attachment to the sediments or in hatching.

Moreover, **Yoon, (1998)** has reviewed the possible function of buoy-like structure on the egg appendage of *Entobdella hippoglossi* seems to be needed for preventing egg from being covered by mud or particles and thus maintaining the eggs in an oxygenated environment.

It seems likely that the behavior of the oncomiracidia observed for *Diplectanum aequanus* in the present study were very similar to those reported for other monogeneans **(Khidr and Abu-Samak, 1998; Whittington et al., 2000 and Albelda, et al., 2012)**: firstly a period of vertical swimming, which might lead to contact with hosts in the water column, and then period near the substrate, a “sit and wait” strategy which is less energy consuming. **Gannicott and Tinsley (1998)** reported that swimming ability, rather than survival, is related to infectivity, as oncomiracidia are able to find new hosts mostly while they can swim in the water column. **Kearn (1980)** highlighted the importance of the vertical swimming in *Entobdella. soleae*, which was probably related to a search pattern for a bottom-dwelling host. The vertically swimming of *Diplectanum aequanus* oncomiracidia after emerging are especially infectious while, this in which to find a new host.

The coordination between parasite development and fish habits has been reported in other species as a useful strategy to increase location of hosts by parasites. As swimming speeds of oncomiracidia and hosts differ considerably, coordination between the behavior of host and parasite becomes essential for successful transmission of oncomiracidia **(Kearn, 1986)**. Hatching rhythms in some monogeneans have been related to the behavior of the host. **Macdonald (1975)** discovered that various species of *Diclidophora* emerged rhythmically, that the rhythms differed from one species to another and appeared to be adapted to specific host behavior patterns. In *D. sagittata*, the egg hatching rhythm is apparently coordinated with the resting habits of the trout host. The present study has revealed that the oncomiracidium swimming near the bottom of the glass dish could aid host finding and attachment of the larvae.

A striking feature of the larva of *Diplectanum aequans* in the present study is that possessing four bands of cilia: (one anterior; two symmetrical regions around the median zone of the body and one posterior on the opisthaptor in the form of a ciliated cone). This manner resembles the majority of monopisthocotyleans, monocotylid oncomiracidia have four bands of cilia and two pairs

of eyes have been regarded as a distinctive feature of the most monopisthocotylinean oncomiracidia **(Whittington et al., 2000)**.

The oncomiracidia of *Diplectanum aequanus* possess two pairs of eyes with crystalline-like lens. This configuration suggests that the worms may be capable of detecting the direction of a light source. Assuming that oncomiracidia of *Diplectanum aequanus* are photopositive and that the eyespot is phototactic and allows orientation to a light source, oncomiracidia with eyespots could position themselves at appropriate levels in the water column in order to maximize the probability of an encounter with a fast moving host. Pigment-shielded eyes probably endow the free swimming larva (oncomiracidia) in most monogeneas with a directional response to light **(Whittington and Kearn, 1989, 1990)**, oncomiracidia that lack pigment-shielded eyes fail to respond in a directional sense to light, e.g. *Diclidophora* spp**. (Frankland, 1955). Kearn and Whittington (1992)** demonstrated that the presence of four eyes in the oncomiracidia is one of two synapomorphies used previously to support the monophyly of the Monogenea. They reported that pigment-shielded eyes were absent in the oncomiracidia, although juvenile and adult specimens are known to possess them. Furthermore, adult specimens of *E. caballeroi* are known to display a strong response to light on the pharyngeal tooth pads of their fish hosts.

The distribution of gland-cells in the oncomiracidia of *Diplectanum aequanus* appears to be consistent throughout the family, with the majority of monocotylid larvae having gland-cells in the antero-lateral region, posterior to the pharynx. The function of these glands in the oncomiracidia is unknown, although they probably have an adhesive role **(Chisholm and Whittington, 1996b)**. **Kearn (1975)** speculated that the anteromedian gland-cells may aid hatching by producing a secretion which digests the opercular cement of the egg or that they may facilitate attachment to the host after hatching. Furthermore, **(Chisholm and Whittington, 1996a, b)** suggested that the anteromedian gland-cells probably have an adhesive role. The present study supports **Chisholm and Whittington, 1996a, b** hypothesis, in that some of the oncomiracidia of *Diplectanum aequanus* in the present study was observed to attach themselves by the anterior end to the bottom of the glass egg-dishes. Since the lateral gland-cells that open anteriorly, it seems likely that some or all of these secretions have adhesive properties.

The morphological features of the opisthaptor of *Diplectanum aequanus* oncomiracidia are seven pairs of marginal hooklets and two pairs of well-developed hamuli. These features were similar to other diplectanids described previously by **Lambert (1980) Kearn (1980)** and **Khidr and Abu-Samak, (1998)**.

The contribution of the marginal hooklets in the process of attachment of monogenean parasites seems to be minor if compared with that of the relatively massive hamuli and it is difficult to understand why they persist. **Llewellyn (1963)** and **Kearn (1998)** suggested that the marginal hooklets of most monogeneans play an important role in attachment in the larval stage.

Finally it can concluded that simple life cycle of monogenean gill parasite of *Diplectanum aequanus* masks many wonderful adaptations to parasitism, especially egg and regarding the time of larval emergence (rhythmical hatching, or hatching stimulated by host-generated cues. Furthermore, larval behaviours in response to environmental and host factors and the pigmented eyes; lateral gland cells and haptoral sclerites can direct oncomiracidia to the habitat of their specific host fish species.

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Study of domestic violence against

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Abstract: Violence against women is a subtle phenomenon, because many women in different societies refrain from expressing it; for reasons such as lack of stiff penalties and legal support. Today even in the capitalist world, citizens have been exploited especially violence against women. Violence against women is not limited to sexual harassment and physical and emotional, but the phenomenon is far more frightening; Capitalism increases patriarchy and violence against women in various ways in the community. Violence against women cannot be separated from the struggle with the capitalist system and we cannot ignore the historical roots of women's oppression. Analysis of liberal and fight against the inequality of women, distort the historical oppression of women, and secondly, to limit women's rights reforms within the capitalist system. Violence, discrimination and oppression of women, the emergence of the class system in history entered a new phase. In the past, women as men have involved in everything. Nowadays, due to the fact that capitalism is dominant in the whole world and currently is the dominant trend. But there are many forms of violence against women, including governmental violence and violence in the family, workplace, streets and various forms of violence in society. Violence against women, despite claims by the government and international institutions based on reducing violence against women, as well as the phenomenon is widespread and blatant and obscene. Women in different countries under various violations, such as: state repression, social and family beating, humiliation, mutilation, sexual and economic deprivation to the victims of honor killings, threats and insults. United Nations Population Fund estimates that 5,000 women each year worldwide are killed by their families for reasons of honor. 20% of women have been raped in their life or threatens it.
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Keywords: violence, Domestic Violence, violence against women, violence in society

1. Introduction

Family, a relative of the child's socialization and collaboration with other individuals is responsible role in society. However, with the development of urbanization and industrialization in many countries, the structure, function and authority of the family has changed, But the family as a social institution in the regulation of sexual behavior and reproduction, rearing of children, their socialization, social status, care and protection of children and the elderly, providing economic and psychological security, has an important role(Hoyle, Carolyn (2002) . this violent behavior, with specific characteristics, can contribute to the damage. And may cause a rupture in relations between family members and achieve the desired goals. In all countries, the cost of gender-based violence is very high. For example, according to a survey conducted in Canada in 1995, violence against women in Canada this year totaled $ 1.5 billion ($ 1.1 billion America) by reducing the cost and increasing the efficiency of labor medical and social services. According to another study in Canada, violence against women is greater than the cost of social services, employment and health care systems. The results of this study, physical and sexual violence against girls and women in Canada will cost $ 4.2 billion annually, and more than 90 percent of these costs will be funded by the government.

Studies on Nicaragua, America and Zimbabwe show that women in early childhood or adult physical or sexual violence have been used more than any other women's health services. The average visit to the doctors, chemists, surgeons, hospitals and mental health centers in the violence-affected women more than other women. Need to receive health care services, significantly raises the cost of health care. For example, the health maintenance organization in Washington, in his report estimated the costs associated with violence against children, totaling over $ 8 million annually.

1.1. Definition of Domestic violence and violence

Domestic violence also is known as domestic abuse, spousal abuse, battering, dating abuse and intimate partner violence (IPV). Family violence when one tries to family members or other members of the family to dominate the physical or mental violence arises. In other words, violence is intentionally brought harm to another person. Or simply, violence is the intentional physical or psychological harm to another person. This behavior not only between spouses and other family members may also be noticed. For example, recent reports indicate that in many cases children are victims of violence, direct or indirect result of the violence,

including divorce, financial constraints, mental disorders, etc. Domestic violence exists in all cultures, societies, races, classes, religions, and between men and women, but the incidence of different types of violent behavior and the behavior of communities. According to global statistics, about 90 percent of domestic violence victims are women and 10 percent men. A comparison between modern and traditional societies or societies in transition from tradition to modernity suggests more violence among traditional communities is highlighted. The social and cultural background influences the incidence of violence and Due to the complex problems (Fisher Sarah. 2010).

1.2. Background

Many hypotheses and theories have been proposed about the violence. Religious leaders, philosophers, psychologists, sociologists and others have expressed the large number of ideas and solutions in this field. For example, many religious leaders have been invited to tolerance. On the other hand, a number of philosophers have another aspect to this topic. In response to the question of whether humans are inherently violent or peaceful, John Locke, English philosopher, believes that before entering into society, has been in the normal situation. In this situation, attempt to preserve human life and property and should respect the lives and property of others. Locke says: "The natural state of the natural law hat control and direct it, and everyone is obliged to follow it. It is the same reason all people who wanted to consult with it, teaches that all are equal. Someone should not damage other people's lives, people's health, liberty or property of others. Because all human beings are created with a constructor, While Freud and his followers have considered violence as a biological instinct, but those such as Albert Bandura, Aggression and violent behavior as a result of the learning community. Rousseau also says that the natural man, being kind, cheerful and good, but the social constraints that forced him to aggression and corruption. The theory and other aspects of learning acquired emphasize aggression. However, Locke's theory in the Western countries to respect human rights and protect them. Life is a natural right of man and others have no right to pass it. Evidence suggests that humans are equally inclined to violence and peace. In other words, man has fluctuated between violent and peace-oriented (Kocacik F, Dogan O. 2006)

1.3. World report on violence against women

International Day for the Elimination of Violence against Women, UNIFEM (United Nations Development Fund for Women) has published has published the following report: Violence against women and girls is epidemic problem. At least one in three women worldwide battering, sexual abuse or harassment in their lifetime, so it is physically located. Worldwide, at least 30 percent of the women in their lives have been beaten sexually abused or harassed as physical. In most of these cases, the abuse is carried out by a relative or family. Nowadays, the problem of violence against women is probably one of the most pervasive human rights violations. Violence against women has destroyed families and thus, prevents the development of society. The statistics of violence against women draws a marvelous illusion of social and health consequences. Based on the research that has been done around the world, half of all women murdered are killed by their husbands or former partners. Violence is a major cause of death and disability for women between ages fifteen to forty-four years. In the survey in 1994, was based on data from the World Bank, among dozens of threatening the lives of women, death rates due to rape and violence is greater than the rate of death from cancer, car accidents, war or illness malaria. Violence against women is weakened individuals, families, communities and economic development will reduce (Straus, M. A. (1999).

Australian, Canadian, Israel, South Africa and America have the highest numbers of female violence. Violence against women in Islamic countries has the lowest and violence against women in Iran is very low. According to John Ellison, vice president of the UN Secretary-General on violence against women is the highest in Australia, Canada, Israel and South Africa. And women make up 40 to 70 percent of homicide victims. The practice of forced marriage is still practiced in the parts of South Asia, East Asia and Africa and among immigrants to the West from these regions (YODANIS, C.L. and GODENZI, A. (1999).

1.4. The Consequences of violence against women

1.4.1. The Violence in the family

Violence that occurs within the family may be involved in addition to the negative impact of wife, children and family members.

1.4.2. The Impact on wife

Feelings of inadequacy in managing family, Physical or mental disability, Woman inefficiency, Damage to the integrity, Reduction in social status of women, Use of psychotropic medication, Alcohol and drug abuse, gambling and divination, suicide, feelings of helplessness, chronic depression, feelings of inferiority and failure, personality, severe depression, paralysis in decision-making capability deprivation, suffering from mental illness - ton, male pessimism, negative impact on body image. The negative effects of violent are behavior of men against women in the family.

1.4.3. Impact on children

Parental violence is negative role model for children. The children learn that violence is the only solution. Domestic violence impairs healthy development of the child's personality and violence creates an environment full of tension and anxiety. Observing aggressive behavior can lead to the failure of the education of their children and they will encounter a variety of psychological trauma, such as anxiety, depression and emotional problems and Education and the uncertainty in the relationship between parents and children. Such children will indulge in excessive affection or so, seeking affection.

1.4.4. The Violence in Society

Social insecurity, feeling the need to support, care for fear of violence, The loss of energy to deal with violence, disrupting normal social relationships, sense of uncertainty and public distrust, absence from work, reduced efficiency, The high cost of treatment, etc. All the adverse consequences of violence against women are in society

1.5. What are causes of violence against women?

Violence is one of aggression that it is hypocritical in many forms. And perpetrators, especially at the moment of committing violence lose their balance. Violence is different types according to the character of the offender and the victim's age and relationship violence and the victims. Violence is sometimes verbal that person uses profanity and insults to humiliate. The second type of violence, emotional violence.in fact emotional violence is Neglect. If this situation is prolonged, this may affect the long-term effects, especially in children and in family relationships. The third type of violence is physical violence. Violence associated with assaults and beatings, and even lead to murder. This type of violence, due disease or abnormalities, usually due to a broken relationship for purposes such as power, revenge, intimidation, forced to obey or be taken advantage. Other types of violence are such as: economic violence, religious violence. Domestic violence, especially violence against women, must be considered to human history. Violence has suffered due to the current state. War, as the pinnacle of human violence is to change the fate of mankind. Thus Human violence has violated the rights of others and social life.

Women compared with men, for various reasons and life history were able to force. Characteristics of women and the pressures that society has forced her, she is forced to accept violence.

In the modern era, women were responsible for stronger and more effective role in social and family relationships Due to increased education and greater participation in social life. This role is not only to accept responsibility but should be presented with options. Limit the authority of the husband. Men and women will share the strength of legal rights is a cause of conflict. A new campaign begins to gain power in the family. First argument leads to revenge and retaliation finally, the physical engagement occurs.

Physical, the conventional method is not for women. Women are usually mechanisms such as verbal assaults and occasional huff of reactive toward their rights and act against oppression. So when they get married instead of reacting to violence with violence that counteracts the acceptance or rejection of violence and the use of other mechanisms. Furthermore, the economic condition of the family (COUNCIL OF EUROPE (2006).

Men because of economic problems, particularly inflation, rising prices, unemployment is caused by a kind of spiritual and intrinsic involvement. The man lowers the pressure threshold conditions. so the pressure is expressed through violence towards family members, particularly his wife. Another factor in male violence is such as: air pollution, noise and traffic and the problems of social relationships with colleagues and employees.

The next factor is related to sexual problems between couples. The result was disagreement between the couple. From this point onward the men who are having this problem and show the effect of various reactions such as depression, suicidal tendencies, divorce, acts of violence.

An important part of family disputes, particularly violence that leads to divorce, sexual problems are rooted in a lack of coordination and understanding between couples sex.

2. Methods and Suggestions

International community and international conventions have presented recommendations on combating violence against women, which is largely supportive (establishment of special centers, shelters, social, financial support, community emergency services), legal - legal and Training – Consulting. The Global solutions have been proposed by Western countries. But it should be noted: firstly this strategy is consistent with Western culture. Secondly, despite the efforts ,the rate of violence against women in Western nations has not decreased. Some people seem to solve the problem of domestic violence in the shortest time and with the least trouble, go to the center and support facility, such centers after being identified by the individual, are no longer safe and the person leaving the center to be at risk again. However the family is the most important place for solutions to educational, clinical and counseling to

prevent violence against women and the long-term effect will be better.

On the advice of a group of victims, against violent behavior in the family and society , The correct behavior of the violence, domestic violence and ways to prevent a repeat of the violence, their experience and knowledge to make the correct decisions to be taken. The Couples therapy is a counseling technique. In this way, couples are faced with the problem of domestic violence, preventing violence and learn to deal with it under the supervision and guidance of psychologists.

Individual counseling is also teaching proper strategies to prevent violence. The important point is that violence is not always the result of spirit of violent and aggressive but this condition can be caused by psychological problems, empathy, compassion, moral skills. . Affection and kindness gone and violent behavior take place so to curb the violence, we need to restore social behaviors such as compassion and mercy. Purposes of preventing violence, the family can be considered a social strategy, listening to the issues, stressed-out, interesting, admirable, and he could use some positive signs and strengthen the spirit of solidarity.
The other global strategies that have been neglected, the influence of mass media that No recommendation has been provided about the ban on violent hits Such as murder, rape and brutal murder scene that provides sexual and aggressive behaviors(Typically pornography and sex). Religion and morality are other factors reducing violence against women. Religious beliefs, encouraging the observance of religious orders, would be effective in reducing violence against women (Somach, S.D. and AbouZeid, G. (2009).

3. Discussions

The results of such violent behavior: Physical incorrect, distrust in the individual and society, physical and psychological harm, failure to normalize relations with family. Deal with violence and problems related to it, because it is important that Violence is something that is learned in the socialization process. Nonviolence is related to the level of civilization of society, culture and lifestyles and respect to the personality and ideas and the emerging of a democratic culture in the community. Violence cannot be reduced unless all formal and informal (for example: school and home) institutions of society should be a fundamental transformation. Violence in society can be prevented by the Wise approach Otherwise, the other way will lead to violence.

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Utilization of the Root Bisection Computational Physics Method in the Determination of Roots of Non-Linear Equations using Java

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Abstract: Advancement in programming and language development has made possible improved efficiency and accuracy in solving numerical problems and hence the numerical computation of physical problems as used in Computational Physics. Hitherto, languages such as Basic, Fortran, C, among others, have commonly been employed in solving numerical problems. In this work, Java, a modern object oriented language was deployed in solving some physical problems, specifically, determination of roots of non-linear equations using the Root-Bisection Method. A comparison between results obtained showed faster convergence and greater accuracy using Java than as obtained using Fortran.

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1. Introduction

Java is a modern object oriented language which facilitates disciplined approach to program design (Deitel and Deitel, 2007). It has features that make it suitable for modern day computation which include multithreading (parallel programming), object orientation, support for internet, among others.

Computational Physics seeks numerical solutions to physical problems. It involves the use of numerical analysis methods to provide approximate solutions to problems in Physics. As described by Gerald and Wheatley (1999) numerical analysis is the development and study of procedures for solving problems with a computer. The term "algorithm", used for a systematic procedure that solves a problem, is defined as a step by step solution to a problem in terms of the actions to be taken and the order in which they are to be taken. A computational physicist or numerical analyst often is interested in determining which of several algorithms that can solve the problem is, in some sense, the most efficient. Efficiency may be measured in many ways some of which include the number of steps in the algorithm, the time taken by the computer to execute the algorithm, the amount of computer memory used, among others. A major advantage of numerical analysis is that a numerical solution can be obtained even when a problem has no analytical solution.

It is important to realize that numerical analysis of a problem always give numerical solution. Analytical methods usually give a result in terms of mathematical functions that can then be evaluated for specific instances (Gupta, 2010). Furthermore, a numerical solution is an approximation whose results can be made as accurate as desired (Arfken et. al., 2012).

Solving for the roots of non-linear equations is one of the numerous operations that numerical analysis can do (Gerald and Wheatley, 1999). It can also be applied in solving large systems of linear equations; obtaining the solutions of a set of non-linear equations, interpolating to find intermediate values within a table of data, finding efficient and effective approximations of functions, among others.

Pang (2006) used Java extensively to implement computational methods in his bid to introduce students to computational physics and to show the suitability of Java to computational science. Stroud and Booth (2001, 2003), enumerated the numerous ways in which computational methods can be adapted to solve numerical problems.

In this work, Java was used to implement the computational methods because

i) much of the work that had been done in the field of computational physics used FORTRAN and C;
ii) these two languages, although still powerful and efficient, tend toward becoming old languages in that they do not provide fully for the needs of the modern day computational physicists.
iii) Java is a modern object oriented language which facilitates a disciplined approach to program design.

Some of the other features of Java that make it suitable for modern day computation include

multithreading (parallel programming), object orientation, support for the internet among others.

2. Objectives

The objectives of this work include:

i) Implementation of the root bisection method for practically simple equations, using Java in the determination of roots of non-linear equations.
ii) Testing the implemented method with examples obtained from academic sources.
iii) Evaluating the Java implementation of the computational physics methods by comparing them with similar implementations done with other programming languages.

3. Methodology

Determination of the roots of non-linear equations

What does it mean to find the root of an equation?

Consider a function f(x); if f(x) = 0, then the values of the variable x that satisfies f(x) = 0 are called the *roots* of the equation. They are also known as the *zeros of f(x).*

Some equations are very easy to solve, that is, to find the roots. For example, if the function f(x) is linear in nature and given as f(x) = 6x - 12, then by making f(x) = 0, that is, 6x - 12 = 0, the equation is solved simply by rearranging the terms of the equation to make the variable x stand alone on the left-hand side of the equation, giving 6x = 12 or x = 12 / 6, that is, x = 2.

Also, if f(x) is quadratic, that is, $f(x) = ax^2 + bx + c$, in which the highest power of the variable x in the function is 2, a formula exists to find the roots of the equation - the well known quadratic formula given as: $x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$. However, as we move higher in the power to which the variable x is raised, finding the roots of the equation becomes more tedious.

According to Gerald & Wheatley (1999), it has been proved that no general formula exists for polynomials of degree greater than four meaning that there is no way to exhibit the roots in terms of "ordinary" functions. Usually, such polynomials are solved by successive approximations and some of the methods employed include: Root Bisection (or Interval Halving), Secant Method, Regula Falsi method, Fixed-Point Iteration method, Newton's method, Muller's method, among others (Dass, 2010).

Theory of the Root Bisection Method

The root bisection method is an ancient but effective method for finding a zero of f(x). Out of the common methods, the root bisection method is almost the simplest to understand and the easiest to implement.

To find a root of f(x), the root bisection method begins with two values $x = x_1$ and $x = x_2$ that bracket (enclose) a root. It is known that a root is enclosed if the function changes sign at the endpoints, that is, at $f(x_1)$ and $f(x_2)$; this is true if $(f(x_1)*f(x_2)) < 0$ (Kreszig, 2006). It is certain that there is at least one root in the interval $[x_1, x_2]$ as long as f(x) is continuous in $[x_1, x_2]$. The method then successively divides the interval in half and replaces one endpoint with the midpoint so that again the root is enclosed. Known in advance is that the error in the estimate of the root must be less than $|(x_2 - x_1)*(^1/2^n)|$ where n is the number of iterations performed (Gupta, 2010).

In implementing the Root Bisection Method, the pseudocode was written to set the bracket values and algorithm for implementation. The pseudocode for the Root Bisection algorithm is stated thus.

```
To determine a root of f(x) = 0 that is accurate
within a specified tolerance value, given values
X1 and X2 such that f(X1) * f(X2) < 0.
 REPEAT
Set X3 = (X1 + X2) / 2
 IF (f(X3) * f(X1) < 0):
      Set X2 = X3
      ELSE
      Set X1 = X3
      END IF
UNTIL (|X1 - X2| < 2 * tolerance value) or f(X3) = 0
```

NOTE: The method may give a false root if *f(x)* is discontinuous in $[X_1, X_2]$. The final value of X_3 approximates the root within the accuracy of the specified tolerance value (Gerald & Wheatley, 1999).

Implementation

Implementation of the root bisection method was achieved by creating a Java class called *RootBisection*. This class consists of six private fields and fifteen public methods which includes a constructor and the corresponding *set* and *get* assessors for each of the fields. The method called *getRoot()* implements the algorithm for the root bisection method.

A driver class called *RootBisectionMethod* (Adesina, 2010) was created to collect the data to satisfy the preconditions of the root bisection algorithm and to execute the *getRoot()* method of the RootBisection class which is the method that implements the root bisection algorithm. The *RootBisectionMethod* class is an application class because it contains a method called *main()* which is the entry point for all Java programs. The code listing for the *getRoot()* method is shown next.

```
1	public double getRoot() {
2		int iterate = 0;
3		double mid, x1, x2, oppSign, fxmid;
4		x1 = lowerLimitOfInterval;
5		x2 = upperLimitOfInterval;
6		setOutput("");
7		compileOutput(String.format("\n%15s%15s%15s%15s%15s\n", "ITR NO","X1", "X2", "X3",
		"F(X3)"));
8		do {
9			iterate += 1;
10			mid = (x2 + x1) / 2;
11			fxmid = Function.getFofX(mid, coefficients);
12			compileOutput(String.format("\n%15d%15.7f%15.7f%15.7f
			%15.7f", iterate, x1, x2, mid, fxmid));
13			oppSign = fxmid * Function.getFofX(x1, coefficients);
14			if ( oppSign < 0 ) {
15				x2 = mid;
16			} else {
17				x1 = mid;
18			}
19		} while ( !((Math.abs(x1 - x2) < ( 2 * tolerance )) ||
		(fxmid == 0) || (iterate >= maxIteration)) );
20		compileOutput(String.format("\n\n%s\n\n", "Program output for x1 = " + lowerLimitOfInterval +
		", x2 = " + upperLimitOfInterval + ", tolerance = " + tolerance));
21		return mid;
22	}
```

Code Listing 1: The *getRoot()* method of *RootBisection* class.

Lines 2 and 3 of Code Listing 1 declare variables that are used within the *getRoot()* method. Integer variable *iterate* (which is initialized to zero) keeps track of the number of iterations. Variables *mid, X1, X2, oppSign,* and *fxmid* are of double data-type and they store double precision floating point numbers; *.mid* stores the mid-point of the interval [X1, X2], while *X1, X2* stores the lower and upper limits of the interval respectively.

The root bisection algorithm starts from line 8 and ends at 19 of Code Listing 1. Line 11 calls method *getFofX()* of class Function which contains only two methods -*getFofX()* and *getDerivativeFofX()*. *getFofX()* computes the value of the polynomial function using the value of x and the coefficients vector passed to it as arguments; *compileOutput()* is one of the methods of the root bisection class; it stores the formatted output, which will be displayed to the user, passed to it as arguments as it is used in lines 7 and 20. Line 13 performs the operation *f(X3) * f(X1)* and stores the result in the variable *oppSign*. Line 14 begins an *if-else* structure that tests whether *f(x)* changes sign at the endpoints *X3* and *X1*. If the function changes sign then *mid* replaces *X2* in line 15 else *mid* replaces X1 in line 17. The condition in line 19 checks if the absolute value of *X1* - X2 (*Math.abs(X1 - X2)*) is not less than 2 times the tolerance value given or if *f(X3) (fxmid)* is not equal to zero or if the iteration number is not greater than or equal to the maximum given. If this complex logical condition is true, that is if any one of the tests is true, then the iteration continues otherwise lines 20 and 21 execute. Line 21 returns the value of X3 that is *mid.*

In order to obtain the roots therefore, parameters are first set as input to the program thus: For a polynomial of order n,

$f(x) = A_n x^n + A_{n-1}x^{n-1} + A_{n-2}x^{n-2} + \ldots\ldots + A_2x^2 + A_1x + A_0$

For example, if $f(x) = x^2 – 2$; then $A_2 = 1$, $A_1 = 0$, and $A_0 = - 2$

if $f(x) = x^3 + x^2 - 3x – 3$, then $A_3 = 1$, $A_2 = 1$, $A_1 = - 3$, and $A_0 = - 3$

if $f(x) = x^4 – 2$; then $A_4 = 1$, $A_3 = 0$, $A_2 = 0$, $A_1 = 0$, and $A_0 = - 2$

```
WELCOME TO THE ROOT BISECTION METHOD
THIS PROGRAM IMPLEMENTATION ALLOWS
YOU TO FIND THE ROOT(S) OF A POLYNOMIAL
OR NON-LINEAR EQUATION
	Enter the lower limit of the interval x1:
	Enter the upper limit of the interval x2:
	Enter the degree of the polynomial: n
	Now, enter the elements of the coefficient vector
	one after the other.
	Enter A0:
	Enter A1:
```

Enter A_2:
.
.
Enter A_{n-1}:
Enter A_n:
Enter the tolerance value: 0.00001
Enter the maximum number of iterations in case tolerance is not met: 20

In order to determine other roots, either of two approaches can be made: i) to reset the interval limits to new values of [x1, x2] which bracket the second root; ii) to set an all encompassing enclosing limits [X1, X2] from the outset.

