WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 16, 1544-1557. Research Article ISSN 2277– 7105

ANALYSIS OF ANTIBACTERIAL ACTIVITY AND WATER CLARIFICATION USING SELECTED SEED EXTRACTS

J. Renuka[1]* and J. Karunyal[2]

[1]Assistant Professor, Department of Biochemistry, The American College, Madurai, Tamilnadu, India.

[2]Associate Professor & Dean (Retd), Department of Botany, The American College, Madurai Tamilnadu, India.

Article Received on
15 July 2018,

Revised on 05 August 2018,
Accepted on 26 August 2018,

DOI: 10.20959/wjpr201816-13287

***Corresponding Author**
J. Renuka
Assistant Professor, Department of Biochemistry, The American College, Madurai, Tamilnadu, India.

ABSTRACT

Drinking water is currently a scarce world resource, the processing of which requires complex treatment that include clarification of suspended particles and disinfection. Plant extracts have been proposed as an environment friendly alternative due to their traditional use for the clarification and purification of drinking water. In the present investigation, various locally available seeds were screened for the clarification of water and its antibacterial activity at different pH range from 1, 5, 8 & 12 and at various temperatures 4^0C, 37^0C, 50^0C, 100^0C, 121^0C in order to find the stability of seed extracts. The study included 12 plant species, of that 7 of them exhibited antibacterial activity *namely Eugenia jambolana, Moringa loeifera, Murraya koenjii, Peltophorum ferrugineum, Phyllanthus emblica, Terminalia chebula and Vitis vinifera. Moringa oleifera* showed highest antibacterial activity than other seed extracts. The pH of seed extract of *Moringa oleifera* was found to be neutral showed antibacterial activity at altered pH by 12. Extracts of *Eugenia jambolana, Phyllanthus emblica, Peltophorum ferrugineum* exhibited antibacterial activity only at their natural pH. Seed extract of *Moringa oleifera* is thermoresistant and remained active after 5 hours treatment at 95.C.whereas other seeds doesn't exhibited antibacterial activity at altered temperatures. Drastic decrease in turbidity was observed for *Moringa oleifera* within a day, seeds of *Peltophorum ferrugineum* and *Phyllanthus emblica* took a week for clarification. Microbial quality of water after clarification was analysed, seed powder of *Moringa oleifera* showed lesser number of bacteria (120 colonies) compared to that control (300 colonies). Coagulation was observed in

the seed extracts of *Moringa oleifera* and *Peltophorum ferrugineum* subjected to 121^0C due to presence of coagulant proteins. Seeds of *Moringa oleifera, Peltophorum ferrugineum* and *phyllanthus emblica* as a potential water clarifying agent which could replace the harmful chemical coagulants.

KEYWORDS: Moringa oleifera, Peltophorum ferrugineum, Antibacterial activity, Coagulation.

I. INTRODUCTION

Water is becoming increasingly important as a natural resource of our planet. As drinking water is one of the basic needs and as prerequisite for life, its quantity is of utmost importance. Inadequate sanitation and water services to the urban poor population are among the most serious challenges gracing the developing world. About one million people lack safe drinking water and more than six million people (2million children) die from diarrhea every year (*Post note, 2002*). As stipulated in the millennium development goals (MDGs), provision of safe drinking water to the poor is one of the priorities in the agenda. The acuteness is such that during 2025 there may be a water emergency era where less than 1000 cu.m of precipitation is considered critical for human survival. Poor water quality is a key cause of poor livelihood and health. Water treatment usually comprises water clarification and disinfection. In developing countries treatment plans are expensive, the ability to pay for services is minimal and the availability of skills as well as technology is scarce. The cost involved in achieving the desired level treatment depends among other things on the cost and availability of water treatment materials. In most cases the commonly used water treatment chemicals are expensive and they have to be imported in hard currency. In developed countries, salts of aluminium and other metals are often used despite the concern that they induce Alzheimer's and other carcinogenic effects (Martyn *et.al.*,1989). Natural polyelectrolytes of plant origin have been used for many centuries in developing countries for clarifying turbid water (Schulz & Okum, 1984) most seeds contain other polysaccharides and have some industrial utility. The seeds of the tropical tree *Moringa oleifera* have been traditionally used for the clarification of drinking water in rural areas of sudan and Malawi (Okuda et al., 2001). *Moringa oleifera* seeds possess effective coagulation properties and they are not toxic to human beings and animals. Various other seeds of *Cassia angustifolia, Strychnous potatorum* were also used. In addition to this the seed extracts are known to exhibit anti- bacterial activity (Olsen *et al*., 2002). The present study aims at the clarification

of water using selected seeds in the form of pulverized powder and extracts. Attempts will also be taken to analyze the purity of water in terms of microbial load.

II. MATERIAL AND METHODOLOGY

The 12 plant seed coagulants which are used in this study are *Eugenia jambolana, Moringa loeifera, Murraya koenjii, Peltophorum ferrugineum, Phyllanthus emblica, Terminalia chebula, Vitis vinifera, Pyrus malus, Myristica fragrans, Tamarindus indica, Annona squamosa, Citrus urantium*. The natural coagulants are collected from the local areas of in and around Madurai, Tamilnadu, India

II. A. PREPARATION OF NATURAL COAGULANTS

After collection, pulp material was removed, seed along with seed coat were collected and they were washed thoroughly and dried over sunlight for few days to kill microorganisms. Then they were pulverized and stored at room temperature in air tight containers. Known quantity of powdered material was extracted with water and centrifuged before performing antibacterial assay.

II. B. ANALYSIS OF ANTIBACTERIAL ACTIVITY OF SEED EXTRACT BY DISC DIFFUSION METHOD

Antibacterial activity of selected seed extracts was examined following