Tests and Results

Example 1: As an example, consider the following function from Gerald & Wheatley (1999):

$$f(x) = x^3 + x^2 - 3x - 3 = 0.$$

It can almost be seen by inspection that a root is √3, that is, square root of 3. Although the function is simple enough to be easily solved by hand, it is a good example to show how successive iterates converge on the value √3, that is, 1.732050808.The result obtained by Gerald & Wheatley (1999), who implemented the Root Bisection Method using FORTRAN 90 is given next in table 1:

Table 1: Finding the root of $f(x) = x^3 + x^2 - 3x - 3 = 0$ starting with $X1 = 1, X2 = 2,$ and tolerance 1E-4 by root bisection method (Adapted from Gerald & Wheatley, 1999) Tolerance met

ITR NO	X1	X2	X3	F(X3)	MAXIMUM ERROR	ACTUAL ERROR
1	1.000000	2.000000	1.500000	- 1.875000	0.500000	- 0.232051
2	1.500000	2.000000	1.750000	0.171875	0.250000	0.017949
3	1.500000	1.750000	1.625000	- 0.9433594	0.125000	- 0.107051
4	1.625000	1.750000	1.687500	- 0.409424	0.062500	- 0.044551
5	1.687500	1.750000	1.718750	- 0.124786	0.031250	- 0.013301
6	1.718750	1.750000	1.734375	0.022030	0.015625	0.002324
7	1.718750	1.734375	1.726563	- 0.051756	0.007813	- 0.005488
8	1.726563	1.734375	1.730469	- 0.014957	0.003906	- 0.001582
9	1.730469	1.734375	1.732422	0.003512	0.001953	0.000371
10	1.730469	1.732422	1.731445	- 0.005728	0.000977	- 0.000605
11	1.731445	1.732422	1.731934	- 0.001109	0.000488	- 0.000117
12	1.731934	1.732422	1.732178	0.001202	0.000244	0.000127
13	1.731934	1.732178	1.732056	0.000045	0.000122	0.000005

The result obtained in the implementation of the root bisection algorithm using Java (Code Listing 1) is given next in table 2:

Table 2: Finding the root of $f(x) = x^3 + x^2 - 3x - 3 = 0$ starting with $X1 = 1, X2 = 2,$ and tolerance of 1E-4 by root bisection method using Java

Approximate root found: 1.732056

ITR NO	X1	X2	X3	F(X3)
1	1.0000000	2.0000000	1.5000000	- 1.6750000
2	1.5000000	2.0000000	1.7500000	0.1718750
3	1.5000000	1.7500000	1.6250000	- 0.9433594
4	1.6250000	1.7500000	1.6875000	- 0.4094238
5	1.6875000	1.7500000	1.7187500	- 0.1247864
6	1.7187500	1.7500000	1.7343750	0.0220299
7	1.7187500	1.7343750	1.7265625	- 0.0517554
8	1.7165625	1.7343750	1.7304688	- 0.0148572
9	1.7304688	1.7343750	1.7324219	0.0035127
10	1.7304688	1.7324219	1.7314453	- 0.0057282
11	1.7314453	1.7324219	1.7319336	- 0.0011092
12	1.7319336	1.7324219	1.7321777	0.0012013
13	1.7319336	1.7321777	1.7320557	0.0000460

Program Output for X1 = 1.0; X2 = 2.0; tolerance 1.0E-04

Table 1 and Table 2 show that it takes the root bisection method thirteen iterations to find the approximate root within the accuracy of the tolerance value. X3 is the mid-point of the interval while *f(X3)* gives the value of the function at *X3*.

It was observed in the tables that the estimate of the root may be better at an earlier iteration than at later ones. The second iterate in Table 1 is closer to the true root than are the next two, that is, iterates 3 and 4. Also, it is closer at iterate 6 than iterate 7. In this example, we have the advantage of knowing the answer, but this is never the case in real world applications. However, the values of *f(x)* themselves show that these better estimates are closer to the root. Although, this may not always be an absolute indicator due to the fact that some functions may be nearly zero at points which are not so near the root, but for smooth functions, a small value of the function is a good indicator that we are near the root; this is especially true when we are quite close to the root.

Example 2: Consider another example: $f(x) = x^4 - 2 = 0$

This function is a fourth degree polynomial and a root is the fourth-root of 2 which is 1.189207115.

Using the Java implementation of the root bisection method, the following results shown in Table 3 was obtained.

Table 3: Finding the root of $f(x) = x^4 - 2 = 0$ starting with $X1 = 1$, $X2 = 2$, and tolerance of 1E-4 by root bisection method using Java

Program output for x1 = 1.0, x2 = 2.0, tolerance = 1.0E-4

ITR NO	X1	X2	X3	F(X3)
1	1.0000000	2.0000000	1.5000000	3.0625000
2	1.0000000	1.5000000	1.2500000	0.4414063
3	1.0000000	1.2500000	1.1250000	- 0.3981934
4	1.1250000	1.2500000	1.1875000	- 0.0114594
5	1.1875000	1.2500000	1.2187500	0.2062693
6	1.1875000	1.2187500	1.2031250	0.0952845
7	1.1875000	1.2031250	1.1953125	0.0413893
8	1.1875000	1.1953125	1.1914063	0.0148350
9	1.1875000	1.1914063	1.1894531	0.0016555
10	1.1875000	1.1894531	1.1884766	- 0.0049100
11	1.1884766	1.1894531	1.1889648	- 0.0016293
12	1.1889648	1.1894531	1.1892090	0.0000126
13	1.1889648	1.1892090	1.1890869	- 0.0008085

It can also be observed in Table 3, that earlier estimates of the root may be better as reflected in iterate 4 being closer to the root than the next two. It took thirteen iterations for the root bisection method to converge to an approximate root within the accuracy of the tolerance value, that is, 0.0001. From the foregoing, it is evident that the root bisection method is indeed slow to converge.

Root Bisection Method Applied to Quadratic Equations

Hitherto, all examples taken were non-quadratic. To elucidate its applicability to quadratic equations, two quadratic equations are here taken as further examples.

Example 3: Consider the equation $f(x) = x^2 – 2 = 0$ (Adapted from Stroud and Booth, 2003)

Results obtained from the Root Bisection Method Java program is given as follows:

Approximate root found: 1.414200

ITR NO	X1	X2	X3	F(X3)
1	1.0000000	2.0000000	1.5000000	0.2500000
2	1.0000000	1.5000000	1.2500000	- 0.4375000
3	1.2500000	1.5000000	1.3750000	- 0.1093750
4	1.3750000	1.5000000	1.4375000	0.0664063
5	1.3750000	1.4375000	1.4062500	- 0.0224609
6	1.4062500	1.4375000	1.4218750	0.0217285
7	1.4062500	1.4218750	1.4140625	- 0.0004272

8	1.4140625	1.4218750	1.4179688	0.0106354
9	1.4140625	1.4179688	1.4160156	0.0051003
10	1.4140625	1.4160156	1.4150391	0.0023355
11	1.4140625	1.4150391	1.4145508	0.0009539
12	1.4140625	1.4145508	1.4143066	0.0002633
13	1.4140625	1.4143066	1.4141846	- 0.0000820
14	1.4141846	1.4143066	1.4142456	0.0000906
15	1.4141846	1.4142456	1.4142151	0.0000043
16	1.4141846	1.4142151	1.4141998	- 0.0000388

Program output for x1 = 1.0, x2 = 2.0, tolerance = 1.0E-5

Example 4::f(x) = $2x^2 - 9x + 5 = 0$

The first root can be found in the interval [1, 4] and the results obtained from the RootBisectionMethod Java program are given as follows:

Enter the lower limit of the interval x1: 1
Enter the upper limit of the interval x2: 4
Enter the degree of the polynomial: 2

Now, enter the elements of the coefficient vector one after the other.

Enter A0: 5
Enter A1: -9
Enter A2: 2
Enter the tolerance value: 0.00001
Enter the maximum number of iterations in case tolerance is not met: 20

Approximate root found: 3.850780

ITR NO	X1	X2	X3	F(X3)
1	1.0000000	4.0000000	2.5000000	- 5.0000000
2	2.5000000	4.0000000	3.2500000	- 3.1250000
3	3.2500000	4.0000000	3.6250000	- 1.3437500
4	3.6250000	4.0000000	3.8125000	- 0.2421875
5	3.8125000	4.0000000	3.9062500	0.3613281
6	3.8125000	3.9062500	3.8593750	0.0551758
7	3.8125000	3.8593750	3.8359375	- 0.0946045
8	3.8359375	3.8593750	3.8476563	- 0.0199890
9	3.8476563	3.8593750	3.8535156	0.0175247
10	3.8476563	3.8535156	3.8505859	- 0.0012493
11	3.8505859	3.8535156	3.8520508	0.0081334
12	3.8505859	3.8520508	3.8513184	0.0034410
13	3.8505859	3.8513184	3.8509521	0.0010956
14	3.8505859	3.8509521	3.8507690	- 0.0000769
15	3.8507690	3.8509521	3.8508606	0.0005093
16	3.8507690	3.8508606	3.8508148	0.0002162
17	3.8507690	3.8508148	3.8507919	0.0000696
18	3.8507690	3.8507919	3.8507805	- 0.0000037

Program output for x1 = 1.0, x2 = 4.0, tolerance = 1.0E-5

Now, to find the second root, the interval limits are reset to new values of [-1, 1] which bracket the second root. The results obtained from the RootBisectionMethod Java program are given below:

Enter the lower limit of the interval x1: -1
Enter the upper limit of the interval x2: 1
Enter the degree of the polynomial: 2

Now, enter the elements of the coefficient vector one after the other.

Enter A0: 5
Enter A1: -9
Enter A2: 2
Enter the tolerance value: 0.00001
Enter the maximum number of iterations in case tolerance is not met: 20

Approximate root found: 0.649216

ITR NO	X1	X2	X3	F(X3)
1	-1.0000000	1.0000000	0.0000000	5.0000000
2	0.0000000	1.0000000	0.5000000	1.0000000
3	0.5000000	1.0000000	0.7500000	- 0.6250000
4	0.5000000	0.7500000	0.6250000	0.1562500
5	0.6250000	0.7500000	0.6875000	- 0.2421875
6	0.6250000	0.6875000	0.6562500	- 0.0449219
7	0.6250000	0.6562500	0.6406250	0.0551758
8	0.6406250	0.6562500	0.6484375	0.0050049
9	0.6484375	0.6562500	0.6523438	- 0.0199890
10	0.6484375	0.6523438	0.6503906	0.0074997
11	0.6484375	0.6503906	0.6494141	- 0.0012493
12	0.6484375	0.6494141	0.6489258	0.0018773
13	0.6489258	0.6494141	0.6491699	0.0003139
14	0.6491699	0.6494141	0.6492920	- 0.0004677
15	0.6491699	0.6492920	0.6492310	0.0000769
16	0.6491699	0.6492310	0.6492004	0.0001185
17	0.6492004	0.6492310	0.6492157	0.0000208

Program output for x1 = -1.0, x2 = 1.0, tolerance = 1.0E-5

In another way round, the interval could be set at [-1, 4] form onset. For this example, doing that would yield the result as given next:

WELCOME TO THE ROOT BISECTION METHOD

THIS PROGRAM IMPLEMENTATION ALLOWS YOU TO FIND THE ROOT OF A POLYNOMIAL OR NON LINEAR EQUATION

Enter the lower limit of the interval x1: -1

Enter the upper limit of the interval x2: 4

Enter the degree of the polynomial: 2

Now, enter the elements of the coefficient vector one after the other.

Enter A0: 5

Enter A1: -9

Enter A2: 2

Enter the tolerance value: 0.00001

Enter the maximum number of iterations in case tolerance is not met: 20

First Approximate root found: 0.649227

ITR NO	X1	X2	X3	F(X3)
1	-1.0000000	4.0000000	1.5000000	- 4.0000000
2	-1.0000000	1.5000000	0.2500000	2.8750000
3	0.2500000	1.5000000	0.8750000	- 1.3437500
4	0.2500000	0.8750000	0.5625000	0.5703125
5	0.5625000	0.8750000	0.7187500	- 0.4355469
6	0.5625000	0.7187500	0.6406250	0.0551758
7	0.6406250	0.7187500	0.6796875	- 0.1932373
8	0.6406250	0.6796875	0.6601563	- 0.0697937
9	0.6406250	0.6601563	0.6503906	- 0.0074997
10	0.6406250	0.6503906	0.6455078	0.0237904
11	0.6455078	0.6503906	0.6479492	0.0081334
12	0.6479492	0.6503906	0.6491699	0.0003139
13	0.6491699	0.6503906	0.6497803	- 0.0035937
14	0.6491699	0.6497803	0.6494751	- 0.0016401
15	0.6491699	0.6494751	0.6493225	- 0.0006631
16	0.6491699	0.6493225	0.6492462	- 0.0001746
17	0.6491699	0.6492462	0.6492081	0.0000696
18	0.6492081	0.6492462	0.6492271	- 0.0000525

Second Approximate root found: 3.850769

ITR NO	X1	X2	X3	F(X3)
1	0.6492462	4.0000000	2.3246231	-5.1138628
2	2.3246231	4.0000000	3.1623116	-3.4603753
3	3.1623116	4.0000000	3.5811558	-1.5810486
4	3.5811558	4.0000000	3.7905779	- 0.3782395
5	3.7905779	4.0000000	3.8952889	0.2889514
6	3.7905779	3.8952889	3.8429334	- 0.0501263
7	3.8429334	3.8952889	3.8691112	0.1180420
8	3.8429334	3.8691112	3.8560223	0.0336152
9	3.8429334	3.8560223	3.8494779	- 0.0083412
10	3.8494779	3.8560223	3.8527501	0.0126156
11	3.8494779	3.8527501	3.8511140	0.0021319
12	3.8494779	3.8511140	3.8502959	- 0.0031060
13	3.8502959	3.8511140	3.8507049	- 0.0004874
14	3.8507049	3.8511140	3.8509095	0.0008222
15	3.8507049	3.8509095	3.8508072	0.0001674
16	3.8507049	3.8508072	3.8507561	- 0.0001600
17	3.8507561	3.8508072	3.8507816	0.0000037
18	3.8507561	3.8507816	3.8507688	- 0.0000782

Program output for x1 = 0.6492462, x2 = 4.0, tolerance = 1.0E-5

4. Conclusion

Scientific computing is today becoming the third pillar of scientific inquiry alongside the more traditional theory and experimentation pillars. For example, scientists today do not have to brave the risks of hazardous or dangerous chemical experiments, rather they use computational methods implemented with programming languages such as Java to simulate and model such experiments.

The relevance that computational physics, numerical analysis or computational science in general has today, is as a result of a lot of work that had been done in the implementation of several computational methods using computer programming languages.

FORTRAN, which was developed by IBM, is essentially a computational tool; it has been used extensively to develop programs in both the defense and geophysical fields (Chapman, 1998). Chapman (1998) implemented computational methods using FORTRAN 90/95. C, a language developed by Dennis Ritchie in the 1960s, is another language that has found extensive use in computational science. C is most suitable for High Performance Computing (HPC) because of its speed of execution (Chow, 2000). However, it is very susceptible to errors especially if used by a not so skillful programmer.

The scale of modern day problems being solved by computational physicist requires the use of programming languages that are very easy to use; provide features which make it possible to re-use existing codes; is capable of specifying different operations to be executed simultaneously by the computer; and that enable distributed programs to be easily developed (Kiusalaas, 2005; Jeffrey, 2002)). Java is such a programming language, and has been used in this work to determine roots of non-linear equations as set out, and for adaptability in training students.

One pertinent question is, having found one of the roots, how do we obtain the other root(s)? The solution to that problem is simply that to find all roots, the limits are reset to new values within the expected range x1 < x < x2, or a broad all enclosing limits [x1, x2] is chosen from inception with the necessary codes included. Either of these procedures brings out clearly the other roots of the equation being solved.

The main advantage of root bisection is that it is guaranteed to work if f(x) is continuous in $[x_1, x_2]$ and if the values $x = x_1$ and $x = x_2$ actually bracket a root. Another advantage is that the number of iterations required to achieve a specified accuracy is known in advance (DeVries, 1993). To find all roots, the limits are reset to new values within the expected range $x_1 < x < x_2$, or to choose a broad all enclosing limits $[X_1, X_2]$ from inception.

The major drawback of root bisection is that it is slow to converge. Other methods such as the Newton's method require fewer numbers of iterations to achieve the same level of accuracy.

In spite of arguments that other methods find roots with fewer iterations, root bisection is nevertheless an important tool in the computational physicist's arsenal. It is generally recommended that root bisection be used for finding approximate root which can then be refined by more efficient methods. The reason is that most other methods require a starting value near to a root which, if not available, may cause them to fail completely.

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Implementation of the False Position (Regula Falsi) as a Computational Physics Method for the Determination of Roots of Non-Linear Equations using Java

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Abstract: Computational Physics cuts across all branches of Physics, Engineering, and Sciences in general. Determination of roots is one of the most common areas/topics that show up in various disciples where Computational Physics is applied or utilized. In the computation and determination of the roots of non-linear equations, various methods such as Root Bisection method, Regula Falsi method, Newton's method among others have been implemented using FORTRAN, C, Basic, among other programming languages. This work considered the implementation of the False Position Method, otherwise known as the Regula Falsi method for the determination of roots of non-linear equations using Java. Comparison between results obtained showed that there is faster convergence and greater accuracy in the results obtained using Java than as in the results obtained using FORTRAN. Hence a good working knowledge of Java might end up being advantageous to an average physicist.
[Makinde, V., Akinboro, F.G., Okeyode, I.C., Mustapha, A.O., Coker, J.O., and Adesina, O.S. **Implementation of the False Position (Regula Falsi) as a Computational Physics Method for the Determination of Roots of Non-Linear Equations using Java.** *Nat Sci* 2013; 11(9):68-75]. (ISSN: 1545-0740). http://www.sciencepub.net/nature. 10

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1. Introduction

Computational Physics aims at obtaining numerical solutions to physical problems in which numerical analysis methods are used to provide approximate solutions to problems in Physics (Gupta, 2010). The scale of modern day problems being solved by computational physicist requires the use of programming languages that are very easy to use; provide features which makes it possible to re-use existing codes; efficient; is capable of specifying different operations to be executed simultaneously by the computer; and that enable distributed programs to be easily developed (Arfken et. al., 2012).. Java is such a programming language.

The relevance that computational physics, numerical analysis or computational science in general has today, is as a result of a lot of work that had been done in the implementation of several computational methods using computer programming languages. Chapman (1998) extensively implemented computational methods using FORTRAN 90/95. FORTRAN, which was developed by IBM, is essentially a computational tool; it has been used extensively to develop programs in both the defense and geophysical fields (Chapman, 1998). C, a language developed by Dennis Ritchie in the 1960s, is another language that has found extensive use in computational science. C is most suitable for High Performance Computing (HPC) because of its speed of execution but is very susceptible to errors especially if used by a not so skillful programmer.

Java is a modern object oriented language which facilitates disciplined approach to program design (Deitel and Deitel, 2007). It has features that make it suitable for modern day computation; these include multithreading (parallel programming), object orientation, support for internet, among others.

Pang (2006) used Java extensively to implement computational methods in his bid to show the suitability of Java to computational science as well as in introducing students to computational physics.

In this work, Java was used to implement the computational methods because

i) it is a modern object oriented language which facilitates a disciplined approach to program design.
ii) it is suitable for modern day computation as it provide fully for the needs of the modern day computational physicists which include parallel programming, object orientation, support for the internet, among others.
iii) FORTRAN and C have been used extensively in the implementation of computational physics problems.

2. Objectives

The objectives of this work include:

i) Implementation of the Regula Falsi method using Java.
ii) Testing the implemented method with examples obtained from other academic sources.

iii) Evaluating the Java implementation of the computational physics methods by comparing them with similar implementations done with other programming languages.

3. Determination of the Roots of Non-Linear Equations

Given a function f(x), if f(x) = 0, then the values of the variable x that satisfies the condition f(x) = 0 are called the *roots* of the equation. These are also known as the *zeros of f(x).*

It is quite easy to find the roots of some equations.

For example, if the function f(x) is linear in nature may be given as f(x) = 3x - 12, then 3x - 12 = 0, is solved simply to obtain x = 4.

In a situation where f(x) is quadratic, then, there exists a standard formula, the well known quadratic formula, given by $x = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$. that can be used to obtain the roots of the equation.

However, as the power to which the variable x is raised increases, finding the roots of the equation becomes more difficult. It has been proven that no general formula exists for polynomials of degree greater than four meaning that there is no way to exhibit the roots in terms of "ordinary" functions (Gerald & Wheatley, 1999),. Such polynomials are usually solved by successive approximations. Some of the methods employed include: Root Bisection (or Interval Halving), Secant Method, Regula Falsi method, Fixed-Point Iteration method, Newton's method, Muller's method, among others. This work therefore focuses on solving these higher order polynomials numerically to obtain one or more of the roots of such equations.

The False Position (Regula Falsi - in Latin) Method

Theory

The technique employed in the False Position method is such that each next iterate is taken at an arbitrary point between the pairs of x-values that is, the two starting values rather than the midpoint as in other methods such as the root bisection method. This may result in an advantage of faster convergence than some other methods, but at the expense of a more complicated algorithm.

In achieving the goals of this work, pseudocode for the False Position algorithm (*regula falsi*) was developed and is given next:

To determine the root of $f(x) = 0,$ given values X_1 and X_2 that bracket a root, that is, $f(X_1)$ and $f(X_2)$,

```
REPEAT
    Set X3 = X2 - f(X2) * (X1 - X2) / (f(X1) - f(X2))
    IF f(X3) of opposite sign to f(X1)
        Set X2 = X3
    ELSE
        Set X1 = X3
    END IF
UNTIL |f(X3)| < tolerance value
```

NOTE: The method may give a false root if *f(x)* is discontinuous on the interval. The final value of X_3 approximates the root within the accuracy of the specified tolerance value (Gerald & Wheatley, 1999).

Implementation

In implementing the False Position method, classes *RegulaFalsi* and *RegulaFalsiMethod were* created. Class *RegulaFalsi* extends class *RootBisection* (Adesina, 2010). This feature of Java is called Inheritance and it is a technique for enhancing code reusability and for establishing what is known as a "*is-a*" relationship between the inheriting classes and the inherited class. The inheriting class is called the subclass while the inherited class is called the superclass.

By allowing *RegulaFalsi* to inherit from *RootBisection*, all the public methods of class *RootBisection* are automatically available in the *RegulaFalsi* class and can be called from within any method in *RegulaFalsi*. Class *RegulaFalsi* overrides the*getRoot()* method of class *RootBisection* from which it inherits by providing its own implementation. The term "override" in the sense that because *getRoot()* is declared and defined in *RootBisection* - the superclass, the *getRoot()*, of the *RegulaFalsi* class, that implements the False Position algorithm.

```
1    public double getRoot() {
2        int iterate = 0;
3        double mid, x1, x2, oppSign, fxmid;
4        x1 = getLowerLimitOfInterval();
5        x2 = getUpperLimitOfInterval();
6        setOutput("");
7        compileOutput(String.format("\n%15s%15s%15s%15s%15s\n", "ITR NO","X1", "X2", "X3",
         "F(X3)"));
8        do {
9            iterate += 1;
```

```
10                      mid = x2 - (Function.getFofX(x2, getCoefficients()) * ((x1 - x2) / Function.getFofX(x1,
                        getCoefficients()) - Function.getFofX(x2, getCoefficients()))));
11                      fxmid = Function.getFofX(mid, coefficients);

12                      compileOutput(String.format("\n%15d%15.7f%15.7f%15.7f
                        %15.7f", iterate, x1, x2, mid, fxmid));
13                      oppSign = fxmid * Function.getFofX(x1, coefficients);
14                      if ( oppSign < 0 ) {
15                              x2 = mid;
16                      } else {
17                              x1 = mid;
18                      }
19              } while ( !((Math.abs(x1 - x2) < getTolerance() ) || (iterate >= maxIteration)) );
20              compileOutput(String.format("\n\n%s\n\n", "Program output for x1 = " +
                getLowerLimitOfInterval() + ", x2 = " + getUpperLimitOfInterval() + ", tolerance = " +
                tolerance));
21              return mid;
22      }
```

Code Listing 1: The *getRoot()* method of *RegulaFalsi* class.

Adesina (2010), implemented the Root Bisection Method using a similar algorithm. It could be observed in Code Listing 1 that the lines 10 and 19 are quite different from lines 10 and 19 of similar listing for the Root Bisection method (Adesina, 2010). Line 1 of Code Listing 1 shows how the False Position method differs from the Root Bisection method algorithm developed by Adesina (2010). Line 19 of Code Listing 1 compares the absolute value of the difference between X1 and X2 directly with the tolerance value rather than twice the tolerance value as was done in the Root Bisection method.

Tests and Results

Illustration 1: Gerald and Wheatley (1999) implemented the function $f(x) = x^3 + x^2 - 3x - 3 = 0$ with FORTRAN 90/95. The result obtained is as shown in table 1.

Table 1: Finding the root of $f(x) = x^3 + x^2 - 3x - 3 = 0$ starting with $X1 = 1, X2 = 2,$ and tolerance of 1E-4 as implemented by Gerald and Wheatley (1999) using FORTRAN 90/95

ITR NO	X1	X2	X3	F(X3)
1	1.000000	2.000000	1.500000	- 1.875000
2	1.500000	2.000000	1.750000	0.171875
3	1.500000	1.750000	1.625000	- 0.943359
4	1.625000	1.750000	1.687500	- 0.409424
5	1.687500	1.750000	1.718750	- 0.124786
6	1.718750	1.750000	1.734375	0.022030
7	1.718750	1.734375	1.726563	- 0.051756
8	1.726563	1.734375	1.730469	- 0.014957
9	1.730469	1.734375	1.732422	0.003512
10	1.730469	1.732422	1.731445	- 0.005728
11	1.731445	1.732422	1.731934	- 0.001109
12	1.731934	1.732422	1.732178	0.001202
13	1.731934	1.732178	1.732056	0.000045

When the function $f(x) = x^3 + x^2 - 3x - 3 = 0$ obtained from Gerald and Wheatley (1999) was solved using the Java implementation of the method of False Position, the following results were obtained.

Table 2 reveals that the method of False Position is faster to converge as can be seen in the values of X3; it converges at iterate 9. The values of X3 approach the true value of the root, which is √3 (1.732050808) as the number of iterations increase unlike the Root Bisection method which is irregular in that earlier estimates may be better than later ones. However, one should note that the method of False Position converges to the root from one side, which slows it down, especially if that end of the interval is farther from the root.

Table 2: Finding the root of $f(x) = x^3 + x^2 - 3x - 3 = 0$ starting with $X1 = 1, X2 = 2,$ and tolerance of 1E-4 by the method of False Position

ITR NO	X1	X2	X3	F(X3)
1.	1.0000000	2.0000000	1.5714286	-1.3644315
2.	1.5714286	2.0000000	1.7054108	-0.2477451
3.	1.7054108	2.0000000	1.7278827	-0.0393306
4.	1.7278827	2.0000000	1.7314049	-0.0061107
5.	1.7314049	2.0000000	1.7319509	-0.0009459
6.	1.7319509	2.0000000	1.7320353	-0.0001463
7.	1.7320353	2.0000000	1.7320484	-0.0000226
8.	1.7320484	2.0000000	1.7320504	-0.0000035
9.	1.7320504	2.0000000	1.7320508	-0.0000005
10.	1.7320508	2.0000000	1.7320508	-0.0000001
11.	1.7320508	2.0000000	1.7320508	-0.0000000
12.	1.7320508	2.0000000	1.7320508	-0.0000000
13.	1.7320508	2.0000000	1.7320508	-0.0000000

Program output for x1 = 1.0, x2 = 2.0; tolerance = 1.0E-4

Illustration 2: The function $f(x) = x^4 - 2 = 0$ obtained from Gerald & Wheatley (1999), was implemented with Java for the Root Bisection Method. The following results, shown in table 3, were obtained.

Table 3: Finding the root of $f(x) = x^4 - 2 = 0$ starting with $X1 = 1, X2 = 2,$ and tolerance of 1E-4

ITR NO	X1	X2	X3	F(X3)
1	1.0000000	2.0000000	1.5000000	3.0625000
2	1.0000000	1.5000000	1.2500000	0.4414063
3	1.0000000	1.2500000	1.1250000	- 0.3981934
4	1.1250000	1.2500000	1.1875000	- 0.0114594
5	1.1875000	1.2500000	1.2187500	0.2062693
6	1.1875000	1.2187500	1.2031250	0.0952845
7	1.1875000	1.2031250	1.1953125	0.0413893
8	1.1875000	1.1953125	1.1914063	0.0148350
9	1.1875000	1.1914063	1.1894531	0.0016555
10	1.1875000	1.1894531	1.1884766	- 0.0049100
11	1.1884766	1.1894531	1.1889648	- 0.0016293
12	1.1889648	1.1894531	1.1892090	0.0000126
13	1.1889648	1.1892090	1.1890869	- 0.0008085

When the function $f(x) = x^4 - 2 = 0$ obtained from Gerald & Wheatley (1999) was solved using Java implementation of the method of False Position, the following results were obtained.

Table 4: Finding the root of $f(x) = x^4 - 2 = 0$ starting with $X1 = 1, X2 = 2,$ and tolerance of 1E-4 by the method of False Position Approximate root = 1.189207115

ITR NO	X1	X2	X3	F(X3)
1.	1.0000000	2.0000000	1.0666667	- 0.7054617
2.	1.0666667	2.0000000	1.1114413	- 0.4740298
3.	1.1114413	2.0000000	1.1405419	- 0.3078263
4.	1.1405419	2.0000000	1.1590327	- 0.1953924
5.	1.1590327	2.0000000	1.1706082	- 0.1222133
6.	1.1706082	2.0000000	1.1777857	- 0.0757337
7.	1.1777857	2.0000000	1.1822096	- 0.0466595
8.	1.1822096	2.0000000	1.1849261	- 0.0286439
9.	1.1849261	2.0000000	1.1865903	- 0.0175454
10.	1.1865903	2.0000000	1.1876085	- 0.0107327
11.	1.1876085	2.0000000	1.1882308	- 0.0065598
12.	1.1882308	2.0000000	1.1886110	- 0.0040073

13.	1.1886110	2.0000000	1.1888432	- 0.0024473
14.	1.1888432	2.0000000	1.1889849	- 0.0014943
15.	1.1889849	2.0000000	1.1890715	- 0.0009123
16.	1.1890715	2.0000000	1.1891243	- 0.0005569
17.	1.1891243	2.0000000	1.1891566	- 0.0003400
18.	1.1891566	2.0000000	1.1891763	- 0.0002075
19.	1.1891763	2.0000000	1.1891883	- 0.0001267
20.	1.1891883	2.0000000	1.1891956	- 0.0000773

Program output for x1 = 1.0, x2 = 2.0; tolerance = 1.0E-4

Regula Falsi Method applied to Quadratic Equations

The Regula Falsi has already been implemented and shown to be realizable for non-linear polynomials of order greater than 2. In order to buttress its applicability to all non-linear polynomials in general, further examples elucidating its applicability to quadratic equations are shown in the following two examples:

Illustration 3: $f(x) = x^2 - 2 = 0$ (Adapted from Stroud and Booth, 2003)

WELCOME TO THE REGULA FALSI METHOD
THIS PROGRAM IMPLEMENTATION ALLOWS ONE TO FIND THE ROOT OF A POLYNOMIAL OR NON-LINEAR EQUATION
Enter the lower limit of the interval, x1: 1
Enter the upper limit of the interval, x2: 2
Enter the degree of the polynomial: 2
Now, enter the elements of the coefficient vector one after the other.
Enter A0: -2
Enter A1: 0
Enter A2: 1
Enter the tolerance value: 0.00001
Enter the maximum number of iterations in case tolerance is not met: 20

Approximate root found: 1.414214

ITR NO	X1	X2	X3	F(X3)
1	1.0000000	2.0000000	1.3333333	-0.2222222
2	1.3333333	2.0000000	1.4000000	-0.0400000
3	1.4000000	2.0000000	1.4117647	-0.0069204
4	1.4117647	2.0000000	1.4137931	-0.0011891
5	1.4137931	2.0000000	1.4141414	-0.0002041
6	1.4141414	2.0000000	1.4142012	-0.0000350
7	1.4142012	2.0000000	1.4142114	-0..0000060
8	1.4142114	2.0000000	1.4142132	-0.0000010
9	1.4142132	2.0000000	1.4142135	-0.0000002
10	1.4142135	2.0000000	1.4142136	-0.0000000
11	1.4142136	2.0000000	1.4142136	-0.0000000
12	1.4142136	2.0000000	1.4142136	-0.0000000
13	1.4142136	2.0000000	1.4142136	-0.0000000
14	1.4142136	2.0000000	1.4142136	-0.0000000
15	1.4142136	2.0000000	1.4142136	-0.0000000
16	1.4142136	2.0000000	1.4142136	-0.0000000
17	1.4142136	2.0000000	1.4142136	-0.0000000
18	1.4142136	2.0000000	1.4142136	-0.0000000
19	1.4142136	2.0000000	1.4142136	-0.0000000
20	1.4142136	2.0000000	1.4142136	-0.0000000

Program output for x1 = 1.0, x2 = 2.0, tolerance = 1.0E-5

Illustration 4: $f(x) = 2x^2 - 9x + 5 = 0$

Results obtained from Java implementation Regula Falsi Method for this equation are as follows:

First Root

Enter the lower limit of the interval, x1: 1
Enter the upper limit of the interval, x2: 4
Enter the degree of the polynomial: 2
Now, enter the elements of the coefficient vector one after the other.
Enter A0: 5
Enter A1: -9
Enter A2: 2
Enter the tolerance value: 0.00001
Enter the maximum number of iterations in case tolerance is not met: 20

Approximate root found: 3.850781

ITR NO	X1	X2	X3	F(X3)
1	1.0000000	4.0000000	3.0000000	-4.0000000
2	3.0000000	4.0000000	3.8000000	-0.3200000
3	3.8000000	4.0000000	3.8484848	-0.0146924
4	3.8484848	4.0000000	3.8506787	-0.0006552
5	3.8506787	4.0000000	3.8507765	-0.0000292
6	3.8507765	4.0000000	3.8507809	-0.0000013
7	3.8507809	4.0000000	3.8507811	-0.0000001
8	3.8507811	4.0000000	3.8507811	-0.0000000
9	3.8507811	4.0000000	3.8507811	-0.0000000
10	3.8507811	4.0000000	3.8507811	-0.0000000
11	3.8507811	4.0000000	3.8507811	-0.0000000
12	3.8507811	4.0000000	3.8507811	-0.0000000
13	3.8507811	4.0000000	3.8507811	0.0000000
14	3.8507811	4.0000000	3.8507811	0.0000000
15	3.8507811	4.0000000	3.8507811	0.0000000
16	3.8507811	4.0000000	3.8507811	0.0000000
17	3.8507811	4.0000000	3.8507811	0.0000000
18	3.8507811	4.0000000	3.8507811	0.0000000
19	3.8507811	4.0000000	3.8507811	0.0000000
20	3.8507811	4.0000000	3.8507811	0.0000000

Program output for x1 = 1.0, x2 = 4.0, tolerance = 1.0E-5

Second Root:
Enter the lower limit of the interval, x1: -1
Enter the upper limit of the interval, x2: 1
Enter the degree of the polynomial: 2
Now, enter the elements of the coefficient vector one after the other.
Enter A0: 5
Enter A1: -9
Enter A2: 2
Enter the tolerance value: 0.00001
Enter the maximum number of iterations in case tolerance is not met: 20

Approximate root found: 0.649219

ITR NO	X1	X2	X3	F(X3)
1	-1.0000000	1.0000000	0.7777778	-0.7901235
2	-1.0000000	0.7777778	0.6941176	-0.2834602
3	-1.0000000	0.6941176	0.6646267	-0.0981829
4	-1.0000000	0.6646267	0.6544741	-0.0335943
5	-1.0000000	0.6544741	0.6510076	-0.0114465
6	-1.0000000	0.6510076	0.6498273	-0.0038946
7	-1.0000000	0.6498273	0.6494258	-0.0013245
8	-1.0000000	0.6494258	0.6492893	-0.0004503
9	-1.0000000	0.6492893	0.6492429	-0.0001531

10	-1.0000000	0.6492429	0.6492271	-0.0000521
11	-1.0000000	0.6492271	0.6492217	-0.0000177
12	-1.0000000	0.6492217	0.6492199	-0.0000060
13	-1.0000000	0.6492199	0.6492193	-0.0000020
14	-1.0000000	0.6492193	0.6492190	-0.0000007
15	-1.0000000	0.6492190	0.6492190	-0.0000002
16	-1.0000000	0.6492190	0.6492190	-0.0000001
17	-1.0000000	0.6492190	0.6492189	-0.0000000
18	-1.0000000	0.6492189	0.6492189	-0.0000000
19	-1.0000000	0.6492189	0.6492189	-0.0000000
20	-1.0000000	0.6492189	0.6492189	-0.0000000

Program output for x1 = -1.0, x2 = 1.0, tolerance = 1.0E-5

4. Conclusion

The scale of modern day problems being solved by computational physicist requires the use of programming languages that are very easy to use; provide features which make it possible to re-use existing codes; is capable of specifying different operations to be executed simultaneously by the computer; and that enable distributed programs to be easily developed (Dass, 2010). Java is such a programming language, and has been used in this work to determine roots of non-linear equations as set out. The relevance that computational physics, numerical analysis or computational science in general has today, is as a result of a lot of work that had been done in the implementation of several computational methods using computer programming language (Stroud and Booth, 2001).

Implementation of the Regula Falsi method using both FORTRAN and Java implemented and compared in this work has shown that

i) Java implementation is more robust than FORTRAN implementation;
ii) Java is more adaptable in shorter listings than FORTRAN;
iii) Regula Falsi is fast and regular in convergence.

It therefore means that Java, a modern object oriented language which facilitates disciplined approach to program design with features that make it suitable for modern day computation is highly effective in the implementation of basic computational physics methods in such a way that makes the realization of computational objectives easy to achieve (Chow, 2000; DeVries, 1993). It is also robust in adaptation and implementation (Kiusalaas, 2005); and therefore a veritable tool in the implementation of various computational physics methods.

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Changes in Morpho-Physiology of *Jatropha curcas* grown under different water regimes

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Abstract: *Jatropha curcas*, a drought resistant perennial species has come into prominence as an important biodiesel crop. The investigation was aimed at studying the extent to which *Jatropha curcas* seedlings can resist water stress. During the experimentation, the response of *J. curcas* to water stress in terms of growth attributes, photosynthesis, fluorescence characters and leaf and soil water potential was studied. All the growth attributes showed drastic reduction in stressed plants in comparison to control plants. A significant reduction in fresh and dry biomass was observed with increasing water stress level. Minimum leaf and soil water potential was shown by stressed plants as compared to daily watered plants. When compared to control plants, photosynthesis decreased in water stressed plants and Fv/Fm, a parameter of PSII efficiency was maximum in control plants as compared to stressed plants.

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Key words: Water stress; Growth attributes; Photosynthesis; *J. curcas*

1. Introduction

Water is one of the major and essential abiotic components that regulate plant growth and development and its deficiency alters the morphological, physiological and biochemical properties of plants. Decreasing the external water potential by only -0.1 MPa or less results in a perceptible decrease in cellular growth (which is irreversible cell enlargement). The quantity and quality of plant growth depend on cell division enlargement, and differentiation and all of these events are affected by water stress (Cabuslay *et al.*, 2002, Correia *et al.*, 2001). The Severe water stress may result in the arrest of photosynthesis, disturbance of metabolism and finally the death of plant (Jaleel *et al.*, 2008c). When the water status in a leaf falls below a threshold value, stomata respond by closing with consequent reduction in CO_2 assimilation as well as transpiration. Stomatal closure decreases the carbon dioxide influx which limits photosynthesis and supports photoinhibition under high irradiance. Non-stomatal photosynthesis limitation has been attributed to the reduced carboxylation efficiency (Jia and Gray, 2004), reduction in ribulose 1-5, biphosphate regeneration, reduced amount of functional Rubisco (Kanechi *et al.*, 1995) and decrease in electron transport chain activities. Water stress caused negative effect on seedlings of Mediterranean water saver *Pinus halepensis* and water spenders *Quercus coccifera* and *Q. ilex.*, As a result stomatal conductance, CO_2 assimilation and transpiration rate reduced (Baquedano and Castillo, 2006). Similarly Mokhatar *et al.* (2011) evaluated effect of different water regimes on *Hevea brasiliensis* and concluded that *Hevea brasiliensis* cannot withstand water stress at nursery stage and replanting in dry areas.

Jatropha curcas L., a drought resistant perennial shrub or small tree is one of the most promising sources of bio-fuel today. It is known to grow in drier sites but can grow under a wide range of rainfall regimes from 250 to 1200mm per annum (Katwal and Soni, 2003). In low rainfall areas and in prolonged dry spell periods, the plant sheds its leaves to counter drought. However, its tolerance to water stress at the seedling stage is not known. Therefore the objective of present study was to analyze the response of *J. curcas* to water-deficit at seedling stage in respect of the growth behaviour and photosynthesis.

2. Material and Methods

J. curcas seeds were sown in seedling Styrofoam trays in mid March and after 15 days of their radical emergence, the seedlings were transferred to large plastic pots of 33cm diameter and 35cm height (approx capacity- 25 kg of soil, containing equal proportion of garden soil, sand and farmyard manure) and kept in a glass house.

The study was conducted in randomized block design with five replications. In each pot five seedlings were planted and for each treatment five pots were used. After 3 weeks of planting in pots (allowing seedlings to establish), the plants were subjected to water stress treatment. Before subjecting the plants to water stress treatment, mean leaf number per plant, leaf area and shoot length were recorded randomly. Three levels of water stress were given i.e. daily watering (control), watering after two days (2-D) and watering after five days (5-D). Watering of every pot was done

with 1000ml of water. Before the initiation of experiment, water holding capacity of soil (Goel and Trivedi, 1992) was determined, which was 25%. The plants were maintained in these water regimes for two months and subsequently observations were made on their growth, bioproductivity (capacity to produce biomass) and photosynthesis.

For growth measurements five plants from each water regime were randomly selected and their shoot length, number of leaves and leaf area were determined at fortnightly intervals. After 60 days, these plants were uprooted, washed thoroughly, blotted dry and the shoot length, root length, shoot thickness, number of leaves and leaf area were determined. Subsequently the root, shoot and leaves were separated and their fresh weight, dry weight and water content were determined. For dry weight determination, the plants were dried in an electric oven at 80^0 C for 48 hours (Evans, 1972). Leaf area was measured with portable leaf area meter (model Li 3000, Li COR, USA). Stem thickness was measured with the help of digital vernier calliper. Before the harvest, plants from all the treatments were studied to observe the changes in physiological parameters under stress condition. Leaf and soil water potential measurements of control and stressed plants were done using the WP4 Dew point Potentiometer. Gas exchange characteristics were measured using Li-COR photosynthetic system (Li-6400 portable photosynthetic system). To measure the chlorophyll fluorescence parameters in different treatments Plant Efficiency Analyzer PEA (Hansatech Ltd. U.K) was used.

The data were statistically analyzed using mean and standard deviation. ANOVA was applied to test the variations and least significant difference (Lsd at 5% level) was also determined to compare treatment mean. Simple correlation (Karl Pearson's) matrix between water regimes and morpho-physiological parameters were also prepared.

3. Results and Discussion

The results clearly indicated that all the aspects of plant growth and productivity were adversely affected by water stress. As shown in table 1, growth measurements recorded during the termination of experiment (after 60 days), were at best in daily watered plants while these were least in plants with five days watering cycle. Figure 1 shows the effect of water stress on shoot length, leaf number and leaf area from the date of initiation of stress upto 60 days. The growth of the plant was significantly inhibited under water stress condition. Data obtained that there was an inverse relationship between increasing level of water stress and the number of leaves and leaf area from all the treatments. The leaf number and leaf area were recorded maximum in daily watered plants (19±2.59 and 155.25±8.16 cm^2 respectively) and minimum in plants with five days watering cycle (11±2.30 and 55.72±6.85 cm^2 respectively). The first sign of water shortage in the field usually is a restriction in foliage growth (Fischer and Hagan, 1965, Jordan *et al*., 1975), attributable to the high sensitivity of expansive growth to water stress. The reduction in leaf growth may be due to the sensitivity of process of cellular growth to water stress, which is reduced long before photosynthesis and stomatal conductance (Hsiao, 1973). Similar results were recorded in soybean (Zhang *et al*., 2004) and Populus (Wullschleger *et al*., 2005), where water deficit stress mostly reduced leaf growth and in turn the leaf area. Hence, leaf area can be considered as an important parameter indicative of the level of stress.

Table 1. Effect of water stress on growth measurements of *J. curcas* after 60 days of stress treatment

	Control	2-D	5-D	P value	LSD
Growth attributes					
Leaf number	19	13	11	<0.001	3.01
Leaf area (cm^2)	155.25	77.54	55.72	<0.001	9.92
Shoot length (cm)	44.20	26.00	21.10	<0.001	3.86
Root length (cm)	25.00	19.60	14.60	<0.001	3.77
Shoot thickness (cm)	1.31	0.97	0.72	<0.001	0.13

The shoot length and shoot diameter were also affected adversely by water stress conditions. The shoot length and shoot thickness was maximum in daily watered plants (44.2± 3.96 cm and 1.31±0.11cm respectively) while it was minimum in plants with five day watering cycle (21.10±1.14 and 0.72±0.09 respectively) and the differences among different water regimes were highly significant (P< 0.001). Similar results were obtained by Aref and Juhany (2005) and Abdalla and Khoshiban (2007).The process of cell division, cell enlargement and differentiation which together are responsible for cell growth and development, are very much sensitive to water stress because of their dependence upon turgor (Jones and Lazeby, 1988), and this sensitivity must have resulted in the decreased growth of water stressed plants. Moreover, apical meristem which is responsible for shoot growth, is very much sensitive to water stress (Husain and Aspinall, 1969). According to Abe and Nakai (1999) lowering of water potential during early

phase of water stress, directly affects cell expansion, whereas in later stages rate of cambial cell division declines because of hormonal regulation.

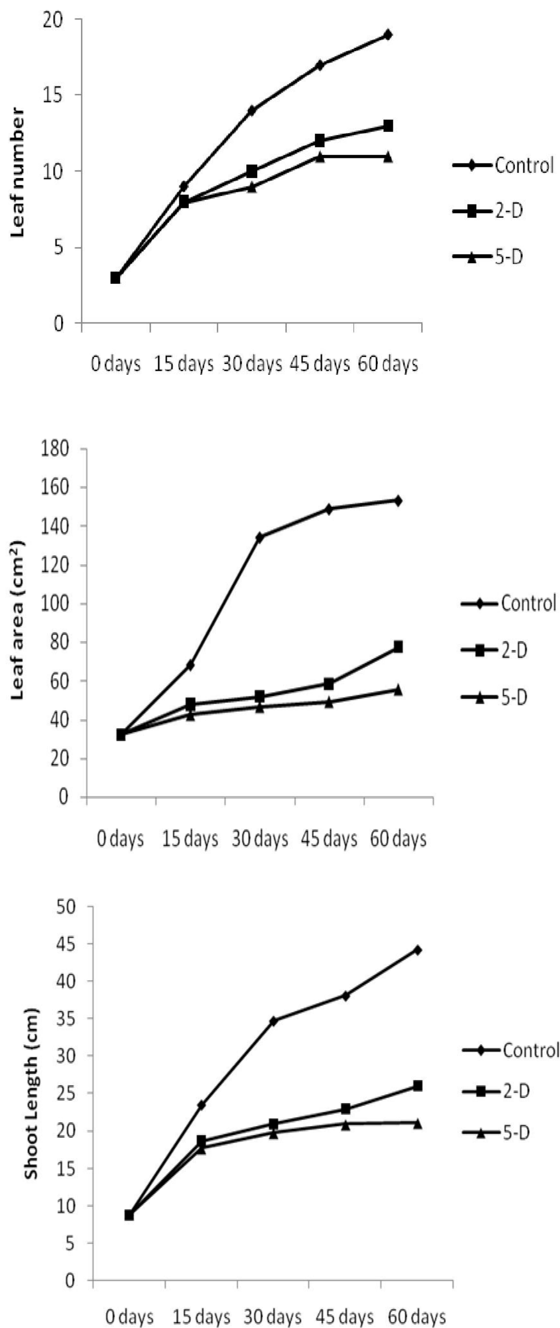


Figure 1. Changes in leaf number, leaf area and shoot length of *J.curcas* grown under different water regimes

Under limited supply of water plants usually develop extensive root system which prevent or postpone drought injury. But in the present study, increasing stress level also hindered root growth. However, the magnitude of reduction of root length was lower than that of shoot length. This means that the plant was able to maintain its internal root system to access soil water better. The results are in accordance with the results of Kuhns *et al.* (1985), Waring and Schlesinger (1985), Seiler and Cazell (1990), Pokhriyal *et al.* (1997), Kumari *et al.* (1999), where water stress caused decrease in root growth. The reduced extensibility of the root tip tissue due to hardening of the expanding cell walls might cause decline in root growth (Neumann *et al.* 1994). The maximum root length was recorded in daily watered plants i.e. 25.00±2.55 cm whereas minimum was recorded in plants with five day watering cycle 14.60±2.95 cm.

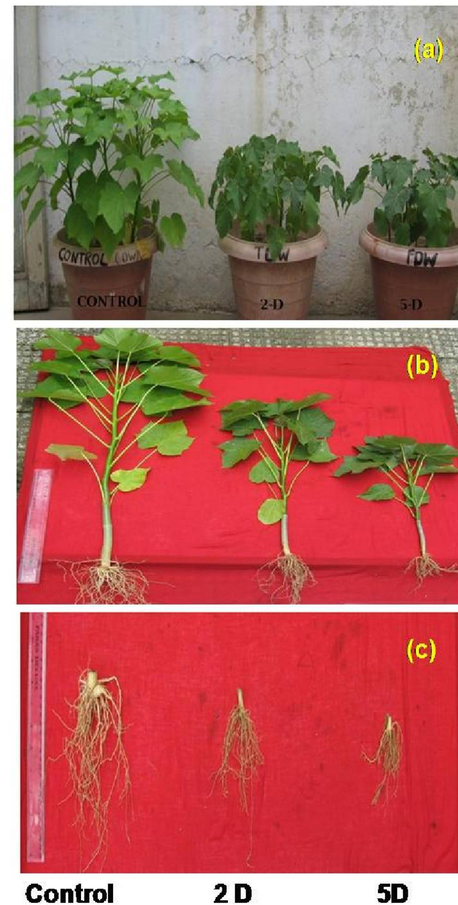


Plate 1. (a) *J. curcas* plants grown under different water regimes, (b) Changes in growth pattern under different water regimes, (c) Changes in root growth under different water regime

Water stress affects the soil moisture thus soil water potential and leaf water potential are also affected. Therefore, tissue water potential is the best parameter to measure water stress in studies of plant responses to water stress. Drought is a situation that

lowers plant water potential and turgor to the extent that plants face difficulties in executing normal physiological functions. Water stress caused reduction in leaf water potential from -0.49 MPa in control plants to -1.95 MPa in 5-D plants. A similar trend was also recorded in case of soil water potential (Figure 2). Similar observations where water stressed plants showed lowest value of water potential have also been reported by Yadav *et al*. (2001) in wheat, Chartzoulakis *et al*.(2002) in two avocado cultivars, Klamkowski and Treder (2006) in strawberry. As the primary effect of dehydration on plants, the rapid lowering of leaf water potential is associated with relatively modest losses of water from the leaf tissue, which ultimately cause the loss of turgor. When soil water is inadequate for plant supply, the transpirational water loss reduces the water potential in the tissue. Under stress there is a reduction in turgor, leading to the narrowing of stomatal aperture and a progressive reduction in photosynthetic activity.

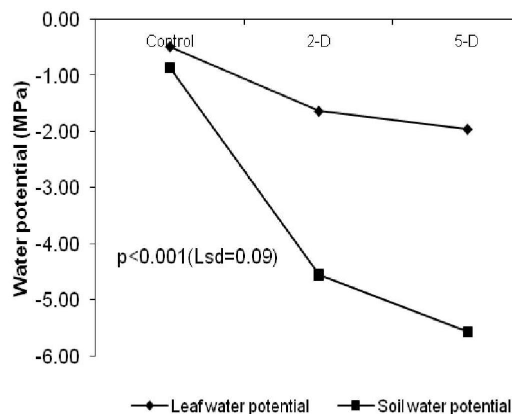
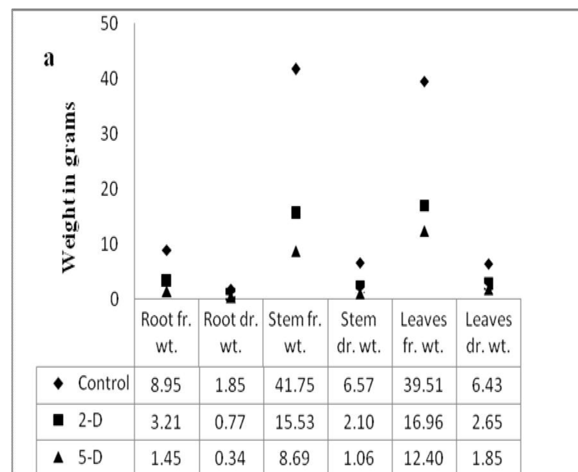


Figure 2. Effect of water stress on leaf and soil potential of *J. curcas* after 60 days of stress treatment

There was a reduction in whole plant fresh weight and dry weight with the increase in water stress level. Daily watered plants accumulated higher biomass than stressed plants. There was a significant difference (P<0.001) among the different water regimes in fresh and dry weight of plants (Figure 3). Decreased plant height, total fresh weight and dry weight of *Trachyspermum ammi* under increasing water stress level have also been reported by *Azhar et al*. (2011).Our results confirm the finding of Ghassemi-Golezani *et al*. (2008) who reported a decrease in fresh and dry biomass of *Anethum graveolens* under water stress condition. Since the growth is especially sensitive to water stress, this could have occurred due to impairment of cell division process: cell elongation and cell expansion (Hussain *et al*., 2008), as a result yields decreased noticeably even with moderate stress.



	Root fr. wt.	Root dr. wt.	Stem fr. wt.	Stem dr. wt.	Leaves fr. wt.	Leaves dr. wt.
◆ Control	8.95	1.85	41.75	6.57	39.51	6.43
■ 2-D	3.21	0.77	15.53	2.10	16.96	2.65
▲ 5-D	1.45	0.34	8.69	1.06	12.40	1.85

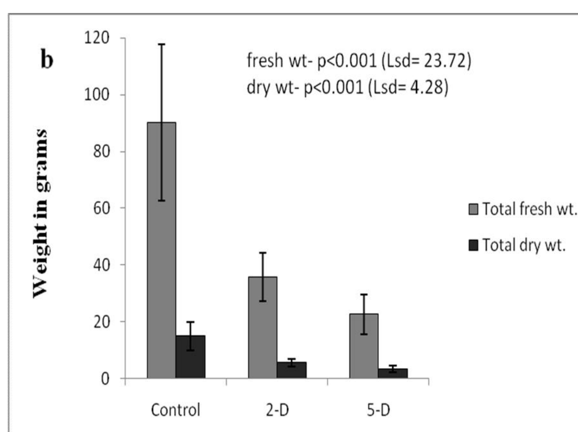


Figure 3. Effect of water regimes on (a) fresh weight and dry weight of root, stem and leaves and (b) total fresh weight and dry weight in *J. curcas*

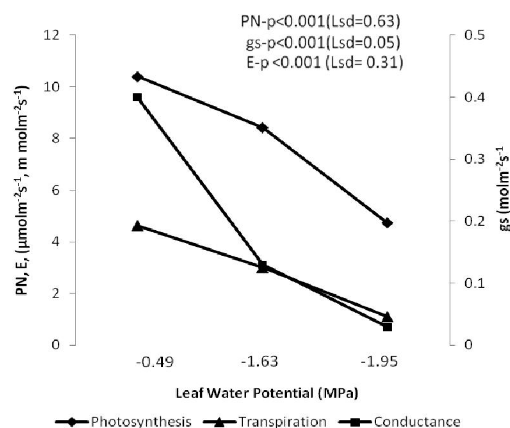


Figure 4. Variation in photosynthetic rate, conductance and transpiration with respect to variation in leaf water potential in *J. curcas* under different water regimes

A considerable decrease in rate of photosynthesis from 10.4 µmol m^{-2} s^{-1} in daily watered plants to 4.74 µmol m^{-2} s^{-1} in 5-D plants was recorded (Figure 4). Our results are in accordance with those of Klamkowski and Treder (2006), Yang *et al*. (2010) and Mokhatar *et al*. (2011), where photosynthesis had inverse relationship with increasing water stress. Decreased photosynthesis in water stressed plants could be due to closed stomata, decreased hill reaction or due to reduced leaves which are photosynthetically less efficient as also reported by Marani *et al*. (1985).

When plants are rapidly stressed stomata close very effectively and the depression of photosynthesis can be almost totally ascribed to isolation from external CO_2 supply. Boyer and Bowen (1970) reported convincing data indicating that even moderate stress on sunflower leaves inhibit the activity of Photosystem II in chloroplast fragments isolated from these leaves. The inhibition of Photosystem II in sunflower was well correlated with the inhibition of CO_2 assimilation by intact leaves for the range of leaf water potential from the beginning of to complete stomatal closure. Mohanty and Boyer (1976) found that the quantum yield of photosynthesis in intact leaves as well as Photosystem II activity in isolated chloroplast fragments were reduced by stressing the plants. These data indicate that basic aspects of light- harvesting and energy conversion were altered by stress. Like photosynthesis, transpiration and conductance rate were also higher in well watered plants than in stressed ones (Table 2). Statistical data indicated a significant effect of water stress on WUE (A/E). WUE was increased with increasing water stress level i.e. from 2.26 mmol/mol in control plants to 4.41mmol/mol in 5-D plants. Wie Hua *et al*. (2003) also reported an increase in WUE in *Hippophae rhamnoides* seedlings under drought conditions. According to Heitholt (1989) and Jensen (1976) moderate water stress could improve the WUE, but this improvement is at the cost of reduced growth. Transpiration depends upon opening and closing of guard cells. Decrease in rate of transpiration might have occurred due to lose of turgidity of guard cells under soil moisture deficit conditions causing stomatal closure. This limits the rate of CO_2 diffusion through stomates causing decline in the photosynthetic rate (Luvaha, 2005). The plants suffering with water stress are comparatively warmer than well watered plants because of reduced transpiration (Begg, 1980). Kirnak *et al*. (2001) reported that in egg plant water stress increased the leaf temperature upto 3-4^0 C as compared to control plants. Similar was the case in the present study in which decrease in soil water also caused sequential decrease in the rate of transpiration which causes increase in leaf temperature.

Table 2. Effect of water stress on physiological parameters of *J. curcas* after 60 days of stress treatment

	Control	2-D	5-D	P value	LSD
Physiological Parameters					
Photosynthesis	10.40	8.43	4.74	<0.001	0.63
Conductance	0.40	0.13	0.03	<0.001	0.05
Transpiration	4.63	3.01	1.12	<0.001	0.31
Leaf Temperature	29.89	31.76	34.00	<0.001	0.30
Water Use Efficiency	2.26	2.80	4.41	<0.001	0.66

Among the different fluorescence parameters studied in *J. curcas* under different water regimes, the Fv/Fm, a parameter of PSII efficiency, in general was higher in well watered plants in comparison to stressed plants (Figure 5). Similar results were obtained by Jeyaramraja *et al*. (2003) who observed a clear reduction in Fv/Fm ratio with increasing soil moisture deficit, indicating a loss in the primary photochemical efficiency of the stressed leaves. The reduction of Fv/Fm must be due to the reduced efficiency of PSII apparatus and under water stress photosynthetic electron transport through PSII is inhibited (Chakir and Jenson, 1999). Hamidou *et al*. (2007) also reported a similar trend in cowpea and asserted that decrese in photochemical activity under water stress condition was mainly due to stomatal process.

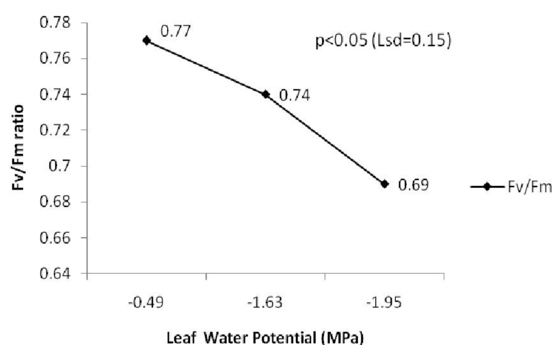


Figure 5. Changes in Fv/Fm ratio in daily watered plants (Ψ= -0.49 MPa), 2-D plants (Ψ= -1.63MPa) and 5-D plants (Ψ= -1.95 MPa)

Table 3. Correlation matrix among different parameters of *J. curcas* after 60 days of stress treatment

	WR	*PN*	*gs*	*E*	*Fv/Fm*	*LWP*	*SWP*	*LN*	*LA*	*SL*	*RL*	*ST*
WR	1											
PN	-0.99***	1										
Gs	-0.87**	0.94***	1									
E	-0.97***	0.99***	0.97***	1								
Fv/Fm	-0.82**	0.82**	0.76*	0.81**	1							
LWP	-0.83**	0.91***	0.99***	0.94***	0.71*	1						
SWP	-0.83**	0.91***	0.99***	0.94***	0.72*	0.99***	1					
LN	-0.70*	0.77**	0.84**	0.80**	0.72*	0.81**	0.84**	1				
LA	-0.85**	0.92***	0.99***	0.95***	0.74*	0.98***	0.98***	0.87**	1			
SL	-0.78**	0.87**	0.96***	0.90***	0.72*	0.96***	0.97**	0.92***	0.98***	1		
RL	-0.89***	0.91***	0.85**	0.90***	0.71*	0.82**	0.83**	0.80**	0.83**	0.85**	1	
ST	-0.88***	0.92***	0.93***	0.93***	0.89***	0.90***	0.91***	0.88***	0.91***	0.93***	0.92***	1

Note: * = $p<0.05$, ** = $p<0.01$ ***= $p<0.001$ significance level, WR- water regime, PN-photosynthesis, gs-conductance, E-transpiration, LWP-leaf water potential, SWP-soil water potential, LN-leaf number, LA-leaf area, SL-shoot length, RL- root length, ST-shoot thickness

The correlation matrix among the various parameters studied was shown in table 3. The table clearly indicated that all the parameters were negatively correlated with the increasing level of water stress. However all the morpho-physiological parameters studied during the experimentation were highly and positively correlated among themselves. This showed that water stress slowed down all the morpho-physiological characters of *J. curcas*.

Although *J. curcas* is a drought tolerant plant but a close perusal of data obtained in present study indicated that imposition of even a mild water stress would lead to significant reduction in morpho-physiological attributes of *J. curcas*. This was evident from the differences between daily watered and water stressed plants that were of higher magnitude between 2-D and control plants than between the 2-D and 5-D plants. Such a response of a drought tolerant species to water stress could be an area of further research.

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Studies on the effect of clindamycin on experimental infection with *Staphylococcus aureus* in relation to dose and treatment with special emphasis to antibiotic bacterial resistance

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Abstract: present study aimed to investigate and search about the most common causes of bacterial resistance to antiboiotics which are the dose and method of treatment using Clindamycin on experimental infected mice with *Staphylococcus aureus.* ATCC 25923 and study the effect of antibiotic on immune response humeral and cellular. A total number of 100 adult albino male mice were divided into 5 groups (20 mice each.). All groups were injected intra peritoneal I/P with *Staphylococcus aureus* 3 x10^9 cuf. 24 hours after bacterial injection all groups were treated with Clindamycin 500 mg/8h for 8 days. 1st group: control group.: animals apparently healthy untreated.orally received saline sol., 2nd group: orally received single dose of defined prescribed dose of Clindamycin, 3rd group: orally received 5 times the defined dose of Clindamycin then decreased,4th group: orally received 1/5 of defined dose Clindamycin then increased and 5th group: orally administrated the initial dose of Clindamycin along the experiment. Animals were sacrificed, blood samples were collected and divided into 2 portions; first one added to it anticoagulant for determination of phagocytic index and the second one without anticoagulant for Serum collection then keep in -20°C till used for determination of anti body titer in experimental infected mice. Blood samples were collected 6 hours after antibiotic administration for 8 days. Determination of antibotic concentration in blood, determination of antibody titer in serum and phagocytic index in blood for detection of the effect of antibiotic dose on the immune response of experimental infected mice with *S.aureus*. At the end of treatment survived mice scarified and isolation of *S. aureus* was performed, the isolated strain was identified as *Staphylococcus aureus.* ATCC 25923 (same injected strain) using API20E system. Antibacterial susceptibility performed using disk diffusion method, the results displayed that the concentration of anti Clindamycin in serum was correlated with anti body titer and phagocytic index, all the obtained results were discussed.

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Key words: Clindamycin; *Staphylococcus aureus;* immune response; humeral; cellular; antibody titer; phagocytic index; disk diffusion

Introduction:

Disease-causing microbes that have become resistant to antibiotic drug therapy are an increasing public health problem. Antibiotic resistance is a serious and growing phenomenon in contemporary medicine and has emerged as one of the major public health concerns, particularly if it is included pathogenic organisms (**Drusano, 2004; Finley, *et al.*,2013).** Nowadays, about seventy percent of the bacteria that cause infections are resistant to at least one of the drugs most commonly used for treatment. Some organisms are resistant to all approved antibiotics and can only be treated with experimental and potentially toxic drugs (**Handel, *et al*.,2009**).

Although there were low levels of preexisting antibiotic-resistant bacteria before the widespread use of antibiotics **(Caldwell & Lindberg 2011; Nelson 2009**) evolutionary pressure from their use has played a role in the development of multidrug resistance varieties and the spread of resistance between bacterial species.(**Hawkey & Jones, 2009,**) In medicine, the major problem of the emergence of resistant bacteria is due to misuse and overuse of antibiotics. (**WHO**, **2002).**

In some countries, antibiotics are sold over the counter without a prescription, which also leads to the creation of resistant strains. Other practices contributing towards resistance include the addition of antibiotics to livestock feed. (**Okeke, *et al.*,1999; Ferber, 2002; Mathew, *et al.*,2007**) The use of antimicrobials in farming (**Witte, 2000; Teuber, 2001**), together with the practice of raw sewage discharge into receiving waters, has resulted in a significant increase of antibiotic resistant bacteria in aquatic environments. Household use of antibacterials in soaps and other products, although not clearly contributing to resistance, is also discouraged (as not being effective at infection control) (**CDC,2009**) Unsound practices in the pharmaceutical manufacturing industry can also contribute towards the likelihood of creating antibiotic-resistant strains.

(**Larsson and Fick, 2009** ;**Novo,** ***et al*****.,2013**). Present study aimed to investigate and search about the most common causes of bacterial resistance to antiboiotics which are the dose and method of treatment using clindamycin on experimental infected albino mice with *Staphylococcus aureus* and study the effect of antibiotic on immune response humeral and cellular.

Material and methods:

Bacterial strain:

Staphylococcus aureus. ATCC 25923, lyophilized strains Kindly, supplied from King Fahd Hospital (armed forces) in Jeddah.

Antibiotics:

Antibiotic used in the experiment was purchased from local pharmacy in Jeddah, commercial Clindamycin with defined dose 500 mg /8 h. for 8 days. Clindamycin is a good choice to treat a variety of *Staphylococcus aureus* infections.

experimental design:

A total number of 100 adult albino male mice (55 - 65 gm) body weight were divided into 5 groups (20 mice each.). All groups were injected intra peritoneal I/P with *Staphylococcus aureus* 3 x10^9 cuf. 24 hours after bacterial injection all groups were treated as in the table 1.

Table 1: showing experimental groups administrated clindamycin

groups	treatment
1st group	control group: animals apparently healthy untreated.orally received saline solution.
2nd group	orally received single dose 500mg/8h. of defined prescribed dose of clindamycin *
3rd group	orally received 5 times the defined dose of clindamycin then decreased **
4th group	orally received 1/5 of defined dose clindamycin then increased ***
5th group	orally administrated the 500mg/8h. of c clindamycin along the experiment (+ control)

*Normal dose 500 mg/8h.

**Initial dose 2500 mg decreased to 2000 mg, 1500mg,1000mg,500mg,50mg,25mg and10mg

***Initial dose 50 mg increased to 100 mg, 200mg,300mg,400mg,500mg,600mg and700mg

Sampling:

Animals were sacrificed, blood samples were collected and divided into 2 portions with first one added to it EDTA as anticoagulant for determination of phagocytic index and the second one without anticoagulant and placed in a slope in 4°C for 24 h. then centrifuged 1000 xg for 10 min at 4ºC. Serum was collected and keep in -20°C till used for determination of anti body titer in experimental infected mice. Blood samples were collected 6 hours after antibiotic administration for 8 days.

determination of antibodies concentration in blood:

Serum samples containing unknown concentrations of antibiotic were diluted 1:2, 1:4, and 1:5 in sterile pooled human serum. Four seeded agar plates were used, each with two discs saturated with the reference standard and two discs saturated with the patient's undiluted or diluted serum. For maximal accuracy, duplicate plates were set up with discs saturated with diluted serum. The plates were incubated for 4 hr at 37 °C. Zone diameters of the reference standards and of the patient's serum were measured and averaged. Correction of the zone diameter was accomplished by adding or subtracting the difference between the mean zone diameter of the reference standard obtained in the assay plate and the zone diameter of the correction point of 2.0 µg/ml in the standard curve. The final result, in micrograms per milliliter, was extrapolated from the standard curve and multiplied by the dilution of the original specimen. **(Warren,** ***et al*****.,1972)**

Determination of antibody titer:

Antibody titer determined using Thermo Scientific Easy-Titer Antibody Assay Kits (23300) which, enable accurate determination of antibody concentration in mice serum in brief Prepare standards (5 to 500 ng/mL) by diluting purified antibody in Kit Dilution Buffer. Prepare samples by diluting at least 20-fold in Dilution Buffer to within assay range (8 to500 ng/mL).Vortex vial of microsphere beads to create homogeneous suspension. Pipette 20µL of bead suspension and 20µL of each sample and standard into 96-well microplate wells. Incubate microplate for 5 minutes with vigorous mixing. Add 100 µL of Kit Blocking Reagent. Incubate microplate for 5 minutes with vigorous mixing. Measure absorbance on standard plate reader (340 nm). Plot standard curve and interpolate samples to determine concentration.

Phagocytic assay:

Macrophage monolayers were obtained and prepared it were harvested from the peritoneal cavities of mice and were resuspended in Hanks' balanced salt

solution (HBSS) (Sigma Chemical Co., St. Louis, MO). The number of viable cells was determined by trypan blue dye exclusion and the coverslips were washed with HBSS. *Candida albican* particles were added to the monolayers in a 5:1 (particle: macrophage) ratio and the coverslips incubated at 37°C in humidified atmosphere. After 30 min, the coverslips were washed with HBSS, fixed in methanol, and stained with Giemsa. After drying, the coverslips were mounted on glass slides and examined microscopically. The percentage of cells with ingested particles was multiplied by the average number of particles per macrophage to calculate phagocytic index. At least 100 macrophages were counted per cover slip (**Belline, *et al*.,2004)**

Antibacterial susceptibility and disk diffusion method:

Before and after clindamycin treatment course each experimental group, mice were scarified and bacterial strain was isolated from liver and spleen in nutrient broth incubated at 37°C for 24h. isolated strain was identified using API20E Antimicrobial resistance patterns of isolates were determined by the agar disk diffusion method **(Bauer, *et al*., 1996**). *S. aureus* was suspended in sterile saline to a turbidity to match a McFarland No. 2 standard (bioMe´rieux, Marcy l'Etoile, France), diluted 1:20, and streaked on Mueller-Hinton agar (Difco Laboratories, Detroit, MI). Clindamycin disks. Plates were incubated at 37 °C for 24 h. Characterization of strains as sensitive, intermediate or resistant was based on the mean size of the inhibition zones around each disk according to the National Committee for Clinical Laboratory Standards criteria (**NCCLS, 1999).**

Statistical:

The mean values of antibiotic concentration in serum and phagocytic index in blood. All the experimental groups comparing to the control (5th group orally administrated the inital dose of clindamycin along the experiment) were subjected to statistical analysis, using the "F" test.

Results:

concentrations of antibiotic in serum:

present study revealed that in the 2nd group which subjected for one dose only of clindamycin 500 mg/12 h for 8 days, the antibiotic concentration decreased from the 1st day to be 0 concentration at day 7 and 8, while the 3rd group which subjected for 5 times the normal prescribed dose the concentration decreased along 8 days of the experiment but did not rich to concentration zero. 4th Group which subjected to1/5 defined dose then decreased the antibiotic nearly found along the course in suitable high concentration but not constant concentration the higher concentration in the day 5 and the lowest concentration in the day 8. Group 5 which subjected to 500 mg/8h for 8 days, concentration of clindamycin was nearly constant along 8 days of treatment. Concerning the mortality percent in all treated groups, the highest mortality was in 2nd group 46% (orally received single dose of defined prescribed dose of clindamycin) while the lowest mortality was in 5th group 3% (orally administrated the inital dose of clindamycin along the experiment) table 2.

Table 2: Showing concentrations of clindamycin in serum during 8 days after experimentally infected mice with S. *aureus* (mg) and mortality%

group	**0 +6h.**	**12+6h**	**24+6h**	**36+6h**	**48+6h**	**60+6h**	**72+6h**	**84+6h**	**Mort.**
1st gp	00	00	00	00	00	00	00	00	00.00
2nd gp	465±23	411±19	373±18	216±11	134±8.3*	11±0.5*	00±00*	00±00*	46%*
3rd gp	2224±89*	1732±86*	1234±61*	789±39	471±23	211±12	56±2.8*	21±1.0*	33%*
4th gp	46±2.5*	92±4.6*	164±8.2*	215±10	377±17	283±14	76±3.8*	35±1.7*	13%*
5th gp	478±21	495±24	488±22	495±20	499±25	479±19	498±19	497±21	3%

Mean of 2 samples ± SD * significance

Antibody titer serum of infected mice with *S.aureus*:

Results showed that the antibody titer in 2nd group was the highest titer 1/64 was in day 5 then decreased to 1/16 in the day 8 while 3rd group showed that the highest titer was in day 2 and 3. 4th group displayed that the highest titer was in day 3 while 5th group showed that the highest titer was in day 8 and lowest titer was in day1 to day 7 table 3.

Table 3: showing antibody titer of experimentally infected mice with *S.aureus*

group	0+6h.	24+6h	36+6h	48+6h	60+6h	72+6h	84+6h	96+6h
1st gp	00	00	00	00	00	00	00	00
2nd gp	1/8	1/16*	1/32*	1/32*	1/64*	1/32*	1/16	1/16*
3rd gp	¼ *	1/8	1/8	¼*	½*	½*	½*	00*
4th gp	1/16*	1/16*	1/32*	1/16	1/8	1/8	¼*	00*
5th gp	1/8	1/8	1/8	1/16	1/8	1/8	1/16	1/32

* significance

Phagocytic index of infected mice with *S.aureus:*

Present study displayed that the phagocytic index in 2nd group was low then increased, the highest phagocytic index was in day 8 while the lowest was in day1. 3rd group revealed that the highest phagocytic index was in day 8 while the lowest was in day 6 in comparison 4th group showed that the highest phagocytic index was in day1 while the lowest one was in day8. Group 5th showed that the highest phagocytic index was in day 6 while the lowest was in day 8 but the phagoctic index nearly in the same level in this group table 4.

Table 4: Showing phagocytic index of experimentally infected mice with *S.aureus*

group	12+6h.	24+6h	36+6h	48+6h	60+6h	72+6h	84+6h	96+6h
1st gp	00	00	00	00	00	00	00	00
2nd gp	23±1.14*	16±0.90	43±2.15*	56±2.80*	77±3.84*	89±4.45*	94±4.72*	98±4.61*
3rd gp	12±0.60	23±1.15*	31±1.55*	11±0.51	8±0.70*	5±0.25*	45±2.25*	54±2.64*
4th gp	87±4.35*	73±3.65*	61±3.05*	63±3.14*	46±2.30*	32±1.40*	13±0.65	5±0.33
5th gp	9±0.45	12±0.60	11±0.55	10±0.60	19±0.96	14±0.63	11±0.33	6±032

Mean of 2 samples ± SD * significance

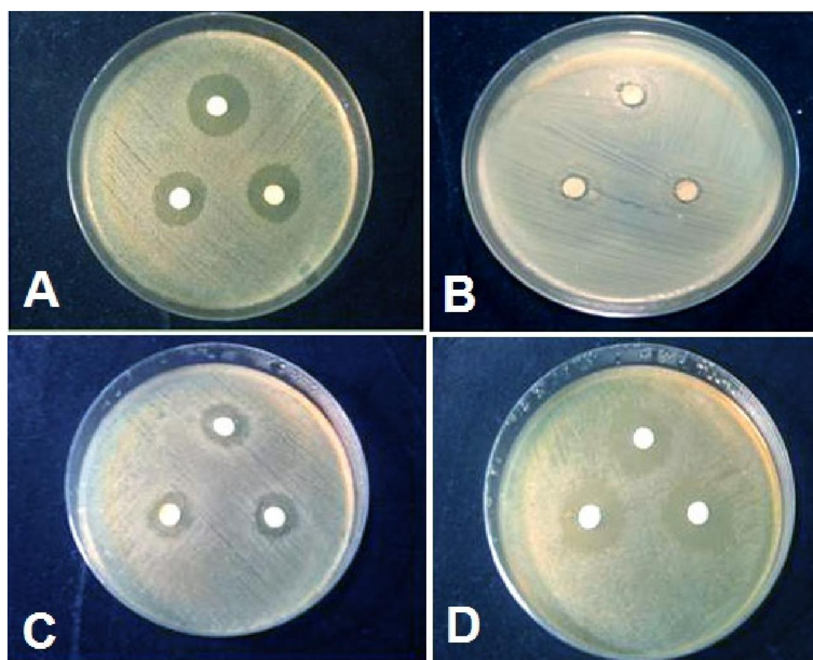

Figure1: Antibacterial susceptibility with disk diffusion A. plate showing large inhibition zone of *S. aureus* against clindamycin before experiment, B. plate showing small inhibition zone of *S. aureus* isolated from group 2(orally received single dose of defined prescribed dose of clindamycin) C. plate showing relatively small inhibition zone of *S. aureus* isolated from group 3 (orally received 5 times the defined dose of clindamycin then decreased) D. plate showing large inhibition zone of *S. aureus* isolated from group 5 (orally administrated the initial dose of clindamycin along the experiment).

Disk diffusion method and antibacterial susceptibility:

Isolated bacterial strain was identified as *S. aureus* and tested for antibiotic resistance using disk diffusion method and inhibition zones was measured for each group it is expressed as mean for 3 replicates ±SD. Present study revealed that the highest inhibition zone was for test before experiment and the lowest group was the second group (orally received single dose of defined prescribed dose of clindamycin) followed by 4th group (orally received 1/5 of defined dose clindamycin then increased) then 3rd group an 5th group table, 5 fig, 1.

Table 5: showing inhibition zone in different experimental group before and after treatment of *S.aureus* with clindamycin (3 replicates±SD)

group	Inhibition zone (mm)
Before treatment	19± 0.95*
2nd group	3±0.15*
3rd group	9±0.45
4th group	7±0.35
5th group	12±0.60

* significance

Discussion:

present study aimed to investigate and search about the most common causes of bacterial resistance to antiboiotics which are the dose and method of treatment using clindamycin on experimental infected albino mice with *Staphylococcus aureus* and study the effect of antibiotic on immune response humeral and cellular.

One of the most terrible problems in medicine is the emergence and spread of antibiotic resistance, which is becoming a serious menace to modern societies. the major problem of the emergence of resistant bacteria is greatly due to misuse and overuse of antibiotics. (**WHO, 2002**). An increasing resistance to antibiotics, which are in daily use for the treatment of infections in both humans and animals, is well documented. Many bacteria have become resistant to several types of antibiotics and there are hardly any new antibiotics available to combat multiresistant bacteria of this kind. It is important to make sure that antibiotics continue to be an effective treatment for the generations to come, but incorrect and excessive use of antibiotics can increase the occurrence of resistant microorganisms.

Present study indicated that un- proper dose and treatment with clindamycin; high then lowed, low then high and single dose along the course of treatment, all make disturbance of clindamycin concentrations in serum along the treatment course, the results nearly coincide with (**Golub, *et al*.,1990; Labro, 2000; Barrie, 2012).**

Antibiotics are typically dosed at levels below the minimum inhibitory concentration (MIC) so as to reduce the bacterial resistance. For instance, some antibiotics, when administered at levels above the MIC inhibit phagocyte function. These effects seem to be independent of their antibacterial effect (**Tacconelli, *et al*., 2009 and CDC, 2009**)

Concerning the effect of antibiotics and immune response. present study displayed that the immune response of experimental infected mice with *S, aureus* both arms of immune response humeral and cellular immunity were affected,the results indicated that antibody titer of all experiment was Results showed that the antibody titer in 2nd group was the highest titer 1/64 was in day 5 then decreased to 1/16 in the day 8 while 3rd group showed that the highest titer was in day 2 and 3. 4th group displayed that the highest titer was in day 3 while 5th group showed that the highest titer was in day 8 and lowest titer was in day1 to day 7,this meaning that there antagonistic effect of antibody titer and dose of clindamycin the results nearly agree with that obtained by **Woo, *et al*. (1999)** reported that clarithrtiomycin and doxycycline suppress the antibody response induced by tetanus toxoid, pneumococcabiol polysaccharide vaccine,

Concerning cellular immunity and effect of clindamycin, present study displayed that the phagocytic index in 2nd group was low then increased, the highest phagocytic index was in day 8 while the lowest was in day1. 3rd group revealed that the highest phagocytic index was in day 8 while the lowest was in day 6 in comparison 4th group showed that the highest phagocytic index was in day1 while the lowest one was in day8. Group 5th showed that the highest phagocytic index was in day 6 while the lowest was in day 8 but the phagoctic index nearly in the same level in this group the results nearly agree with the obtained results by **Williams, *et al*. (2005**) reported that quinolones, including ciprofloxacin and moxifloxacin, are nalidixic acid analogue antibiotics, which exert their bactericidal effect by inhibiting DNA gyrase activity. Ciprofloxacin has been shown previously to have immunomodulatory effects, recently reviewed by **Dalhoff and Shalit (2003)**, and although moxifloxacin is a relatively new drug, it too has been shown to be immunomodulatory in both animals and humans. (**Shalit, *et al*., 2001; Williams, *et al*., 2001**) Clarithromycin is a semi-synthetic acid-stable member of the broad-spectrum macrolide family and has been shown to modulate release of several cytokines.4,5 T helper lymphocytes are important for both cell-mediated and humoral immunity. (**Mosmann and Sad 1999**).

The immune system is natural defense mechanism against illness. It allows your body to fight against the invasion by bacteria, viruses, yeast, fungus etc. Taking antibiotics reduces the level of bacterial infection, but your immune system still has to completely finish fighting the infection (**Shalit, *et al*.,2001**).Once you have a particular infection, and your body fights it without the use of antibiotics, your immune system will develop 'memory T cells' (**Williams, *et al*.,2001**). The next time you contract the same infection, these memory T cells "remember" the previous infection and mounts an immediate immune response to fight it. With the use of antibiotics you are giving the responsibility of fighting infection to the antibiotics instead of your body's immune system. So overtime, and with the overuse of antibiotics your immune system can become less effective (**Morikawa, *et al*.,1996**).

Regarding the antibacterial susceptibility and antibiotic resistance disk diffusion method was used for determination of *S. aureus* resistance for clindamycin, after treatment course, present study revealed that isolated bacterial strain was identified as *S. aureus* and tested for antibiotic resistance using disk diffusion method and inhibition zones were measured for each group. Present study revealed that the highest inhibition zone was for test before experiment and the lowest group was the second group (orally received single dose of defined prescribed dose of clindamycin) followed by 4th group (orally received 1/5 of defined dose clindamycin then increased) then 3rd group an 5th group. The results nearly agree with **Messi, *et al*., (2005)** and **Novo, *et al*.,(2013).**

From present study it was concluded that treatment with antibiotics must be carful and with proper dose and duration to avoid antibiotic resistance which may result from taking antibiotics with wrong way or when they are not needed.. When bacteria survive after a course of antibiotics, another antibiotic must be used with proper dose for complete course even after treatment, to avoid creation of resistant bacteria to antibiotic. During infection using of antibiotics suppress the immune response humeral and cellular so that infected patients must be use probiotics as alternative to antibiotics.

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Study the balance of men's violence against women and Education in Tabriz city

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Abstract today, one of the family phenomena that interest to researchers is the subject of violence in families. Accordingly, the original aim of the current study is to reach the balance of men's violence against women with related variables among families' residing of Tabriz city. to reach the objectives of the study, 400 families (with male and female) were selected with the sampling method for several stages. Given what topic method has been used survey field and correlation. Also, data collection tools include Sigmund and Estate tests to measure depression and anxiety, assertiveness Kambril and Rege test, 57 questions Eysenck test and questionnaire build by researcher. The results show that 55% mental violence, social violence 30 % and physical violence have 28.5%. Male violence against women as well as women with some demographic variables such as education, social class, family income, education level males and psychological variables such as anxiety, depression, and assertiveness is related to men and women. Given the results of the study, it is necessary for families to know how cope with the challenges of life so that they can control with anger, depression and anxiety to find them an alternative to violence, irritating the rest of the cases in the family.

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1. Introduction

The definition of family in the area of study and investigation as fact several dimensions, and can study the different dimensions and themes. One of the phenomena families today that are of interest to investigators, sociologists and psychologists that violence in the family or with the normal convention men's violence against women in the family. Target of violence in this study is the behavior with intent, intention fishing, hide or physical damage (entry, psychological and social to the other individual [1]. But men's violence against women include several multiple dimensions that are in the meantime can be referred to physical violence (damage by members of the body with beatings or wound using hand tools or other), psychological violence (threats, slander, obscenity, reprimand verbal), social violence (control the behavior of women, placed in the social reclusion, prevent to others, and sexual violence. [2] Studies show that men's violence against women in families have a balance large affected by psychological factors social and explain there are different theories such as the theory and realistic use, theory social structure (the act and antibody), Feminist theory, theory sources, system theory, and environment theory, and despair Aggression theory, and that realists users in clarifying family violence believe that the family is a group for a different relationship in this group unequal, are caused by the presence of the implications of the phenomenon or hide. These reflections are the loss of family characteristics and uses appropriate. In the result must be resolved.

The confliction family can be resolved through the use of power in topics including violence [3]. From the point of the theory of culture, the culture of social inheritance of the past that affect the current and future behavior of human beings. Men who deal in bad wives, live in a culture that governs men over women are very large. Social theories of evidence (the act and antibody) can appear violence in the family that whenever the facts or creating sites act that threaten the growing family, the likelihood of the emergence of behavior in that family violence is increasing as well. [4] As that theory Feministic owners believe that violence within the family take shape in more times by men in relation to women and children. They believe that the main causes of violence are a patriarchal power structure in society [5]. Based on William Cod view point, the family system like any system or any other social unit has a capacity of system. Each person has a connection to the interests of other family for the family more than the others it can be for the rest of the membership to continue in orientation activities.[6] In theory the system, the family is a system that takes into account the bound open, closed, midwife to influence or in the vicinity of the parties. This swap, take a positive or negative reflection and hidden objectives can have an impact is reflected in the system. For example, it is possible that violence and effective method in hand to get the goal or the survival of the regime. Violence occurs under the influence of reaction that appears through the method in both inside and outside the family system [7]. Environment theorists believe that if family could not the ability to connect external

vegan Ocean there is a possibility for the emergence of violence (especially to children). Accordingly in the period that there will be no opposite relationship between the child's parents and family and the environment, there is a possibility for the emergence of violence.[8] In the environment theory, that the important thing, which leads to the emergence of violence are showing social interpretations of this work is acceptable and social network in which the family has been affected, they also determine the balance of the emergence of violence. Theory of despair and aggression on the basis of that despair in relation to how long the behavior is not in hand to reach the target leads to find the engine that goal harm others or other things. In this station, the environmental conditions lead to appearance aggression [9]. Besides the theories mentioned, there are many studies in the area of balance and causes of men's violence against women. Eazazi in his study showed domestic violence (violence women) in a large breadth of individuals with different characteristics of social and economic. Families that have spread, including violence, given to factors such as education, income and the type of work there is a growing difference. [10] The rest of the factors involved in the emergence of violence may be referred to thought social about violence, thought for women, the idea for the marriage, the formation of the family, the inability of official organizations to help structure the men of the organizations official, the loss of economic and social protection and lack of awareness of women possibilities.[9] Also, Eazazi concluded in his study that individuals who in their personal lives are a victim of domestic violence or witnesses to the behavior of violent family members to others they have relationship with others because they have many problems in the individual dimension of these individuals that have more tendencies more than others to use drugs and violence. These individuals are not able to find a proper relationship with others because they had down efficiency in the process environment and the social dimension.[11] Baron and Peron showed there are factors related with violence such as; such as non-violence to take care of the emotions of women, individuals in the smoking and alcoholic beverages, haven't taking into account the point of view of women, deal with women like a person vile and ignorant, overlooked women, the lack of reflection, women satirize, coercion to approach or demand more than the applicable limit [7]. The Eazazy study showed there is an efficacious factor of mental and social relation with violence of family such as aggression, role of the level of education, income, employment and health person. Also, There is another research by Estraws and colleagues entitled (behind closed doors: Violence in American families) and based on the results of this study, there are 16% of respondents have shown they tried during the year kinds of physical violence and 38% of women found violence by their spouses during the period of their live [4]. As investigations indicate that 1.6 million American wife battered each year by their husbands. [13.14]. Amir Moradi in his study showed that 43% of them are infected with severe turbulence and 65% of them have the same weak-esteem. [11] Given the conceptual foundations and literature search can be said that there are 16% of families with domestic violence and these phenomenon's are to appear with the theories of sociology and psychoanalysis and that these two factors: psychological and social have a role in the emergence of this phenomenon.

The current study examines three goals, the first goal of the study is balance of men's violence against women in families residing in the city of Tabriz and the second goal of the study was which kind of violence that was heavily affecting families and which variables related with the phenomenon of violence in families. The third goal of the study: Is this phenomenon have accession of relationship to social class family (medium, weak, and high) and the balance of education for women or not. Which one of the psychological variables such as turbulence, anxiety and autism link women and men, balance of education of men and women, the length of time between men and women, the balance of the rights of the family and ostentation women have a relationship with the balance of men's violence against women..

2. Material and Methods

Statistical Society of the present study is families residing in Tabriz city 2003 that was chosen as 400 families in which the minimum shaped women and men with the sampling method for several stages. Tabriz city of was divided into five regions and each region was chosen four streets at random method and from every street was chosen two lane at random method. To gather information was used 14 questions of Sigmund and Estate tests to measure the depression and anxiety. Seven questions to test of depression and seven other questions about anxiety. Coefficient of item-total correlation and inside coefficient for this test reported in the following order: 0.76 and 0.91 [15]. Also, the correlation 7 questions for this depression test with traditional Psyche MMPI test to 0.72 and 7 questions for anxiety test with Estate tests to measure MMPI to 0.69. [16]. Also, for measure the audacity has use Kambril and Rege test of audacity that was placed in 1975 and has 40 questions & and questions scale was five degrees and item-total correlation test used Cronbach's alpha at level 75. To measure introspection and extraversion for women and men

have been using a 57 question of Eysenck test and item-total correlation this test to 80. [80] Besides testing standards have used the questionnaire developed by the researcher, which aims to assess the balance of violence in the family, ostentation women and some psychosocial variables of class standards in the form of 25 questions in the questionnaire. To assess of Face validity in this questionnaire was used the point of view of three scientists and validity with using of Cronbach's alpha test to level 73. Given the objectives of the study and nature the topic has been use descriptive and Correlation method. Data collection method was individually, In place of residence test and through specialty investigators Science Education. For the statistical analysis of the data analysis test was used deviation analysis of one factor and Spearman Coefficient Of Correlation

3. Results

Results of the study were presented and compressed in the form at three tables. In the first table is indicating the types of violence from the point of view of the balance and intensity. In the second table of the results of the study was presented using deviation analysis per one factor and in third table indicating a relationship of 18 anticipator variable with variable principal means of violence in the family.

Table 1: balance and Intensity of male violence against women

Violence type	balance				Intensity							
	Yes		No		Very little		Little		Much		Very much	
	P	%	P	%	P	%	P	%	P	%	P	%
Psychological violence, verbal	222	55.5	178	44.5	66	30	51	23	49	22.1	56	**25.2**
societal violence	128	32	272	68	49	38.4	35	27.3	25	19.5	19	**14.8**
Physical violence	28.5	14	286	71.5	45	39.5	35	30.7	15	13.2	18	**17.8**
Physical violence	12.2	49	351	87.8	42	86	4	8	2	4	1	**2**

The results show in the first table that the balance of psychological violence at 55%, social violence at 32%, physical Violence at 28.5% and sexual violence 12.2%. Among the four types of violence, the most types of violence is psychological violence. Only 38% of families have shown they did not receive any kind of violence on the one hand their spouses. Absence of physical violence at 71.5% which change this ratio when observation other types of violence.

Table 2: Comparison of male violence against women Tabriz city among the four groups of women with respect to education and social classes using ANOVA

Variables Independent	Sources of change	Sum of Squares	Degrees of freedom	Mean-square	F	Significant
Education of Women	Between groups	1719.159	5	343.83	6.562	0.0000
	Within groups	20591.22	393	52.395		
	Total	22310.381	398			
Social classes (low, medium, high)	Between groups	1360.204	2	680.112	16.30	0.0000
	Within groups	12429.677	298	41.710		
	Total	13789.88	300			

In the relationship with the education of women from Table 2 that observed the amount of F is equal to 6.56 significant. it is given with the comparison of the average group, it is noted that the two-two using sequential test with LSD shown high balance of education of women and the low balance of men's violence against women. When compared between balance of men's violence against women among families that their women had infringement middle school or less and women who have their education to diploma level, bachelor or higher are significant and very high. As the results show in Table 2 that the amount of F significant for relationship with social classes at level 5%, that means there is a relationship between violence in the family with social class for families and comparison of the average balance of violence of families shows that the balance of violence among families with social class weak more than families with medium and high social, but balance of violence among families of middle and high classes have non-significant difference.

Table 3: Psychosocial variables associated with men's violence against women in Tabriz

Anticipator variables	Balance of violence		Anticipator variables	Balance of violence	
	Correlation	Significant		Correlation	Significant
Anxiety Female	0.25	0.000	Extroversion Female	0.009	0.86
Anxiety man	0.33	0.000	Extroversion man	-0.11	0.03
Courage Female	0.025	0.62	Introspection Female	0.14	0.005
Depression in women	0.11	0.02	Introspection Man	0.18	0.000
Edu women	0.31	0.000	Failure men	0.56	0.000
Male Depression	0.33	0.000	Age gap between men and women	-0.30	0.55
Edu man	-0.30	0.000	Income	-0.35	0.000
Courage man	0.12	0.03	Ostentation women	0.15	0.006

As noted in table 3 that among 18 anticipator variable there are 13 variable has relationships with the balance of men's violence against women in the family. As notes there is strongest correlation between the desperation of men and balance of violence in the family that mean the balance of correlation at 56.0. Second rank is income balance had reverse correlation and third and fourth rank is depression and anxiety men with the balance of their violence in the family that are relation directly correlation. The balance between men and women's education and age distance among men and women and the balance of violence has reverse correlation and significant. The introspection for men and women, ostentation women and depression and anxiety women with the balance of men violence direct relationship, but extraversion man have an inverse relationship with balance of men's violence against women.

4. Discussions

One of the original questions in the current study is how many balance spread of men's violence against women in the family. As a result of the study shows that psychological violence observed at 55.7% of families and in the second rank of social violence at 32% of the violence and Physical violence in third rank at (28.5%) and last rank is sexual violence at 12.5 but absence any kind of men's violence against women at 38%. This result corresponds with Straus study because this study also provides a report on the balance of Physical Violence in family at 28%. [4] The other question in this current study is: Are men's violence against women related with social class and education of women? Results achieved show that the balance of family violence decrease with rising balance of women's education and significant. The violence in the family related to social class. Also, there is high relationship between violence among families with weak social class more than families with higher or medium social class. But the balance of violence among families with higher or medium social class shows non-significant difference.

This result more corresponds with Sekhawat study that Sekhawat study showed that the education of women level and the rights of the family influence on the balance of men's violence against women. Perhaps it can be said to show the lack of balance of violence Family in families who received higher education to higher education for the women. Also, the higher education wife, awareness and ability increases to use this method. Families have level of social and economic Limited can be behavior alternative for the act and the contrast in the family of hand men [2, 10]

The other question in this letter: What are the variables related social psychologists with the balance of men's violence against women. The study shows that the balance of variable despair of men more correlation with family violence. Also, this result supports the theory of despair aggression because in this hypothesis, especially in a time when no behavior aimed at targets that may lead to the existence of incentives of individuals [2].

Besides the above variables, the balance of income for the family and balance of education has an inverse relationship with family violence. Also, Eazazi study shows that there is a significant relationship between family violence with education and the type of women's job and family rights [10]. To explanation the relationship between the variables above can be said that higher education is the way open for the rest of the family members and families educated with the awareness of the presence on the roads with conflicts in relationships near and violence in the family and face with barriers external or internal methods reasonable because it helps in resolving the issue. In many of the factors despair because of external barriers and limited income is one of external inhibitions.

For this reason, when the family income is low, the likelihood of violence in the family a lot, too. Of course there is a relationship between family

violence with disorder of women and men. Amir Moradi study showed 4% of women who beaten and abused by their husbands have been injured to severe turbulence. Temperament low of women or men or high aggression man or woman is an internal factor that prevents family members to reach their goals and as a result of violence in the family [11]. One of the characteristics of individuals anxiety and aggression are poor self-esteem and notoriously. Amir Moradi study showed that 65% of women who have been subjected to violence with the self-esteem weak. And poor self-esteem of men and women enhance anxiety and aggression. Here are men seeking to resolve reasonable and logical problems, especially a family of methods that rely on agitation (i.e., violence).

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Ecological and Immunological Studies on *Rhinoestrus purpureus* infecting Donkeys in Egypt and Its Control with Doramectin and Ivermectin

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Abstract: The objectives of this study was to determine the prevalence, habitat and control of parasitic larvae of *Rhinoestrous purpureus* in donkeys. Forty donkeys were necropsied to search larvae in their nasal cavities. The results indicated that 26 out of 40 examined donkeys were infected by *R. purpureus* larvae and the percentage of infection was 65%. Female donkeys showed higher infection rate (77.78%) compared to (54.55%) in males. 263 R. purpureus larvae were collected from all investigated donkeys; 143 from females and 120 from males. The mean burden was 10 in male and 10.1 in females. L3 were more common than L2 (149 L3 compared to 114 L2). Most of L2 larvae (86.84%) attack labrynith of ethmoidal bones., moderate number infect sphenopalatine communications and only 2.63% were collected from pharyngeal cavity. On the other hand 53.59 % of L3 was collected from labrynith of ethmoid bones and other half was collected from sphenopalatine communication (23.49%), pharyngeal cavity (8.75%) and common nasal meatus (5.32%). An increase in serum globulin level (6.30 mg/dl) in R. purpures infected animals compared to (4.8 mg/dl) in control was recorded. Gamma globulins in infected animals was (2.97 mg/dl) while it was in (2.53mg/dl) in control. IgM was higher in infected animals (59.20 ng/dl) compared to (23.90 ng/dl) in control. Also IgA showed an increase in its value in infected animals compared to control (191.75, 87.11ng/dl). Concerning control of *R. purpureus* larvae, the results indicated that both doramectin and ivermectin had a larvicidal effect against *R. purpureus* larvae with superiority of doramectin as it caused 100% larval mortalities in vitro within 8 hrs. It was concluded that, *R. purpureus* larvae affect high number of donkeys specially females and the doramectin was selected to be the drug of choice for control in donkeys.

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Key words: *Rhinoestrous purpures*, equine, ivermectin and nitroxynil

1.Introduction

Equines still receive more interest and care in many countries as draft animals, source of leather and other related products. Donkeys have a prominent position in the agricultural systems in many developing countries **(Pearson, 1999).** The myiasis caused by larvae of *Rhinoestrus purpureus* has important factor in the equine medicine since it causes severe respiratory diseases, impair animals welfare and cause severe economic losses. The larvae localize in nasal cavities, sinuses and pharynx of equids **(Zumpt, 1965),** inducing inflammation, dyspnea, sneezing and cough. Moreover, lesions of the upper respiratory tract and lungs, damage the olfactory nerves and encephalomyelitis were reported **(Kaboret *et al.,* 1997, Angulo- Valadeza *et al.,* 2010)**. Adult flies may also eject larvae into human eyes inducing opthalmomyiasis or into nasal cavities or mouth **(Rhichard Wall, 2007)**. Knowledge about ecology, biology, immunology and control of R.purpureus is limited in Egypt **(Zayed, 1992; Zayed and Helali 1993; Zayed *et al.,* 1993).** Therefore, the objectives of this study was to determine these parameters in donkeys.

2. Material and Method

1- Collection of *Rhinoestrus purpureus* larvae:

The animals examined for the larvae of *Rhinoestrus purpureus* were from north Cairo (Kalubyia Governorate) and southern Cairo (Giza Governorate). The study was carried out on a total of 40 donkeys. The animals were necropsied at Zoo slaughter house to search larvae in their nasal cavities. The entire nasal cavities of killed animals were opened and examined for the larvae. The larvae obtained from donkeys were placed in a jar containing 70 % alcohol and brought to the laboratory for identification according to **(Dorchiesa *et al.,* 2003)**. The site of parasitism, larvae burden, number and type of larvae per animal, animal sex were investigated.

2- Blood samples:

Blood samples were taken from the investigated animals before killing from jugular vein, kept without anticoagulant to separate serum which

was used for determination protein fractionation, immunoglobulin M (Ig M) and immunoglobulin A (IgA)..

II-Experimental study to detect the Larvicidal efficacy of ivermectin and doramectin against *Rhinoestrous purpureus* larvae:

Chemicals:

a- Ivermectin
b- Doramectin
c- Physiological saline

2- *R. purpureus* larvae: 45 active L2 *R. purpureus* larvae were chosen to carry out the insecticidal efficacy of ivermectin.

3- Steps of the experiment

Studying the larvicidal efficacy was done according to **(Pamo *et al.,* 2005)**. One dose of ivremectin and another from doramectine with three replications for each were used *in vitro*. Five larvae per replicate were placed in a clean dry plastic cups with a filter paper, Disc of Whatman No.1 filter paper measuring 62.63 cm2 surface areas, impregnated uniformly with the used concentration of ivermctin and doramectin on the bottom. The drug was dissolved in physiological saline. Bioassays were done at 27±2ºC and 75±5% RH. Larvae were considered alive if they exhibited normal behavior when breathed upon or physically stimulated with a wooden dowels. If larvae were incapable of movement, maintaining normal posture, leg coordination, ability to right themselves, they were considered moribund or dead (Panella *et al.,* 2005). The mortality was initially assessed 30 min. after being subjected to the examined drugs followed by mortality assessment at one hour, 2, 4, 8, 10 and 12 hrs. The used concentrations were 0.1% for each medicaments (Drummond, 1984).

3. Results

Results in Tables (1, 2) indicated that 26 out of 40 examined donkeys were infected by *Rhinoestrus purpureus* larvae. The over all percentage of infection was 65%. Female donkeys showed higher infection rate (77.78%) compared to (54.55%) in males. The table showed also that 263 *Rhinoestrus purpureus* larvae were collected from all investigated donkeys; 143 from she donkeys and 120 from males. The mean burden was 10 in male and 10.1 in females. L3 were more common than L2 (149 L3 compared to114 L2). Most of L2 larvae 86.84% attack labrynith of ethmoidlal bones, moderate number infect sphenopalatine communications and only 2.63% were collected from pharyngeal cavity. The common nasal meatus was free from L2. About one half number of L3 53.59 % was collected from labrynith of ethmoid bones and other half was collected from sphenopalatine communication (23.49%), pharyngeal cavity (8.75%) and common nasal meatus (5.32%).

Results in Table (3) and plates (1, 2) revealed an increase in serum globulin level (6.30 mg/dl) in R. purpures infected animals compared to control (4.8 mg/dl). Gamma globulins in infected animals was (2.97 mg/dl) which was also higher than in control (2.53mg/dl). IgM was higher in infected animals (59.20 ng/dl) compared to control (23.90 ng/dl). Also IgA showed an increase in its value in infected animals compared to control (191.75, 87.11ng/dl) in infected and control animals respectively.

Results in table (4) indicated that, both ivermectin and doramectin had a larvicidal effect against *R. purpureus* larvae compared to control. Larval mortalities started after one hr of exposure to doramectin and increased gradually with time and reach maximum (100%) after 8 hrs. On the other hand ivermectin started to kill larvae after 2hrs and the mortality rate reached 100% after 10 hrs of exposure. All tested larvae remain alive and very active during all time of the experiment in control group.

Table (1): Incidence of *Rhinoestrus purpureus* in male and female donkeys

Sex	No. of ex. Animals	No. of positive animals	Mean burden	No of collected larvae	No. of L2	No. of L3
Males	22	12 (54.55%)	10	120 (5.5/animal)	54	66
Females	18	14 (77.78%)	10.2	143 (7.9/animal)	60	83
Total	40	26 (65%)	10.1	263	114	149

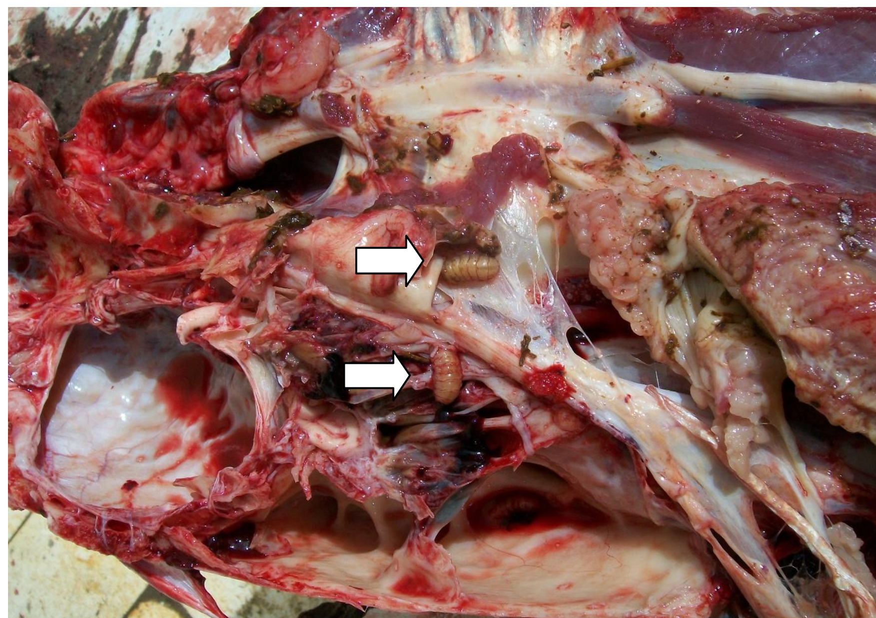

Figure (1): Showing 3rd larval stage of *R. purpureus* inhibit the area of the ethmoid bones.

Table (2): Distribution of Rhinoestrus purpureus in air passages of donkeys

Larvae	Total	Labrynith of ethmoid	Sphenopalatine communication,	Pharyngeal cavity	common nasal meatus
L2	114	99 (86.84%)	12 (10.53%)	3 (2.63%)	---
L3	149	80 (53.59%	35 (23.49%)	20 (13.42%)	14 (12.28%)
Total	263	179 (68.06)	47 (17.87%)	23 (8.75%)	14 (5.32%)

Table (3): Serum protein electrophoresis, IgM and IgA in Rhinoestrus infected donkeys

	Infected	Control
Albumin (gm/dl)	2.3	3.1
Globulin (gm/dl)	6.30	4.8
Alpha1 globulin (gm/dl)	0.24	0.29
Alpha 2 globulin (gm/dl)	0.99	0.96
Beta Globulin (gm/dl)	2.1	1.02
Gamma globulin (gm/dl)	2.97	2.53
IgM (µg/dl)	59.20	23.90
IgA (µg/dl)	191.75	87.11

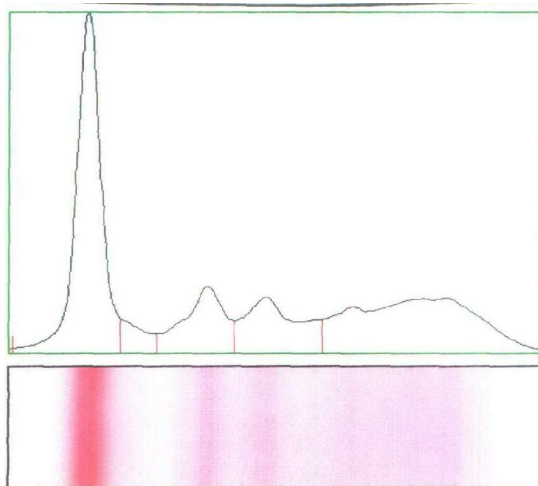

Plate (1): Protein electrophorsis in non infected donkeys

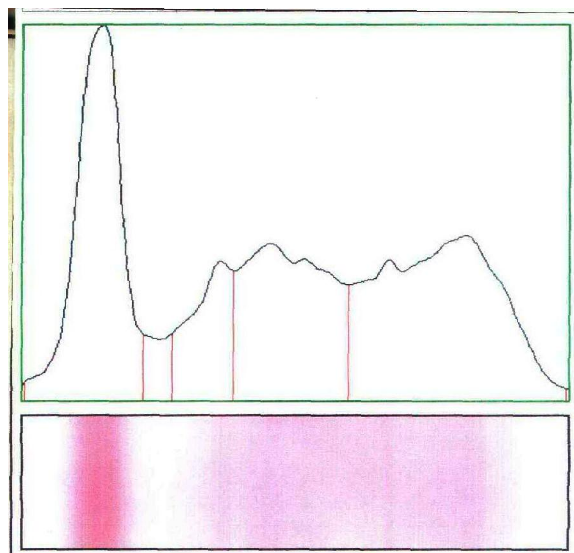

Plate (2): Protein electrophoresis in infected donkeys

Table (3): Mortalities of Rhinoestrous purpureus after treatment with ivermectin and nitroxynil

Drug	30min	1hr	2hrs	4hrs	8hrs	10hrs	12hrs
Ivermectin							
L	5(100%)	5 (100%)	4 (80%)	2 (40%)	2(40%)	0(0.0%)	0(0.0%)
D	0 (0.0%)	0(0.0%)	2 (20%)	3 (60%)	3 (60%)	5(100%)	5(100%
Doramectin							
L	5 (100%)	4 (80%)	2 (40%)	1(20%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
D	0(0.0)	1(20%)	3 (60%)	4 (80%)	5 (100%)	5 (100%)	5(100%)
Control							
L	5(100%	5(100%	5(100%	5(100%)	5(100%)	5(100%	5(100%
D	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)

L= alive larvae; D= dead larvae

4. Discussion

Rhinoestrus purpureus larvae infestation has great importance in the horse medicine since it causes severe respiratory diseases. Therefore, an accurate diagnosis of rhinoestrosis is essential to study its epidemiology and control so, it is crucial to understand the actual incidence and epidemiology of this myiasis in live animals (**Traversa and Otranto, 2006**).

Our results indicated that, the percentage of infection was 65%. This result agreed with the previous study in Egypt which was carried out by (**Zayed *et al.*, 1993)** who examined heads of 144 donkeys at the postmortem during the period from September 1989 to the end of August 1990 and revealed that (61.11%) donkeys were infested with *Rhinoestrus purpureus*. **(Dorchiesa *et al.*, 2003)** recorded 3 species of nasal bots of equids namely; *Rhinoestrus purpureus* and *R. latifrons* in horses and donkeys (paleartic region) and *R. usbekistanikus* in horses, donkeys (palearctic region) and Burchell's zebra (*Equus burchellii*) in Africa south of Sahara (Ethiopian region). Also, **(Otranto *et al.*, 2004)** & **Otranto and Colwell, 2008)** noted myiasis caused by *Rhinoestrus* larvae recently in Europe, specifically in southern Italy, with a prevalence rate up to 6%. The recorded lower incidence in Italy may attributed to changes in environmental conditions

Higher infection rate (77.78%) was recorded in she-donkeys showed compared to (54.55%) in males. Such higher incidence in females may be attributed either to calm habit of she-donkeys that enable *Rhinoestrous* females to larviposit in their nasal cavities or to hormonal differences between male and female donkeys.

In the present study, L3 predominate L2. Such finding confirmed the previous results of (**Zayed *et al.*, 1993)** in Egypt who recorded an increase in larviposition activity of *Rhinoestrous* females from mid-January to mid-April and highest mean larval number during June.

Concerning habitat of *R purpureus* larvae, it was noticed that, most of L2 larvae inhibit labrynith of ethmoidal bones., moderate number infect

sphenopaltine sinus and the lowest number were collected from pharyngeal cavity. These results agreed with (**Zayed and Hilali, 1993)** in Egypt, who studied the localization and migratory route of *Rhinoestrus purpureus* larvae and recorded 93.9%, of 2nd instar larvae, were found in the labrynith of ethmoid bone, 5.8% in the sphenopalatine sinus, and only 0.3% in the pharyngeal cavity. Such observation suggested that, moulting of the 1st instar larvae occurred only in the labrynith of ethmoid bone. On the other hand, (53.59 %) of L3 was collected from labrynith of ethmoid bones and others were collected from sphenopalatine sinus (23.49%), pharyngeal cavity (8.75%) and common nasal meatus. Our results confirmed the suggestion of **(Zayed and Hilali, 1993)** who reported that, the 2nd instar larvae moulted in the labrynith of ethmoid bone, sphenopalatine sinus and pharyngeal cavity.

The mean larval burden was 10 in male and 10.1 in females. Indeed **(Biggs *et al.,* 1998)** suggested that any number of larvae above 10 is potentially harmful to the hosts

Absence of first larval instars together with predomination of L3 indicated that there is no larvipostion activity of adult females during the period of the study (summer months). This may be attributed to higher environmental temperature during Summer months which either kill pupae or resulted in deformities in emerged females. In this respect, **(Zayed,1992)** in Egypt studied the pupal duration of *R. purpureus* under variable degrees of temperature and relative humidity (RH) and found that, at 37°C, the pupated larvae failed to pupate and died and their was a deformities in emerged flies. Some necropsy surveys showed that, kinetics of 1st instar larvae is related to adult mating activity (**Tabouret *et al.,* 2001a,b**). An extended pupariation outside has been observed for O. ovis by **(Biggs *et al.,* 1998)** allowing adults flies to wait for the best time for emergence, mating and larviposition. Choosing the right place for burrowing and pupariation is of high importance in this species and extreme temperatures into the soil (for example above 40 ◦C for long periods) being lethal to pupae or leading to low adult weight with high post-emergence mortality rates **(Cepeda Palacios *et al.,* 1998).** For all species of oestrids, mating and seeking activities occur optimal on warm days, sunny and not windy days at temperatures between 20 and 30 ◦C **(Anderson, 2006).**

The present data revealed an increase in serum globulin specially gamma globulin levels in *R. purpures* infected animals compared to control. This is probably caused by a permanent antigenic stimulation during infection as antigenic and some inflammatory products produced by larvae induce inflammatory and hypersensitive reactions in a trail by the host to expel or destruct the larvae. **(Dorchies *et al.,* 2006)** reported that mesoparasites, oestrids display complex host/parasite relationships. Indeed, the hosts develop numerous but often ineffective strategies of expelling them. Frequently there is interstitial pneumonia associated to emphysema and bronchiolitis with many eosinophils. This lesion is firstly related to the effect of parasitic antigens and proinflammatory products liberated by mast cells and eosinophils from nasal cavities inhaled in the lungs. Secondly, it appears that oestrids antigens have an immunosuppressive effect, allowing the actualization of pathogenic effect of virus inducing interstitial pneumonia.

IgM is the first isotype in response to infection, secreted from mucosal surfaces, good agglutinator and fixes complement. IgA is the major isotype at mucosal surfaces, secreted across the epithelial cells, bind to eosinophils, can activate complement by alternative path way and its total % in serum is twice IgM, **(Wakelin, 1996; Scala *et al.,* 2006).** The higher levels of IgM and IgA in infected animals in the present study was in accordance with previous results of **(Suarez *et al.,* 2005; Angulo-Valadez *et al.,* 2009)** who recorded that *Oestrus ovis* infection elicits an IgM and IgG systemic antibody response in both sheep and goats. **Innocenti *et al.*(1995)** demonstrated that salivary glands proteins are the most antigenic *O. ovis* larvae proteins compared to digestive tube contents or cuticular antigens.. Many serological tests have been developed using a crude L2 somatic extract. Sensitivity, specificity, positive and negative predictive values were improved compared to the previous tests but detection of infected animals remains difficult in winter when L1 arrest their development inside the host. Negative correlations among larval establishment and/or larval development as well as intensity of local and systemic IgG responses were found in naturally infected ewes **(Angulo-Valadez *et al.,* 2008).**

With respect to control of *R.purpureus* in vitro, our experiment is the first preliminary trial in this aspect, it showed that both doramectin and ivermecitin had a strong larvicidal effect against its larvae as it cause 100% mortalities. From the middle of the last century control of oestrids myiasis in livestock, relied on the use of the organophosphate products (OPs) which, however, have produced some unsatisfactory results in terms of animal and human safety (**Charbon & Pfister, 1993**). In the past few years OPs have been superseded by macrocyclic lactones such as doramectin and ivermectin **(Sutherland, 1990),** As ivermectin has proved to be highly effective against *Hypoderma* sp. larvae even at dosages as low as 0.2 µg/kg, which is 1/1000th of the

recommended dose **(Drummond, 1984)** so both drugs was suspected to do the same effect against *R. purpureus* larvae in equine.

Conclusion

It was concluded that *R. purpureus* infection in donkeys at 65 % in Egypt with higher incidence in females. The parasitic larvae of *R. purpureus* stimulate immune system. Both of doramectin and Ivermectin had a strong larvicidal effect against *R. purpureus* larvae *in vitro* with superiority of doramectin. Further studies are necessary to determine *R. purpureus* prevalence in the other equine sp. in Egypt as well as studying the effect of ivermectin and doramecin against it in large number of infested equines

It is hoped that this research will help veterinarians, equine owners and others who interested in providing health and welfare to equine to protect equine from such dangerous larvae.

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8/2/2013

Lichen Flora Of Niti Area From Garhwal Himalaya, Uttarakhand

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Abstract: The paper deals first time with the lichen flora of way of Gamsali to Niti area of Chamoli district, Uttarakhand. A total 43 species belonging to 32 genera and 13 families from the area have been reported. Among the different growth forms of lichen, foliose lichens exhibit their dominance with 21 species followed by 14 species of crustose and 8 species fruticose form respectively. Most of the lichen growing sequence of corticolous < terricolous < saxicolous lichen species followed by 22, 18 and 9 respectively. Total 13 species of lichens are medicinally important. The available information regarding lichen diversity provides baseline data which will be useful in conducting future biomonitoring studies and developing conservation strategies in the valley.
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1. Introduction

Recently Indian lichens were achieved that revealed the occurrence of more than 2300 species from India (Singh & Sinha 2010). The Niti valley area is situated in higher temperate and alpine region of Chamoli district in the Himalayas. Being situated at higher altitudes of 2800-3600m, the Niti valley area exhibit luxuriant growth of many lichens particularly the lichen genera growing on soil (terricolous) and exposed rock (saxicolous). First time lichen flora has recorded from Niti area. The Gamsali, Bampa and bank of river Dhauli Ganga are the major temperate localities within Niti Valley comprised of dense coniferous forest of *Taxus baccata, Cedrus deodara, Pinus wallichiana* and bears luxuriant growth of lichen.

The long stretches of grasslands interspersed with snow streams in higher altitudes of Niti area the characteristic features of alpine meadows. The area bears good growth of many terricolous lichens together with herbaceous plants. Within the alpine regions of Niti the dry arid areas show xerophytic type of vegetation represented by scanty growth of *Juniperus, Jruinea, Artemisia* shrubs together with *Ephedra* and *Hippophae* in riverine and rocky situation. The dry and exposed habitats exhibit growth of some exclusive lichen species on rocks and soil.

2. Materials and Methods

In August-September 2007 more than 450 specimens of lichens were collected from the different available substrates Niti and Gamsali area. The specimens were identified in respect of their morphology, anatomy and chemistry. The chemistry of all the specimens were performed by both colour spot tests (K, C, Pd) followed by thin layer chromatographic (TLC) methods as described by Walker & James (1980).The chromatograms were developed in solvent A (Toluene: 1-4 dioxane: acetic acid 180: 60: 8 ml). The collected specimens were identified with the help of recent literature of Awasthi (1988, 1991, 2000, 2007); Divakar and Upreti (2005); Nayaka, (2004); Joshi, Y., (2008). The specimens are deposited in the herbarium of National Botanical Research Institute (CSIR) Lucknow (LWG).

3. Result

In (Table No.1) 143 species belonging to 32 genera and 13 families of lichens from Niti area. Among the different substrates, the trees host the maximum diversity of lichens represented by 22 species followed by 18 saxicolous and 11 terricolous (soil inhabiting) lichens. The area shows good growth of medicinal lichens, represented by 13 species. In Niti area, *Pinus wallichiana, Taxus baccata* are the common host tree for the lichens.

Niti area dominance of Parmelioid lichens represented by 19 species. The probable resion for scarce or poor growth of lichens on various coniferous trees may be attributed to the factors such as rocky dry area, having thinned out, open forest and stunted growth of trees. The area show good growth of saxicolous lichens as 18 species the common species are *Xanthoparmelia stenophylla* (Ach.) Ahti & D. Hawksw., *Rhizoplaca peltata* (Ramond) Leuck. & Poelt., *Xanthoparmelia conspersa* (Ehrh. ex Ach.) Hale, *Diploschistes scruposus* (Schreb.) Norman, on soil or on soil over rocks recorded from the area.

	Table 1: Total lichen taxa of Niti and Gamsali areas in Chamoli district, Uttarakhand, India						
	Lichen species	**Families**	**Habitat**	**Habit**	**Sali**	**Niti**	**amt**
1	*Amandinea punctata* (Hoffm.) Coppins & Scheid.	**Caliciaceae**	Saxicolous,	Crustose	+	-	-
2	*Chrysothrix candelaris* (L.) J. R. Laundon	**Chrysothricaceae**	Corticolous, Saxicolous	Crustose	-	+	-
3	*Cladonia fimbriata* (L.) Fr.	**Cladoniaceae**	Corticolous	Fruticose	+	-	+
4	*Cladonia furcata* (Huds.) Schrad.	**Cladoniaceae**	Saxicolous, Terricolous	Fruticose	-	+	+
5	*Cladonia pyxidata* (L.) Hoffm.	**Cladoniaceae**	Terricolous	Fruticose	+	-	+
6	*Leptogium burnetii* Dodge	**Collemataceae**	Corticolous, Terricolous	Foliose	+	-	-
7	*Lecanora frustulosa* (Dickson) Ach.	**Lecanoraceae**	Corticolous	Crustose	-	+	-
8	*Lecanora muralis* (Schreb.) Rabenh.	**Lecanoraceae**	Saxicolous,	Crustose	-	+	+
9	*Lobothallia alphoplaca* (Wahlenb.) Hafellner	**Megasporaceae**	Saxicolous,	Crustose	-	+	
10	*Allocetraria nygricascens* (Nyl.) Karnefelt & Thell	**Parmeliaceae**	Terricolous	Foliose	-	+	-
11	*Dolichousnea longissima* (Ach.) Articus	**Parmeliaceae**	Corticolous	Fruticose	-	-	+
12	*Evernia mesomorpha* Nyl.	**Parmeliaceae**	Corticolous	Fruticose	+	+	-
13	*Everniastrum cirrhatum* (Fr.) Hale ex Sipaman	**Parmeliaceae**	Corticolous	Foliose	-	+	+
14	*Flavoparmelia caperata* (L.) Hale	**Parmeliaceae**	Corticolous, Saxicolous	Foliose	+	-	+
15	*Flavopunctelia flaventior* (Stirton) Hale	**Parmeliaceae**	Corticolous	Foliose	+	-	-
16	*Flavopunctelia soredica* (Nyl.) Hale	**Parmeliaceae**	Corticolous	Foliose	+	-	-
17	*Hypogymnia tubulosa* (Schaer.) Hav.	**Parmeliaceae**	Corticolous	Foliose	+	-	-
18	*Melanelia tominii* (Oxner) Essl.	**Parmeliaceae**	Saxicolous	Foliose	-	+	-
19	*Melanelixia fuliginosa* (Fr.ex Duby) O. Blanco Crespo, Divakar, Essl. D. Hawksw.	**Parmeliaceae**	Saxicolous,	Foliose	-	+	-
20	*Melanelixia vilosella*(Essl.) O. Blanco Crespo, Divakar, Essl. D. Hawksw..	**Parmeliaceae**	Corticolous	Foliose	+	+	-
21	*Parmelia sulcata* Taylor	**Parmeliaceae**	Corticolous	Foliose	+	-	+
22	*Parmotrema rampoddense* (Nyl.) Hale	**Parmeliaceae**	Corticolous	Foliose		+	
23	*Rhizoplaca peltata* (Ramond) Leuck. & Poelt.	**Parmeliaceae**	Saxicolous,	Foliose	-	+	-
24	*Xanthoparmelia bellatula* (Kurok. & Filson) Elix & Johnston	**Parmeliaceae**	Terricolous	Foliose	-	+	-
25	*Xanthoparmelia conspersa* (Ehrh. ex Ach.) Hale	**Parmeliaceae**	Saxicolous,	Foliose	-	+	+
26	*Xanthoparmelia stenophylla* (Ach.) Ahti & D. Hawksw.	**Parmeliaceae**	Saxicolous,	Foliose	-	+	-
27	*Usnea perplexans* Stirton	**Parmeliaceae**	Corticolous	Fruticose	+	-	-
28	*Usnea subfloridana* Stirton	**Parmeliaceae**	Corticolous	Fruticose	+	-	-
29	*Vulpicida pinastri* (Scop.) Mattsson	**Parmeliaceae**	Corticolous	Foliose	+	-	-
30	*Peltigera didactyla* (With) J. R. Laundon	**Peltigeraceae**	Terricolous	Foliose	+	-	-
31	*Peltigera praetextata* (Flörke ex Sommerf.) Vain.	**Peltigeraceae**	Corticolous , Terricolous	Foliose	+	-	+
32	*Peltigera rufescens* (Weiss) Humb.	**Peltigeraceae**	Terricolous	Foliose	+	-	+
33	*Anaptychia kaspica* Gyeln.	**Physciaceae**	Corticolous	Foliose	-	+	-
34	*Dimelaena oreina* (Ach.) Norman	**Physciaceae**	Saxicolous,	Crustose	-	+	-
35	*Physcia gomukensis* D. D. Awasthi & S. R. Singh	**Physciaceae**	Saxicolous,	Foliose	-	+	-
36	*Physcia stellaris* (L.) Nyl.	**Physciaceae**	Corticolous	Foliose	-	+	-
37	*Physconia detersa* (Nyl.) Nyl.	**Physciaceae**	Corticolous, Saxicolous	Foliose	+	-	-
38	*Porpidia macrocarpa* (DC.) Hertel & A. J. Schwab	**Porpidiaceae**	Saxicolous,	Crustose	-	+	-
39	*Ramalina sinensis* Jatta	**Ramaliaceae**	Corticolous	Fruticose	+	-	+
40	*Rhizocarpon geographicum* (L.) DC.	**Rhizocarpaceae**	Saxicolous,	Crustose	-	+	-
41	*Caloplaca saxicola* (Hoffm.) Nordin	**Teloschistaceae**	Saxicolous,	Crustose	+	+	-
42	*Xanthoria sorediata* (Vain.) S. Kondratyuk & Karuefelt	**Teloschistaceae**	Saxicolous,	Foliose	+	-	-
43	*Diploschistes scruposus* (Schreb.) Norman	**Thelotremataceae**	Terricolous	Crustose	+	-	+

The sites chosen have subalpine climatic characteristics and lichen vegetation which plays a significant part in the evolution of the soils that it colonizes (Asta 2001). The most common lichen species of the area *Evernia mesomorpha, Peltigera praetextata* (Flörke ex Sommerf.) Vain., *Peltigera didactyla* (With) J. R. *Peltigera rufescens* (Weiss) Humb., and *Xanthoparmelia bellatula* (Kurok. & Filson) Elix & Johnston are terricolous species grow on moist vertical slopes along with mosses indicates the moist and humid condition of forest. The Niti area situated on the top of mountain has frequent landslides due to melting of glaciers. The landslides not only destroy the tree vegetation but also remove the top soil and thus resulted into loss of both terricolous and corticolous lichens. Niti and Gamsali areas total 13 species of lichens having medicinal properties. *Cladonia fimbriata* (L.) Fr., *Allocetraria nygricascens* (Nyl.) Karnefelt & Thell, *Flavoparmelia caperata* (L.) Hale and *Flavopunctelia flaventior* (Stirton) Hale are the commen medicinal lichen species from the area.

The available enumeration of the lichen from Niti area will be helpful in documentation of lichens from the Nanda Devi Biosphere Reserve will also provide status of the diversity of medicinally important lichens of the area. The present number of species, their distribution on different substrate will act as baseline data to carry out biomonitorying studies in the area in future.

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The role Knowledge by presence in Sadra epistemology

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Abstract: Knowledge by presence is one of the most discussed topics in the epistemology. Unlike the conceptual science, the philosophers hadn't enough attention to the Knowledge by presence. In this article we're going to answer to this question that, whether Knowledge by presence can be helpful for people to get the truth or not helpful. the man was always looking for an easier way to unlock the truth, although access to the Knowledge by presence is a difficult way and it seems that it has more hazards than conceptual science but the revelation knowledge in this science is clearly far from wrong and it makes easy to understanding the truth, only by removing the obstacles from your eyes, you can immediately access to this knowledge. As long as people are born based on the talent that God gave to them, have insights and beliefs about themselves and their surroundings, This awareness is in the areas of Knowledge by presence not conceptual science, because conceptual sciences are adventitious and may be associated with the error this sciences must be discovered over the time but at the moment of creation, God gives Knowledge by presence to every creature. In this article we are going to define the conceptual science and Knowledge by presence and then we examined their various types and prove authenticity of the Knowledge by presence.

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Key word: conceptual science, Knowledge by presence, epistemology, sadra

Introduction:

One of the most important issues in the philosophy is Knowledge by presence.

At the most basic divisions of science; it has been divided into two parts, conceptual science and Knowledge by presence. Although there is a whole debate about conceptual science but there has been no detailed discussions on Knowledge by presence. According to the critical role of Knowledge by presence we have a quick look at this science.

Humans have always tried to achieve the most appropriate tools to get the truth and they constantly seek a way for access the properly knowledge. It should be noted that conceptual sciences have instrumental view at knowledge, and they are classified in acquired Sciences, it means that humans must obtain them from the outside world but Knowledge by presence classified as immediacy sciences it means that humans at the same time see the truth. One of the interesting features of this science is that, they pointed to the fact that can be accessed immediately. If you'll look at this issue rationally and without prejudice then you notice that desire for truth in Knowledge by presence is more evident and there is no possibility of error in it, due to this fact we can discover the originality of this science.

Definition of conceptual science

Conceptual science is the science, wherein the reality of science is different from the known fact, as our knowledge of the earth, the sky, the trees and other people. we have knowledge than objects, this means that each object have their own image and according to imagine we understand the facts, so here the reality of science is a picture in our minds and the fact is inherent known which independently of us exists in the outside world. For example, we have a picture of friend's face in our minds that it's a figure not the reality of face.

Knowledge by presence

Knowledge by presence is the science, wherein the reality of science is similar to the known fact. In this science perceived object be understood without subjective image mediation. For example, when we decide or desire to do something or when we are sad or happy, the reality of will, decision and pleasure are known for us and immediately we understand that special circumstances.

When holy imam says: There is no secret for us, this means that our performance is supplied for imam and we have always been in their presence, it should be noted that, we may be unaware of this issue.

In Knowledge by presence, man must look carefully at the issues, now they find the entire data in front of themselves. Many things and facts aren't evident for human beings, because we have created many cover for ourselves, which impedes to see inner truth of things.

Another fundamental difference between conceptual science and Knowledge by presence is

that: in Knowledge by presence for awareness and knowledge achieved a special authority or a private mode doesn't interfere but based on known facts, facts are known. Knowledge by presence a specific power from different branches of ego that it work is illustration, intervening and provides a form that obtained awareness and knowledge.

It should be noted that the Knowledge's by presence aren't related to a specific device of various ego devices but conceptual sciences are related to a device which read as mind or perceptual apparatus. We intend to provide an explanation about this power and its features.

Always sages and other scholars had different opinion on this topic that, from humans birth time, their spirit in connection with the affairs will be with what and how quality of performance.

Some scholars believe that human soul at the time of creation does not understand anything through conceptual science and it hadn't any idea on mind and completely unaware about their situations and their own, virtually at birth time it hadn't mind, because the world of mind is the universe of objects and because at first it hadn't any phantasm so it hadn't mind, gradually the imagery provide image of object and ego states and it form the mind.

Although human soul at the time of creation does not understand anything through conceptual science and it hadn't mind at the beginning, Knowledge by presence will help and by this science, the objects in order to the size and their talent finds Knowledge by presence about themselves, because the conceptual science basis is the imagination activity and imagination formation, and Knowledge by presence basis is the existence of single object from article. The child's has no idea of their own states in fact they has no idea of anything but at the same time they understanding the fact such as hunger, pleasure, grief and will, therefore when they are so hungry they have food demand.

Many scientists hadn't sufficient evaluation and research in the field of Knowledge by presence, they belief that everyone knowledge of himself and his ego states is a part of Knowledge by presence.

Everyone's knowledge of himself and of his ego states has two distinct forms: The primary knowledge, that everyone has received since his creation, in this form his ego conditions not separate from himself and he understands all items without the mediation of perceived image, the other form include ideas that gradually developed. The child's understands much fact such as hunger, pleasure, grief and will, it should be noted that, these feelings are not hidden for them, because their mind sets still weak, therefore they have no idea of these basic material, it means that by ordinary and normal science they will be unaware of their will. Then by development of children's mental apparatus and by providing the forms of foreign objects throw their senses supply the ideas of ego states. Some scholars believe that the entire following item belongs to the Knowledge by presence: the item such as, doubt, certainty, imagination, authentication, error and correction, memory, attention, intellection and Argument.

Intuitive knowledge, introduction of conceptual science

In some cases we can expressed that intuitive knowledge can be as an introduction of conceptual science. If people have no intuitive knowledge they cannot reach to the conceptual science. Because intuitive knowledge allowed us to take advantage of the conceptual science. Actually the intuitive knowledge is considered as the engine of conceptual science. In connection with the conceptual science it should be noted that, everything that man does not know is the mental and conceptual science to the other things that obtained through cognitive tools such as the eyes and ears and goes to the heart and mind, in there it recognize, approved and comes in the form of Science. This is the science that will change at the end of life and faced with declining, but intuitive knowledge never has such attributes and never goes away. The science that man's teaches at school, located in the conceptual science division that the students learns from their teacher through the concepts, words and phrases, this knowledge base is very weak and this is why it is considered as a scientific identity crisis in a period and makes educated person that reject their learning.

Certainty

Certainly refers to the intuitive knowledge, in the Quran, death is defined as the certainty because at the death time go away all of the veils from our eyes, ears and our heart, the veils that prevent us from understanding, seeing and hearing. Actually certainly is an intuition that for some people happens in this world and for a number of people occur after deaths.

Characteristics of Knowledge by presence

1-There are no intermediaries in the Knowledge by presence. In fact, nature has no place in the Knowledge by presence and any provisions of nature cannot log in this Science. Because Knowledge by presence is not a general or slight science. It should be noted that only the concepts can be divided into two components like general or slight.

2- The error having no place in the Knowledge by presence. Because in the Knowledge by presence truth of known revealed to us, in contrast Knowledge

by presence are fallible.it should be noted that Knowledge by presence regardless of their interpretation are a kind of conceptual science that aren't fallible.

3- Knowledge by presence is unspeakable and non-transferable to the others. Knowledge by presence unlike the conceptual sciences aren't public. However, the Knowledge by presence can be transmitted to the others, if it converted to the conceptual science but in this case, the conceptual science transferred not Knowledge by presence. The nature of Knowledge by presence likes personal and non-transferable to the others doesn't contradict with the general aspects of this science.

4- Achieve the truth will be possible through Knowledge by presence.

5- States of conscience at the first is usually transient, but it does not take much time. If by doing a large range of exercises the man took intuitive knowledge this state will not last long and shortly the man returned to normal condition and finds himself that surrounded by the conceptual science.it should be noted that this converse is not true for all people.

6- Independence of subjective judgments: Intermediaries such as Suggests, doubt, faith, imagination, affirmations, mind, thinking, defining, understanding and reasoning that are used in conceptual science have no applications in Knowledge by presence, As a result, the laws of conceptual science has no place in it. (3) Knowledge by presence is meta-logic and has its own logic.

7-Unity of science and the nature of the thinker:

In the Knowledge by presence, science is the nature of thinker; As a result they have unity with each other and are not contrasted. (1)

8- Independence of cognition: in conceptual science, especial perception likes external senses, internal senses or intellect are placed as intermediaries between the soul and known and soul by assisted of this power would achieve to the image or concept of known. As a result conceptual science needs to the special device that called the mind whereas Knowledge by presence does not need to the special devices such as mind. (2)

9- Recognition of existence not quiddity: known in Knowledge by presence is availability of objects not their nature this topic is in contrast with conceptual science. (1). Knowledge by presence than an object does not require that we have knowledge about the nature of the object.

10- The doubts: In the Knowledge by presence, there are varying degrees of skepticism

Mulla Sadra view on referrals the conceptual science to the other sciences:

Presence, attainment and discovery are the truth of Science (3). Conceptual science isn't a real science, but it's a credit science.

The types of Knowledge by presence:

Philosophers have considered some case for the Knowledge by presence that some of these cases are clear and some of them are provable by reasoning.

1- The science of incorporeal creatures about their nature which is divided into three types: A- Knowledge of the soul about itself B- Knowledge of the obligatory about itself C- Knowledge of the other singles about themselves

2-Soul knowledge about mental form:

Human knowledge about the outside world is possible through mental form but human knowledge about this form cannot be possible from the other case, so the human knowledge about the outside world is possible through conceptual science and human knowledge about mental form is immediately.

3- Soul knowledge about own actions:

Soul has the actions such as will and decree. Soul has Knowledge by presence about own actions, because cause knowledge about the effect is Knowledge by presence and because these things are effect and cause of soul, so the knowledge of soul about them is Knowledge by presence.

4- Soul knowledge about own forces:

For Soul Including plant, human and animal soul Scientists have considered some forces, these powers are generally divided into two categories include perceptual and excitatory that each of them have their own actions and works.

5- Cause knowledge about the Effect:

Under this type of Knowledge by presence human can understand the rights, as a result, theology found Philosophical justification.

6- Effect knowledge about the Cause:

This type of Knowledge by presence just like the next type has been developed based on Supreme wisdom.

7- Soul knowledge about own situation:

Soul has modes such as pleasure, pain, sadness and happiness and has deep understanding about these matters.

Which Science has more originality? Conceptual science or Knowledge by presence?

In this context, there are two views: The first view believed that Knowledge by presence has originality

First exposition: Shaykh al-Ishraq believes that Sensory science is equal to the presence (4).

In sensory science he believes that Knowledge by presence are in superiority and denied conceptual science in the case of sensory science.

Second exposition: this exposition is attributed to the alameh Tabatabai, he believes that sciences are conceptual and credit. (5)

He believes that divided the sciences into two categories like conceptual and presence isn't a scientific work, he considered this work as a traditional function; he binds the end and the destination science to the Knowledge by presence.

He believes that all humanities are solved in the Knowledge by presence, because scientific forms are abstract and immaterial. It can be argued that:

Firstly, there is no power and no variation in these scientific forms and because changes are the properties of the material so scientific forms aren't the matter type.

Secondly, Material has properties, such as location, time and divisibility but because science is science have none of these properties, so science is not materially.

The Second view believed that conceptual science has originality:

Some contemporary philosophers believe that there isn't an experience that should not be interpreted and human gain to all intents, beliefs and descriptions in the shadow of conceptual science. Consequently, intuition and mystical experience are inaccessible or do not exist.

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Bioefficacy of acrystalliferous *Bacillus sphaericus* M3 against field collected mosquito larvae

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Abstract: The present investigation was directed to determine the pathogenicity; bioefficacy and larvicidal activity of *Bacillus sphaericus* M3 against *Anopheline* and *Culicine* mosquitoes. *B. sphaericus* M3 showed high pathogenicity of 85.0% and 98.8% to *Anopheline* larvae, using vegetative cells or spores, respectively. In contrast, pathogenicity toward *Culicine* larvae was 45.0% and 48.8% for vegetative cells and spores, respectively. *Bacillus sphaericus* M3 possesses high larvicidal activity with LC_{50} values 6.11 x 10^3, 1.71 x 10^4 spores/ ml or 0.872, 24.4 mg/l against *Anopheline* & *Culicine* respectively.

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1. Introduction

Mosquitoes transmit some of the world's most life threatening and debilitating and viral diseases and dengue fever (principally *Aedes aegypti*). Alarmingly, these diseases are on the rise in many tropical and subtropical areas (Miller, 1992; Monath, 1994; Priest, 1992) including malaria (*Anopheles),* fialariasis (*Culex*, *Mansonia* and some *Anopheles* spp). Approaching to reducing the incidence of malaria have focused largely on controlling mosquito population with chemical insecticides (Priest, 1992) and by physical barrier methods (impregnated nets), or by using drugs to prevent infection with malarial parasites (*Plasmodium* spp.). Limited trials of a candidate malaria vaccine have received much attention, but it may be some time before this type of vaccine is adopted (Maurice, 1995). Likewise, various candidate dengue virus vaccines are being developed, but it is not known when an effective vaccine will be available (Monath, 1994; Brandt, 1990).

Biological control of mosquito larvae with naturally occurring bacteria that synthesize potent mosquitocidal toxin (Hofte and Whiteleym, 1989) has received much less attention, despite the fact these bacteria have been used safely in the field for many years (Priest, 1992). Commercial control of lepidopteron and coleopteron pests with entomopathogenic strains of *Bacillus thuringeinsis* (*Bt)* is now well accepted and its usefulness established. Moreover, control of the aquatic larvae of blackflies (the vector of the filarial parasite *Onchocerca volvulus*) with *B. thuringeinsis* subsp. *israelesis* (*Bti*) in West Africa has been hugely successful, eradicating onchocerciasis for many areas (Priest, 1992).

Realization of insecticidal resistance and environmental impact of spraying on a global basis has led to considerable resources being devoted to the search for biological control agents. However, greater success has been achieved with spore formers, especially species of genus *Bacillus*. In fact, four species of *Bacillus* viz., *Bacillus thuringiensis*, *Bacillus popillae*, *Bacillus lentimorbis* and *Bacillus moritai* account for nearly one half of all the trade name microbial products in existence (Ignoffo, 1981). Another species of spore formers evaluated as potential microbial pesticide is *Bacillus sphaericus*, which is effective against gnats and mosquitoes. Barbazan et al. (Barbazan et al., 1998) carried out studies at Maroua (North Cameroon), showed effective control of anophelines using *Bacillus sphaericus* strain 2362 larvicidal treatments at the rate of 10 g/m^2. Due to the gradual development of resistance against commercial products of *Bacillus sphaericus* (Abbott, 1925; Adak et al.,1995; Mc Gaughey and Beeman, 1988; Mc Gaughey, 1985; Tabashnik et al., 1990) in mosquitoes and instability of these products in environmental conditions, further research in this particular area is needed. Promising new formulations of the microbial larvicides *Bacillus sphaericus* (*Bs*) and *B. thuringiensis var. israelensis* (*Bti*) have recently been shown to give excellent control of the major vectors of malaria in Africa (Fillinger et al., 2003; Fillinger and Lindsay, 2006). Use of these biological control agents is better than chemical larvicides since they are very species specific, environmentally safe

(W.H.O., 1990) and appear not to induce resistance when used together (Mulla et al., 2003).

In view of the above limitations, it is desirable to screen potential pathogenic microbes for the potential insect control. The present investigation was, therefore, undertaken to test the pathogenicity & to determine the LC_{50} value of *Bacillus sphaericus* M3 against *Anopheline* and *Culicine* mosquitoes.

2. Material & Methods

Bacterial strain

The *Bacillus sphaericus* M3 is a acrystalliferous (crystal-minus) strain was grown in 'St. Julian' medium consisting of (g/l) glucose – 2.0; KH_2PO_4 – 3.0; tryptone – 5.0; yeast extract – 15.0; Benzyl Penicillin - 10 (µg/ml); Agar – 20.0; pH – 7.2-7.5 (St. Julian et al., 1963).

Mosquito larval culture

Field collected second, third and fourth instar larvae of *Anopheline* and *Culicine* mosquitoes were used for bioassay. Sampling of *Anopheline* and *Culicine* larvae was made during the rainy season from the infected water storage tank in and around Pantnagar and Haldwani in Nainital district of Uttarakhand ($29^0$55' N and $70^0$44'E; 400amsl) (Bisht et al., 1996). Larvae were identified by the courtesy of Dr. B. P. Shukla, O/I, Malaria Research Center, Haldwani in their laboratory. Healthy larvae were collected on the same day of two days prior to the conduction of experimental studies.

Seed inoculum preparation

Seed inoculum of *B. sphaericus* M3 was prepared for bioassay as per Collier (1957) active culture technique and modified by Halvorson (1957) by the resuspension of an inoculating loop of spores from a plate, in 10 ml sterile water, followed by a heat shock at 80°C for 30 min to eliminate vegetative cells. This preparation was then inoculated into 90 ml St Julian medium and grown at 28±2^0C and 200 rpm on gyratory shaker, with samples taken every 4 h to monitor growth phases, and bioassays of representative samples were carried out.

Mass spore production in roux bottle

One ml of the activated seed inoculum of *B. sphaericus* M3 consisting of vegetative cells (100%) was transferred to each 500 ml roux bottle containing St. Julian agar, tilted upward and downward for proper spreading of culture and incubated at 28±2^0C for 48 h for complete sporulation. The spore population of *B. sphaericus* M3 was determined by heat stable counts (HSC) and maintained 10^{10}- 10^{11} spore/ml. For insect feeding one ml spore culture from roux bottle was taken and diluted hundredfold. The undiluted culture consisted of spore in order of 10^{10}-10^{11} HSC/ml.

Preparation of test solution of standard "Spharix" powder

500 mg of Spharix, a carrier based formulation of *Bacillus sphaericus* B101 obtained from ICMR, Govt. of India, New Delhi, was placed in 10 ml sterile distilled water contained in 50 ml flask and thoroughly homogenized by shaking at 200 rpm for 15 minute. From this homogenate a stock solution was made in test tube by adding 1 ml of homogenate to 9 ml sterile distilled water and agitated on vortex for few seconds. The concentration in stock solution was 5 mg standard/ml (WHO, 1990).

Preliminary pathogenicity test

Preliminary pathogenicity test was carried out by taking 10 ml of spores and vegetative cells were suspended in 90 ml of water placed in 250 ml beaker, appropriate dilutions were made to achieve a population of spore 10^8-10^{10} and vegetative cells in the range of 10^4-10^7 cfu/ml. For each assay, equal number (20 each) of II, III and IV instar larvae of a susceptible *Anopheline* or *Culicine* colony were placed in 100 ml natural water contained in 250 ml beaker and different bacterial dilutions were added. Each dilution replicated four times with three controls C1-media; C2-Tap water and C3- natural water. At least five concentrations giving mortality between 2 and 98% were tested, and mortality was recorded after bioassays. After the treatment larvae were considered dead if they were unable to return to surface after being forced to the bottom. Mortality was counted after 24 h (Abbott, 1925; Bisht et al., 1996).

Bioefficacy & determination of LC_{50}

Bioefficacy of *B. sphaericus* M3 was performed in quadruplet in 100 ml natural water placed in 250 ml beakers containing equal number (20 each) of II, III, IV instar larvae of *Anopheline sp.* or *Culicine sp.* and incubated at 28+2°C. A small amount of yeast powder was spread on the surface of water as larval diet. Four dilutions of spores of *B. sphaericus* M3 were assayed and undiluted vegetative cells were used for comparing with undiluted spores. The numbers of surviving and dead larvae were counted at an interval of 24 h after inoculation and each treatment was replicated in quadruplets keeping three controls same as in the pathogenicity test (Ramoska and Hopkins, 1981) $LC_{50's}$ were determined using sporulating cultures from roux bottles. For obtaining maximum spore population in a treatment, undiluted cultures were

used subsequently 10- fold dilutions were prepared to achieve the various spore populations. Each test was replicated four times at six different dilutions in addition to the controls. Abbott's Formula (1925) determined the corrected percent mortality. The lethal concentration required to kill 50% of the test population was subjected to probit analysis with the help of log dose and probit mortality. The LC_{50} was determined by dose mortality regression line plotted on log probit paper and the 95% confidence (Litchfield and Wilcoxan, 1949).

3. Results and Discussion

Thus realizing the above merits, the present investigation was undertaken to evaluate a novel, acrystalliferous *B. sphaericus* M3 for pathogenicity to mosquito larvae. Preliminary pathogenicity tests of *B. sphaericus* M3 against *Anopheline* and *Culicine* mosquitoes were conducted during the month of July - September 1996.

The mortality data indicated that spores as well as vegetative cells of M3 were highly pathogenic recording 98.8% and 85.0% mortality respectively to *Anopheline* as compared to *Culicine* showing 48.8% and 45.0% mortality. Mortality ranged from 0 – 7.5% for three controls (Figure 2 (a-d) & Table 1). The data also indicated that a cell population in the range of 10^6 –10^8 cfu/ml to achieved higher mortality.

Table 1. Pathogenicity of *Bacillus sphaericus* M3 against larvae* of *Anopheline* and *Culicine* mosquitoes after 24h at temperature 28±2ºC

Stage of test organism	cfu/ ml	Target mosquitoes	Percent Mortality
Spores	10^8–10^{10}	*Anopheline*	98.8
		Culicine	48.8
VC	10^4-10^7	*Anopheline*	85.0
		Culicine	45.0
C_1	ND	*Anopheline*	0.0
		Culicine	5.0
C_2	ND	*Anopheline*	0.0
		Culicine	3.5
C_3	ND	*Anopheline*	3.8
		Culicine	7.5

VC - Vegetative cells; C1 - Normal water Control; C2 - Distilled water control; C3 - Growth Media control; * - Mixture of equal number (20 each) of II, III & IV instars larvae; ND - Not Detected

On a comparative basis it is concluded that spores were more effective than the vegetative cells. The results obtained from the above preliminary trial demonstrated that *B. sphaericus* M3 spores had significantly higher mosquitocidal activity against *Anopheline* followed by the vegetative cells. These results confirm to the findings of several workers using crystalliferous and noncrystalliferous *B. sphaericus* M3 (Davidson and Yousten, 1990; Davidson, 1984; de Berjac, 1990). It is also reported that the strains of *B. sphaericus* in which parasporal inclusions were not observed, some larvicidal activity was detected which could be due to toxin being localized in cell wall (Meyers and Yousten, 1980) or cytoplasm (Davidson, 1982).

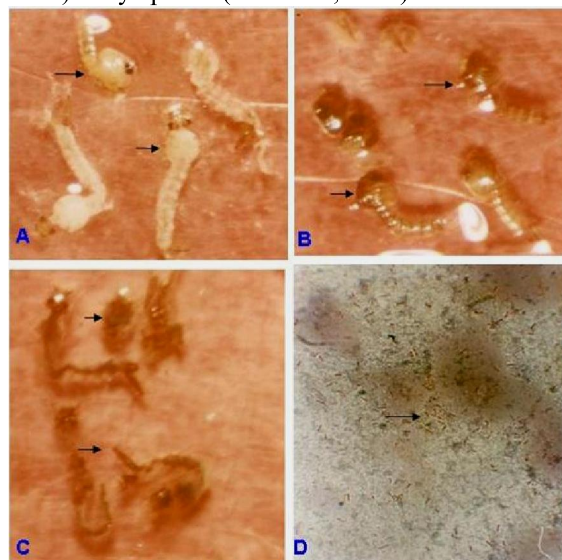

Figure 1. A) Healthy mosquito larvae, B) after 24 hrs of treatment, C) after 48 hrs of treatment, and D) presence of vegetative cells of *B. Sphaericus* M3 in the haemolymph of mosquito larvae

Based on the preliminary trail established the pathogenicity of *B. sphaericus* M3, it was thought worthwhile to examine the period of exposure with a view to achieve maximum mortality of mosquitoes under study.

Bioefficacy of *Bacillus sphaericus* M3 in term of exposure time is presented in Table 2 showed that a considerably higher spore population (10^9 – 10^{11} cfu/ml) of strain R3 resulted in 100 and 82.8% mortality against *Anopheline* and *Culicine* respectively with an exposure time of three days. Even the vegetative cells with a low population of 10^4 – 10^6 cfu/ml were found to be also effective at the end of similar period of exposure as evident from mortality data of 96.3 & 62.3% for *Anopheline* and *Culicine* respectively. Recent literature has revealed that the three mosquitocidal toxin mtx1, mtx2 and mtx3 which are expressed during vegetative growth of *B. sphaericus* are wide spread among various strains, including those with low, moderate and high toxicity (Liu et al., 1993). These two toxins do not display any similarity to each other to crystal protoxin or any other insecticidal proteins (Thanabalu et al., 1991; Thanabalu et al., 1992). The

reported mtx toxin may also be exists in the vegetative cells of *B. sphaericus* M3 which might be responsible for high mortality. There was none or very low mortality (8.8%) in controls. These results also indicated that there was a progressive increase in mortality with the time (1 to 3 day) to both *Anopheline* and *Culicine* mosquitoes in exposure to either spores or vegetative cells. (Table 2)

Table 2. Bioefficacy of *Bacillus sphaericus* M3 against larvae* of *Anopheline* and *Culicine* mosquitoes at different period of exposure.

Stage of test organism	Target mosquitoes	Cell population (cfu/ml)	Percent Mortality Exposure Time (days)**		
			1	2	3
Spores	*Anopheline*	1.96×10^9	91.3	98.8	100.0
	Culicine	1.58×10^{11}	18.8	38.8	82.8
VC	*Anopheline*	4.6×10^6	71.3	85.0	96.3
	Culicine	4.25×10^4	25.0	40.5	62.3
Control	*Anopheline*	ND	0.0	0.0	0.0
	Culicine	ND	5.0	7.5	8.8

VC- Vegetative cells; ND - Not Detected; * - Mixture of equal number (20 each) of II, III & IV instars larvae; ** - Indicative of feeding time of spores or vegetative cells

Toxicity of the *B. sphaericus* M3 was compared with the Standard Spherix powder against *Anopheline* and *Culicine* (Table 3). *B. sphaericus* M3 possesses higher larvicidal activity with LC_{50} values 6.11×10^3, 1.71×10^4 spores/ml or 0.872, 24.41 mg/l against *Anopheline* & *Culicine* respectively as that of Standard Spharix powder.

Table 3. Toxicity of *Bacillus sphaericus* M3 & Standard Spharix powder against larvae* of *Anopheline* and *Culicine* mosquitoes after 3 days of exposure at temperature 28± 2ºC

MI	TM	Heterogeneity		Regression equation	LC_{50}	Fiducial Limit
		d. f.	X^2			
BS	*A*	4	11.4	Y=5.6227 +0.4135x	6.1x 10^3	0.914×10^{-2}– 0.11×10^{-6}
	C	4	9.0	Y=3.655+ 1.329x	1.7x 10^4	0.47×10^{-4}– 0.32×10^{-6}
SSP	*A*	4	16.0	Y=4.6539 +0.6382x	5.9x 10^{-3}	0.59×10^{-3}– 0.55×10^{-6}
	C	4	6.5	Y=4.9635 +0.2474x	4.6x 10^{-3}	0.19×10^{2}– 0.51×10^{-6}
Y = Probit Kill; X = Log (concentration x 10^6)						

MI – Microbial Insecticide; TM- Targeted Mosquito; BS - *Bacillus sphaericus* M3; SSP - Standard Spharix Powder; A- *Anopheline*; C – *Culicine;* d. f. - Degree of freedom; * - Mixture of equal number (20 each) of II, III & IV instars larvae

The results support the hypothesis that the implementation of large-scale application of this test strain can be applied successfully in extended floodplain areas either as liquid or as a carrier based formulation by hand, which leads to a reduction in larval abundance in the natural habitats and could be an additional tool in an IVM programme.

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8/8/2013

Serum Lipid Profile Of Hypertensive Pateints In Hyderabad, Pakistan

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Abstract: A prospective study was conducted in the Hyderabad, Pakistan to investigate the serum lipid profile & the level of total cholesterol (TC), Triglyceride (TG), HDL-cholesterol and LDL-cholesterol of hypertensive patients and compares them with levels of control subjects. The results revealed that serum total cholesterol, triglyceride and LDL-cholesterol were significantly markedly raised (p<0.005) whereas the level of HDL-cholesterol was significantly lower (p<0.005) in hypertensive patients as compared to control subjects. No significant changes of serum lipid profile were found between male and female hypertensive patients, but in control subjects, markedly higher levels of serum lipid profile was observed in male compared to that of female. It was concluded that hypercholesterolemia, hypertriglyceridemia and low density lipoprotein are the main lipid abnormalities on the incidence of hypertension in the study area.

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Keywords: Total cholesterol (TC), triglyceride (TG), HDL- cholesterol and LDL-cholesterol, Hypertension.

1. Introduction

Hypertension is the most common of the cardio-vascular diseases which is the leading cause of morbidity and mortality in the industrial world as well as becoming an increasing common disease in the developing countries (WHO, 1978). Hypertension in adults is arbitrarily defined as systolic pressure to or greater than 150 mm Hg and or/ or diastolic pressure equal to or greater than 90 mm Hg (WHO, 1978). The most important risk factors for the development of hypertension are increased salt intake, obesity, cigarette smoking, and elevated serum level, lack of physical exercise, genetic factors and stress and strain (Williams and Braun Wald 1987). The blood lipids and lipoproteins are closely associated with hypertension. The serum lipid level of hypertensive patients is usually higher and can be lowered either by dietary restriction or by hypolipidemic agents (Lipid Research Clinics Program 1984 and Burke et al. 1991). Hypertension is a risk factor for the development of atherosclerosis. There is increasing evidence that atherosclerosis should be viewed fundamentally as an inflammatory disease. Atherogenic stimuli such as hyperlipidemia appear to activate the inflammatory response by causing expression of mononuclear leukocyte recruiting mechanisms. Hypertension not only is a well-established cardiovascular risk factor but also increases the risk of atherosclerosis. Both of hypertension and dyslipidemia are independent risk factors for the development of atherosclerosis [Janjigian YY, McDonnell K, Kris MG, Shen R, Sima CS, Rizvi NA, et al.2008] the changes in serum lipid profile level on hypertensive patients should be actively investigated. The findings of this study may help to understand the effect of renin-angiotensin system in the regulation of blood pressure. The aim and objectives of the present case-control study were to find out the relationship between serum lipids levels of the hypertensive patients with controls in the study in Hyderabad, Pakistan.

STATISTICAL ANALYSIS

All values are expressed as mean ± SE. For comparison in patient's.

2. Material and Methods

A total number of fifty newly hypertensive patients who have not commenced medication were selected A total numbers of 50 human subjects of age ranging from 30-70 years were included in this study. Out of the 50 subjects, volunteers (10 Males and 10 females) were selected as control (group1). The remaining 30 subjects (20 Males and 10 Females) were grouped as hypertensive (group 2 .Serum total cholesterol levels was determined by enzymatic (CHOD-PAP) colorimetric method (Allain et al.1974) and triglyceride by enzymatic (GPO-PAP) method of (Jacobs and Van demark (1960). HDL-cholesterol and LDL-cholesterol were estimated using precipitant (Gordon and Gordon 1977) and Friedewald formula (Friedewald 1972). Venous fasting blood samples were drawn from all the subjects and analysed for TC, HDLC and TG by enzymatic colorimetric method [Trinder P et al. 1988] above all parameters under investigation were determined in the serum of patients and controls using commercially available reagent kits. All values were expressed as mean ± S.E.Intravenous blood (10mL) samples from fifty patients and healthy

subjects were collected and made to clot before serum was separated by centrifuging. Each metal the serum samples were analyzed using a Hitachi atomic absorption spectrophotometer (Tokyo, Japan). Cholesterol, triglycerides, HDL and LDL were determined using a kit method on Microlab 300.

3. Results

In the present study, maximum numbers of patients of both sexes were between 30-70 years of age and the percentage had declined sharply below these ages (Table 1). The age distribution of hypertensive patients No. Age Group (years) Male (%) Female (%) The age and sex distribution presented in Table 1 revealed that most of the cases belonged to old age group. There was no significant difference (P>0.05) in the means of serum lipid levels in males and females hypertensive patients while there was a significant increase in the levels of serum lipid in males than in female normotensive subjects (Table 2 and 3).

Table 1: Age and sex distribution in hypertensive subjects Age Group (Years) Males (%) Females (%)

Age Group (Years)	Males (%)	Females (%)
30 < 40	5	2
41-50	15	6
61-70	20	2
Total n = 50	40	10

Table 2: Serum lipid profile (mmol/l) of hypertensive patients

Group	TC	LDL	TG	HDL
Hypertensive n = 50	6.0 ± 2.0	4.0 ± 1.5	3.50 ± 1.6	0.90 ± 0.10
P - Value	P<0.05	P>0.05	P<0.05	P<0.05

Table 3. Show Serum lipid profile (mmol/l) of male and female

Group	TC	HDL	LDL	TG
Males n = 40	3.66 ± 2.11	2.50 ± 0.55	0.83 ± 0.43	1.65 ± 1.25
Females n = 10	3.00 ± 2.18	2.12 ± 0.15	0.62 ± 0.34	1. ± 0.82
P - Value	P<0.05	P<0.05	P<0.05	P<0.05

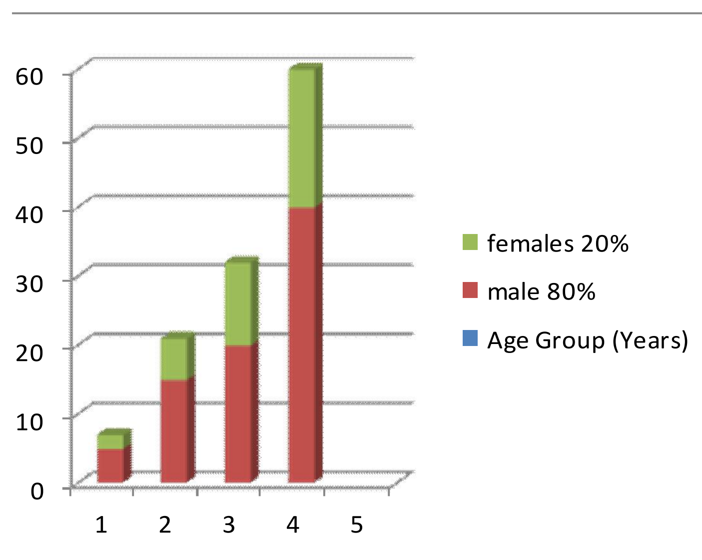

Fig 1. Gender analysis

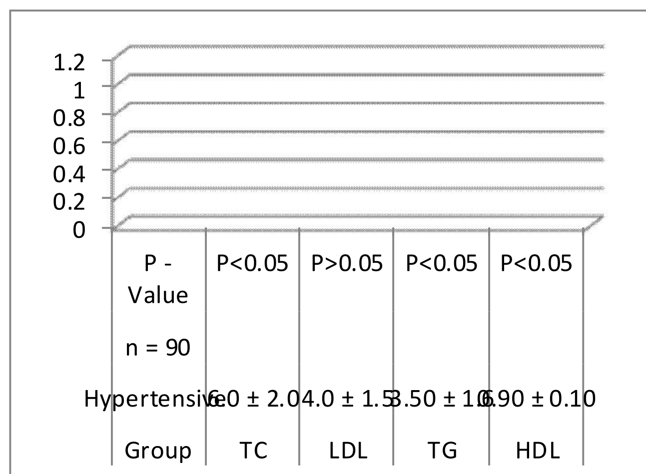

Fig 2 Serum lipid profile (mmol/l) of hypertensive patients

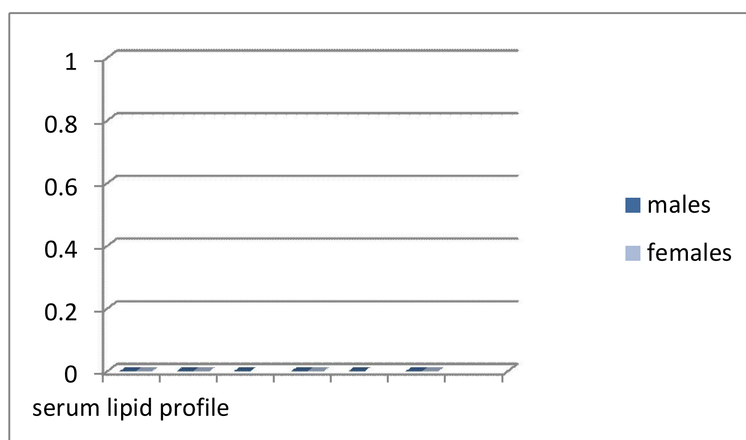

Fig 3. Show Serum lipid profile (mmol/l) of male and female

4. Discussions

The results of our study revealed that the men value of serum cholesterol, triglyceride and LDL-cholesterol was significantly higher and significantly lower HDL-Cholesterol level was found in hypertensive patients than those of the control group. The findings of increased total cholesterol in patients with hypertension are slightly higher than the study of Shahadat et al. (1999). study where they observed that only females have hypertriglyceridemia. Serum HDL-cholesterol level in hypertensive patients was found to be lower than the findings of Shahadat et al. (1999) at home and of the past (Castilli et al. 1977, Wilson et al. 1980, Person et al.1979 and Miller et al.1977) The Framingham Offspring Study (Wilson et al. 1980) and also with the co-operative phenotyping study (Castilli et al. 1977) in U S A, who demonstrated a positive correlation between the level of LDL-cholesterol and coronary risk. In our study, no significant difference of serum lipid profile betweenmale and female hypertensive patients was found but total cholesterol, triglyceride and LDL-cholesterol were significantly higher in male than female controls whereas HDL-cholesterol was vice-versa. Based on the results obtained from the present study, we concluded that serum cholesterol; triglyceride and LDL-cholesterol levels are positively correlated with hypertensive patients whereas HDL-cholesterol has no significant changes with hypertension. The higher level of serum TC, TG and LDL-cholesterol in the study population may be due to genetic factors and increased consumption of dietary animal fat, lack of physical exercise, metabolic disorders like diabetes Mellitus and hypothyroidism, severe stress, increased age, sex as

well as alcohol and tobacco consumption may also be the contributory factors for this phenomenon.

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Isopaches decolorans (Limpr.) H. Buch. (Marchantiophyta: Lophoziaceae) in India, A Noteworthy Rare and Disjunct Liverwort

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Abstract: *Isopaches decolorans* (Limpr.) H. Buch., a rare and disjunct species is being reported from Arunachal Pradesh, India along with a note on its distribution and affinities with plants from other localities. The discovery of male plant also has been clearly authenticating the sexuality of the species (dioecious).
[Rawat K.K., and Verma, P.K. ***Isopaches decolorans* (Limpr.) H. Buch. (Marchantiophyta: Lophoziaceae) in India, A Noteworthy Rare and Disjunct Liverwort.** *Nat Sci* 2013;11(9):119-122]. (ISSN: 1545-0740). http://www.sciencepub.net/nature. 19

Keywords: Arunachal Pradesh, Tawang, Marchantiophyta, Cephaloziaceae, *Isopaches, I. decolorans.*

1. Introduction

Eastern Himalayas, despite of being the adobe of enormous diversity of bryophytes, has been remained underexplored, and consistently provides opportunity to the workers to find some unique information. While working on some plants from Arunachal Pradesh (India), we came across with some beautiful plants of *Isopaches decolorans* (Limpr.) H. Buch., a rare species, and poorly known among Indian bryoflora.

Genus *Isopaches* H. Buch., sometimes being treated as a subgenus of genus *Lophozia* (Dumort.) Dumort. (Schuster, 1995), is represented by four species around the world viz. *Isopaches bicrenatus* (Schmid. ex Hoffm.) H. Buch, *I. pumicicola* (Berggr.) Bakalin, *I. alboviridis* (R.M. Schust.) Schljakov and *I. decolorans* (Limpr.) H. Buch (Zhang *et al*., 2013). Out of these, *Isopaches decolorans* (earlier known as *Lophozia decolorans* (Limpr.) Steph.) is being considered a rare and highly disjunct species (Schuster, 1995, Long, 2005, Zhang *et al*., 2013). The species is distributed mainly in Europe, however also sporadically reported from British Colombia, equatorial Africa, Siberian Arctic, Far-east Russia, Europian Russia, China and Himalayan sector. In India, its first report came through Long (2005), who have reported its presence in Sikkim, however, he did not provide any details of the Indian plants of this rare and poorly known species. Hence it felt necessary to provide the details of Indian plants, which may help future workers to understand this rare species and its phytogeographical details along with the rarity status.

2. Materials and Methods

The specimens were collected from rocks at Tawang town of Arunachal Pradesh and air dried initially then kept in brown paper envelope and preserved in Lucknow University Bryyophyte Herbarium (LWU). Slides of whole mount as well as plants parts and sections were mounted in aqueous glycerin, labeled and stored for further studies, wherever found necessary.

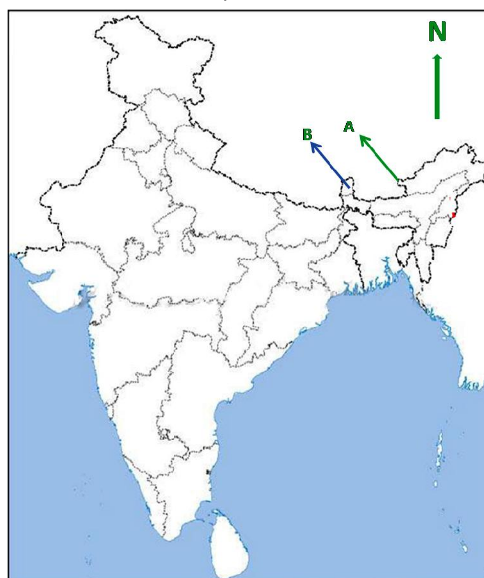

Fig. 1. Map of India showing the distribution of *Isopaches decolorans* (Limpr.) H. Buch.. in Tawang (A) district of Arunachal Pradesh and Sikkim (B).

3. Taxonomic observation

***Isopaches decolorans* (Limpr.) H. Buch.,** *Mem. Soc. F. Fl. Fenn.* **8**: 288 (1932) – *Jungermannia decolorans* Limpr., *Jahresb. Schles. Gesell. Vsterl. Kult.* **57**: 116 (1880) – *Lophozia decolorans* (Limpr.) Steph., *Sp. Hepat*. **2**: 147 (1902).

Plants small, 2.0-4.5 mm in size, robust, succulent, brittle when dry, growing in dense patch, creeping, closely appressed to substratum with numerous rhizoids, except at apices; clear, pale green to bright green when fresh, whitish to yellowish brown when dry; occasionally branched, '*Frullania*-type';

Stem elliptical in cross-section, 147-212 µm across, fleshy, up to 10 cells wide; cells undifferentiated, 16-23 × 13-26 µm in size, thin walled, atrigonous. Rhizoids numerous, scattered along the ventral surface except the shoot apices. Leaves succubous, obliquely inserted, closely imbricate producing somewhat julaceous habit, concave, ventral insertion oblique, dorsal insertion transverse, broader than long, 0.68-1.1 × 0.49-0.85 mm in size, unistratose, apical half and margins hyaline, decolourate, mid basal and middle part bright green due to presence of numerous chloroplasts, bilobed, lobes unequal, dorsal lobe smaller than ventral lobe, rarely with a third small to large lobe, margin almost entire, occasionally with a few one-celled projections appearing as small tooth near subapical margin, usually straight to rarely undulate, rarely with a large 4-6 cells high and 3-4 cells broad tooth or lobe at the antical base of leaf, leaf lobes acute, terminating in a single elongated apical cell, 19-22 × 13-16 µm in size, subapical cells 13-26 × (9.7) 13-16 µm, median cells (13) 19-23 × (13)16-23 µm in size, basal cells 16-29 × 13-16 µm in size; cells somewhat thick-walled in contrast to reportedly thin-walled cells; trigones indistinct except at apices; cuticle smooth. Underleaves variable in shape and size, rounded lobed to elongate. Rhizoids numerous. Gemmae not seen.

Dioecious. Male and female plants are same in size. Male plants very rare in population. Androecia terminal; bracts similar to leaves, with one antheridium per bract; antheridia spherical to elliptical, stalk biseriate, short. Gynoecia terminal on main as well as lateral shoots; bracts larger than leaves, bilobed to trilobed and with or without the antical tooth at distal base; bracteoles smaller, bi-tri lobed. Perianth green, elongate, 5-plicate, mouth lobulate, lobes denticulate, teeth 1-2 celled. Mature sporophytes were not observed.

3.a. Habitat

The plants were growing on partially shady road side slopes with *Solenostoma* sp. and *Diplophyllum nanum*. The locality is a high altitude site in Himalayas near the juncture of borders of India, Bhutan and China and receives heavy snowfall during the winters.

3.b. Specimens examined

INDIA: Arunachal Pradesh – Tawang town; *C.* 2950 mts.; 27 April 2002; KK Rawat and MS Azeem; 15598/2002, 15607/2002 (LWU).

3.c. Range

France, Switzerland, Austria, Norway (Müller, 1954), Italy (Aleffi *et al*., 2008), Scandinavia (Arnell, 1956), Siberian arctic, Europian Russia and Russian Far-east (Ladyzhenskaya and Zhukova, 1971; Schljakov, 1974; Potempkin 1990, 1993; Schuster and Konstantinova, 1996; Bakalin, 2005; Oyesiku, 2008; Potempkin and Sofronova, 2009), Canada (Schuster, 1995) Camerron, Tanzania (Vana, 1982; Bizot *et al*., 1985; Mwasaga, 1991), Uganda (O'Shea *et al*, 2003), Congo (Müller, 1995), Equatorial Guinea (Müller, 2006), Bhutan (Long, 1979), Nepal, India (Long, 2005), China (Long, 2005, 2011; Zhang *et al*., 2013).

3.d. Distribution in India

Sikkim (Long, 2005), Arunachal Pradesh – Tawang (present study).

4. Discussion and Conclusion

The plants are unique in having decolored leaf margins, a characteristic which gave its name. Till now, this plant has been reported mainly from some localities in Europe, Russia and tropical Africa, and only once from Northern America. Due to its rare occurrence, Schuster (1995) considered *I. decolorans* as "one of the periglacial and/or alpine taxa which survived the ice age in local ice free niches" and discussed the link between European plants and Canadian plants. He also added that its presence in western Siberia could be a "post-Pleistocene invasion". However, recent reports of its occurrence in Asian and African elements, particularly in Himalayan sector, suggest a possible disjunct distribution of the species, with some scattered populations. The reports from Nepal, Sikkim, Bhutan and Arunachal Pradesh (present study) clearly indicate that the species is distributed throughout the Himalayan sector, however, in very small pockets. It also indicates possible un-noticed presence of this species in other Himalayan localities, particularly western Himalayan sector, which is having a good representation of other Lophoziaceae elements. Its presence in Chinese territory also suggests its most likely route of dispersal from Siberia to Himalayan region.

The Indian plants were completely lacking gemmae as in Chinese plants (Zhang *et al*, 2013), in contrast to European and American reports where gemmae are well reported. Schuster (1995) and Zhang *et al* (2013), speculated it to be dioecious in contrast to the view of Potempkin (1993) who stated paroecious sexuality. However, presence of male plants in Indian population clearly puts an end to this controversy. The male plants look alike vegetative plants, as the male bracts are similar to normal leaves with only one antheridia per bract, hence may remain unnoticed.

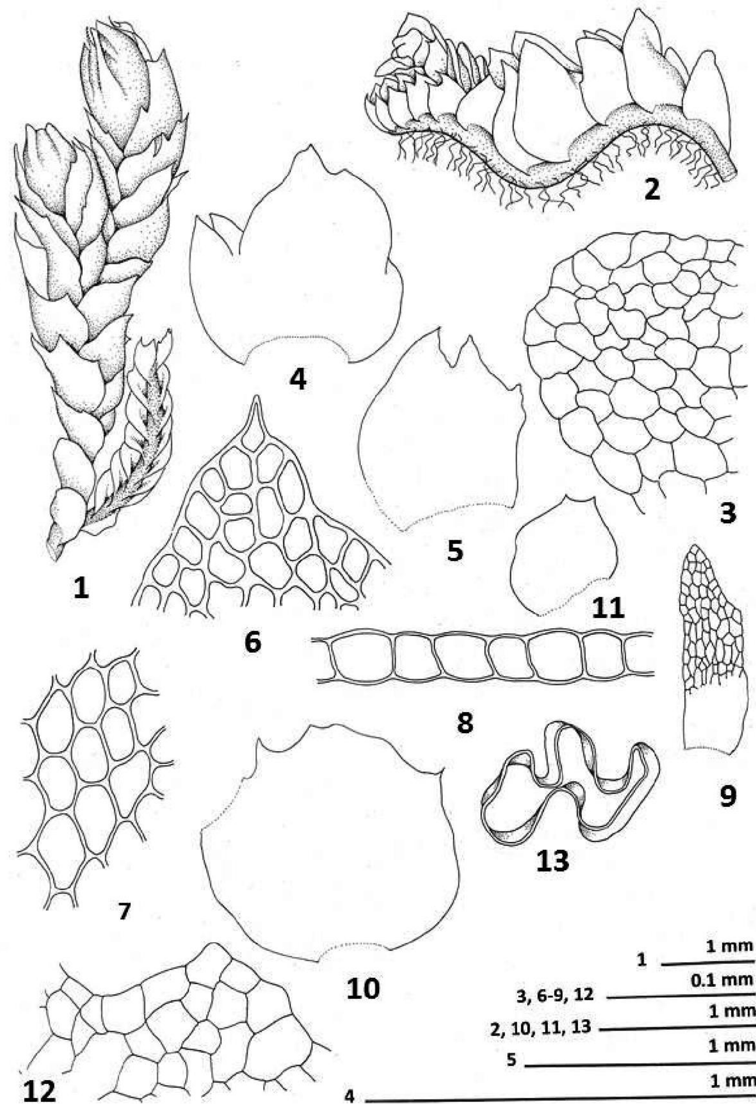

Fig. 2. Plant with perianth, dorsal view, 2. Plant, lateral view, 3. Stem T.S., 4,5. Leaves, 6. Apical and sub-apical cells of leaf, 7. Mid-basal cells of leaf, 8. Leaf T.S., 9. Underleaf, 10. Bract, 11. Bracteole, 12. Cells of perianth mouth, 13. T.S. of perianth (all figures drawn by K. K. Rawat from LWU 15598/2002).

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8/8/2013

Phytochemical Screening and Antimicrobial Properties of the Leaf and Stem Bark Extracts of *Strychnos Spinosa*

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Abstract: The study was carried out to ascertain the antimicrobial properties inherent in the aqueous, ethanol and methanol leaf extracts of *Strychnos spinosa* on *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans*, as well as the phytochemical screening of the extracts. Agar well diffusion method and Macro Broth dilution method were used in determining the antimicrobial activity and minimum inhibitory concentration (MIC) respectively. The extracts showed good inhibitory activities against *E. coli* and *P. aeruginosa*, but minimally against *S. aureus* and *C. albicans.* The inhibitions of the test organisms were measured by the diameter of zone of inhibition. The methanol extracts of the leaves and stem barks were the most potent against *E. coli* while the ethanol extracts, especially the leaf gave the highest potency against *P. aeruginosa* with a minimum inhibitory concentration (MIC) of 125 mg/ml . The extracts gave different MICs against the test organisms using the double-fold dilution method, with concentrations ranging between 31.25 to 500 mg/ml. The minimum lethal concentrations (MLC) of the extracts were 250 mg/ml and 500 mg/ml. The study revealed that the extracts were more bacteriostatic than bactericidal. The results obtained were tested at $P \leq 0.05$ level of significance using the one-way analysis of variance (ANOVA). The phytochemical screening revealed the presence of alkaloids, tannins, saponins and glycosides in the plant parts used.
[Ugoh, Sylvanus Chukwudi and Bejide, Oyeniyi Stephen. **Phytochemical Screening and Antimicrobial Properties of the Leaf and Stem Bark Extracts of *Strychnos Spinosa.*** *Nat Sci* 2013;11(9):123-128]. (ISSN: 1545-0740). http://www.sciencepub.net/nature. 20

Key words: Phytochemicals, Zone diameter of inhibition, Minimum inhibitory concentration, Minimum lethal concentration.

Introduction

Plants are used medicinally in different countries and are a source of many potent and effective drugs (Srivastava *et al*., 1996). According to Ghani, 1990 and Dobelis, 1993, the active principles of many drugs that are found in plants which are responsible for their therapeutic values are secondary metabolites. Some of these active compounds include alkaloids, glycosides, flavonoids and tannins (Chhetri *et al*., 2008). The phytochemical research of plants is considered an effective approach in the discovery of new anti-infective agents from higher plants (Duraipandiyan *et al*., 2006).

The genus *Strychnos* belongs to the family *Loganiaceae. Strychnos* is known by its very toxic substances like strychnine and curare. Originally, these substances have been prepared by cooking the plant bark with water and thickening to a paste. The residue, a brown resinous paste with a bitter taste is used by indigenous people for arrow poisons (Hoet *et al*., 2007).

Strychnos spinosa has been used in different places as remedy for many ailments and disease conditions. The antimicrobial activity of *Strychnos spinosa* against *Candida albicans* and *Aspergillus niger* among other microorganisms was reported by Nwozo *et al*., 2010. Other researchers have also reported the antiplasmodial (Frederich *et al*., 2002, Bero *et al*., 2009)as well as the antitrypanosomal properties of *S. spinosa (*Nwozo *et al*., 2010)

Materials and Methods

Processing of the Plant Materials

This was done according to the methods described by Jigna and Chanda,(2006) and Mann *et al*., 2011. The leaves and stem bark of *S. spinosa* were shade-dried at the temperature of $25^0C \pm 2^0C$ to a constant weight to aid pulverization. The dried samples were then pulverized using a blender. This was done to enhance the maximum penetration of extracting solvents.

Extraction of the Plant Materials

This was done according to the method described by Kubmarawa *et al*., (2007). Exactly 50g of the blended leaves and stem bark were weighed and introduced into 250 ml of distilled water, ethanol and methanol respectively. The mixtures were agitated at 30 minutes interval for 3 hours and then soaked for 72 hours (3 days). Subsequently, the soaked materials were filtered into containers using Whatman's No. 1 filter paper. The filtrates were evaporated to dryness using a vacuum evaporator and

rotary shaker. The extracts were covered and stored in a refrigerator at 4^0C until needed.

Preparation of Standard Drug and Extract concentrations

Three concentrations (100, 200 and 400 mg/ml) of the six extracts: aqueous leaf, aqueous stem bark, ethanol leaf, ethanol stem bark, methanol leaf and methanol stem bark were prepared using methods described by Nascimento *et al*.(2000) and Mallikharjuna *et al*. (2010). In addition, 250 mg/ml of chloramphenicol and 200 mg/ml of ketoconazole were prepared using the same procedures and they served as antibiotic and antifungal controls respectively. The extracts were sterilized using a membrane filter.

Phytochemical Screening

The extracts were analysed for the presence of alkaloids, glycosides, steroids, tannins, reducing sugars, anthraquinones, phlobatannins and saponins as described by Trease and Evans, (1989), Siddiqui and Ali, (1997) and Sofowora (2006).

(i) Alkaloids

To 1 ml of each extract filtrate, 3 drops of Mayer's reagent were added. The mixture was then treated with few drops of 2% hydrochloric acid on a boiling water bath. The presence of turbidity in the observed tube indicates the presence of alkaloids.

(ii) Glycosides

To the solution of the extract in glacial acetic acid, few drops of ferric chloride and concentrated sulfuric acid were added and observed for a reddish brown coloration at the junction of two layers and a bluish green colour at the upper layer.

(iii) Steroids and Terpenoids

1 ml of the extract filtrates was treated with 3 drops of acetic anhydride. Then concentrated sulfuric acid was carefully added to the side of the test tube. The presence of a brown ring at the boundary of the mixture indicates a positive result.

(iv) Tannins

To 0.5 ml of extract solution, 1 ml of distilled water and about 2 drops of 10% ferric chloride solution were added. The mixture was observed for a blue or green-black coloration.

(v) Reducing sugars

To 0.5 ml of extract solution, 1 ml of distilled water was added and about 6 drops of Fehling's solution and warmed. The Fehling's solution was prepared by mixing equal volumes of equimolar concentration of Fehling's solutions A and B. The formation of brick red precipitates is an indication of the presence of reducing sugars.

(vi) Anthraquinones

2 ml of benzene was added to 1 ml of extract solution. Then, 2 ml of ammonia solution was added. The occurrence of turbidity is an indication of positive result.

(vii) Phlobatannins

1 ml of the extract solution was boiled with 1 ml of 1% aqueous hydrochloric acid and was observed for the deposition of a red precipitate to indicate phlobatannins presence.

(viii) Saponins

1 ml of the extract solution was boiled with 5 ml of water for 5 minutes and decanted while still hot. The following tests were performed to detect the presence of saponins.

(a) Frothing test: 1 ml of the mixture was further diluted with 4 ml of distilled water and shaken vigorously. It was then observed on standing for a stable froth.

(b) Emulsion test: This test was performed by adding 2 drops of olive oil to the frothing solution and shaking the mixture vigorously. The mixture was then observed for emulsion.

Preparation of the Test Organisms

Stock cultures of *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans* were obtained from the University of Abuja Teaching Hospital (UATH) and authenticated using cultural and morphological identification, microscopy after Gram's staining as well as biochemical characterization of test organism using protocols described by Cheesbrough, (2002) and maintained in appropriate media in a refrigerator for future use. Suspensions of the test organisms were made in comparison with 0.5 MacFarland standards to give a cell density of 1.0 x 10^8 cells/ml ascertained using the Standard Curve according to Isu and Onyeagba, (1998).

Preparation and sterilization of media

All the media used in this study were obtained in powdered form and constituted in distilled water according to the manufacturers' instructions. The various quantities and volumes of water depended on the particular medium. A weighed quantity of each medium was dissolved in specific volume of de-ionized water in a chemical flask, which was stoppered properly. It was sterilized by autoclaving at 121^oC and 15 p.s.i for 15 minutes and cooled to 45-50^oC before dispensing into pre-sterilized dishes. These were left to gel on the work benches. Glass materials used in this work were also sterilized by autoclaving at 121^oC and 15 p.s.i. 15 minutes. They were then brought out and allowed to cool down properly before use.

Antimicrobial Assay (Agar Well Diffusion Method)

This was done according to the methods described by Mallikharjuna *et al*., 2010. Twenty

milliliters (20 ml) of sterile molten Mueller Hinton agar was poured into a set of sterile Petri dishes under aseptic conditions and was allowed to solidify. Then, each plate was inoculated with 200 µl of pure cultures of the test organisms and was evenly spread with a sterile bent glass rod. After allowing the sensitivity agar surfaces to dry, 4 wells of 8 mm diameter each were made on the seeded agar plates at fairly equidistant positions using a sterile cork borer. Exactly 0.2 ml of the 100, 200 and 400 mg/ml of the extract were placed in corresponding wells for each microorganism. The fourth well contained the control. Chloramphenicol (250 mg/ml) served as the control for the bacteria- *S. aureus, E. coli and P. aeruginosa* while ketoconazole (200 mg/ml) served as control for the fungus- *C. albicans*. The procedure was repeated respectively for the aqueous, ethanolic, methanolic leaf and stem bark extracts of *S. spinosa*. They were allowed to stand for 30 minutes for proper diffusion and incubated at 37^0C for 24 hours. Plates were subsequently observed for zones of inhibition (if any) which were measured (in mm) using a transparent ruler.

Minimum Inhibitory Concentration (MIC)

The MICs of the extracts that showed activity against the organisms were determined according to the macro broth dilution technique as described by Trigg and Hill, (1996). Two drops of standardized suspensions of the test organisms were inoculated separately into a series of sterile test tubes containing 2 ml of nutrient broth each. Then, 3 drops of different dilutions of the extracts were separately added to the tubes. The dilutions were in two folds with these concentrations: 500 mg/ml, 250 mg/ml, 125 mg/ml, 62.5mg/ml and 31.25mg/ml. The tubes were then properly corked and incubated at 37^0C for 24 hours. The MIC was read as the least concentration that inhibited the growth of the test organisms (Isu and Onyeagba, 1998)

Minimum Lethal Concentration (MLC)

The MLC of potent extracts were determined by plating out the tubes that showed no growth (inhibited visible growth) during the MIC determination. Using a heat-sterilized wire loop, a loopful from each of such tubes was sub cultured onto extract-free agar plates and incubated for 24 hours at 37^0C. The MLC was recorded as the least concentration at which no growth was observed.

Statistical Analysis

The Univariate Analysis of Variance (ANOVA) at $P \leq 0.05$ was used to analyse the results obtained, $P \leq 0.05$ was considered to be significant and $P \leq 0.05$ was not significant.

RESULTS

Table 1: Phytochemical constituents of *S. spinosa*

Bioactive Component	Leaf Extracts			Stem bark Extracts		
	A	E	M	A	E	M
Alkaloids	+	+	+	+	+	+
Glycosides	+	+	+	-	+	-
Steroids & Terpenoids	+	+	+	+	+	+
Tannins	+	-	+	+	+	+
Reducing Sugars	+	-	+	+	+	+
Anthraquinones	-	+	+	-	-	+
Phlobatannins	-	-	-	-	-	-
Saponins	+	+	-	+	+	+

Key: += Present, - = absent (not detected), A= Aqueous, E= Ethanolic, M= Methanolic

Table 2: Zone Diameter (mm) of Inhibition of the Aqueous Leaf Extracts of *S. spinosa*.

Test Organisms	Concentration of Extracts (mg/ml)			Control
	100	200	400	
S. aureus	5.0 ± 0.7	7.0 ±0.3	9.0 ± 0.7	33.0 ± 0.3
E. coli	8.0 ± 0.3	9.0 ± 0.7	10.0 ± 0.3	39.0 ± 0.5
P. aeruginosa	6.0 ± 0.7	22.0 ± 0.5	24 ± 0.7	35.0 ± 0.3
C. albicans	8.0 ± 0.5	9.0 ± 0.7	9.0 ± 0.3	33.0± 0.5

Table 3: Zone Diameter (mm) of Inhibition of the Ethanol Leaf Extracts of *S. spinosa.*

Test Organisms	**Concentration of Extracts (mg/ml)**			**Control**
	100	**200**	**400**	
S. aureus	7.0 ± 0.7	9.0 ±0.3	9.0 ± 0.7	32.0 ± 0.3
E. coli	8.0 ± 0.3	9.0 ± 0.7	10.0 ± 0.3	39.0 ± 0.5
P. aeruginosa	14.0 ± 0.7	24.0 ± 0.5	33.0 ± 0.7	37.0 ± 0.3
C. albicans	8.0 ± 0.5	10.0 ± 0.7	10.0 ± 0.3	33.0 ± 0.5

Table 4: Zone Diameter (mm) of Inhibition of the Methanol Leaf Extracts of *S. spinosa.*

Test Organisms	Concentration of Extracts (mg/ml)			Control
	100	200	400	
S. aureus	7.0 ± 0.7	9.0 ±0.3	10.0 ± 0.7	33.0 ± 0.3
E. coli	11.0 ± 0.3	19.0 ± 0.7	27.0 ± 0.3	40.0 ± 0.5
P. aeruginosa	12.0 ± 0.7	23.0 ± 0.5	31.0 ± 0.7	34.0 ± 0.3
C. albicans	9.0 ± 0.5	10.0 ± 0.7	10.0 ± 0.3	33.0 ± 0.5

Table 5: Zone Diameter (mm) of Inhibition of the Aqueous Stem bark Extracts of *S. spinosa.*

Test Organisms	Concentration of Extracts (mg/ml)			Control
	100	200	400	
S. aureus	5.0 ± 0.7	7.0 ±0.3	9.0 ± 0.7	33.0 ± 0.3
E. coli	8.0 ± 0.3	19.0 ± 0.7	25.0 ± 0.3	39.0 ± 0.5
P. aeruginosa	13.0 ± 0.7	25.0 ± 0.5	29 ± 0.7	35.0 ± 0.3
C. albicans	8.0 ± 0.5	9.0 ± 0.7	9.0 ± 0.3	32.0± 0.5

Table 6: Zone Diameter (mm) of Inhibition of the Ethanol Stem bark Extracts of *S. spinosa.*

Test Organisms	Concentration of Extracts (mg/ml)			Control
	100	200	400	
S. aureus	7.0 ± 0.7	9.0 ±0.3	9.0 ± 0.7	32.0 ± 0.3
E. coli	8.0 ± 0.3	13.0 ± 0.7	14.0 ± 0.3	39.0 ± 0.5
P. aeruginosa	13.0 ± 0.7	16.0 ± 0.5	18.0 ± 0.7	34.0 ± 0.3
C. albicans	8.0 ± 0.5	10.0 ± 0.7	10.0 ± 0.3	32.0 ± 0.5

Table 7: Zone Diameter (mm) of Inhibition of the Methanol Stem bark Extracts of *S. spinosa.*

Test Organisms	Concentration of Extracts (mg/ml)			Control
	100	200	400	
S. aureus	7.0 ± 0.7	9.0 ±0.3	10.0 ± 0.7	32.0 ± 0.3
E. coli	11.0 ± 0.3	26.0 ± 0.7	35.0 ± 0.3	39.0 ± 0.5
P. aeruginosa	13.0 ± 0.7	16.0 ± 0.5	18.0 ± 0.7	35.0 ± 0.3
C. albicans	9.0 ± 0.5	10.0 ± 0.7	10.0 ± 0.3	33.0 ± 0.5

The results obtained from the assay of the minimum inhibitory concentration (MIC) revealed the susceptibility of organisms, at different degrees, to extracts that showed antimicrobial activity.

Table 8: Minimum Inhibitory Concentration of Aqueous, Ethanolic and Methanolic leaf Extracts of *S. spinosa*

Test Organism	Solvent	MIC Value (mg/ml)
E. coli	A	500
	E	500
	M	125
P. aeruginosa	A	500
	E	125
	M	250

Key: A= Aqueous, E= Ethanol, M= Methanol

Table 9: Minimum Inhibitory Concentration of Aqueous, Ethanolic and Methanolic stem bark Extracts of *S. spinosa*

Test Organism	Solvent	MIC Value (mg/ml)
E. coli	A	250
	E	500
	M	125
P. aeruginosa	A	500
	E	125
	M	500

Key: A= Aqueous, E= Ethanol, M= Methanol

The minimum lethal concentrations of potent extracts were investigated and shown in Table 10. Since *S. aureus* and *C. albicans* both exhibited resistance to the extracts, the MLC investigation, which in this case, is the investigation of the minimum bactericidal concentration (MBC) was carried out on *E. coli* and *P. aeruginosa*.

Table 10: Minimum Bactericidal Concentration of Aqueous, Ethanolic and Methanolic leaf Extracts.

Test Organism	Solvent	MBC Value (mg/ml)
E. coli	A	NT
	E	NT
	M	250
P. aeruginosa	A	NT
	E	250
	M	NT

Key: A= Aqueous, E= Ethanol, M= Methanol, NT= Not Tested

Table 11: Minimum Bactericidal Concentration of Aqueous, Ethanolic and Methanolic stem bark Extracts

Test Organism	Solvent	MBC Value (mg/ml)
E. coli	A	500
	E	NT
	M	500
P. aeruginosa	A	NT
	E	250
	M	NT

Key: A= Aqueous, E= Ethanol, M= Methanol, NT= Not Tested

DISCUSSION

The qualitative analysis of phytochemicals in *S. spinosa* revealed the presence of alkaloids, steroids and terpenoids, tannins, reducing sugars and saponins in the experimented plant parts. This is in consonance with the findings of Kubmarawa *et al.,* 2007 and Nwozo *et al.,* 2010. However, the detection of glycosides (in leaf) and absence of phlobatannins in the plant parts used in this study is in contrast with the work of Kubmarawa *et al.,* 2007 and Nwozo *et al.,* 2010. These differences may be due to the difference in geographical locations and environmental conditions of the places where the plant was obtained or the use of different levels of extract concentrations Kubmarawa *et al.,* 2007.

The result showed that *S. spinosa* was mildly effective against *Staphylococcus aureus* and *Candida albicans* as both *S. aureus* and *C. albicans* were inhibited, to some extent, by the extracts. The activity of the plant against *C. albicans* is in consonance with the findings of Nwozo *et al.,* (2010) who reported that *S. spinosa* was active against *C. albicans* at concentrations between 20mg/ml and 50 mg/ml. However, the extracts showed marked activities against both *E. coli* and *P. aeruginosa.* The methanol stem bark extract and ethanol leaf extract appeared to be very effective against *E. coli* (Table 7) and *P. aeruginosa* respectively (Table 3). This is in agreement with the report of Verpoorte *et al.,* (1983) and McGaw *et al.,* (2000). However, the result is at variance with that of significant activity of the extracts of *S. spinosa* against *S. aureus*.

The MIC results revealed that methanol extracts of the plant were the most potent against *E. coli* of all the extracts as both extracts had an MIC of 125 mg/ml (Tables 8 and 9). Also, the ethanol extracts of both leaf and stem bark appeared to be the most effective against *P. aeruginosa,* having an MIC value of 125 mg/ml in both cases (Tables 8 and 9). The MBC assay revealed that most of the extracts

were rather more bacteriostatic than bactericidal at the tested concentrations. However, some extracts gave bactericidal actions against the test organisms. Statistically, it was revealed that for most of the plant extracts, there was generally no significant difference ($P \leq 0.05$) in their activities on the test organisms. Some extracts, however, showed significant differences ($P \leq 0.05$).

CONCLUSION

The results of this research have shown the antibacterial properties of *Strychnos spinosa* against certain microorganisms owing to the bioactive substances it possesses. This emphasizes the usefulness of the leaves and stem bark of the plant in the treatment of certain bacterial diseases in the traditional medicine practice and the need to harness this potential in the development of new antibiotics especially with the problem of development of resistance to known antibiotics by bacteria.

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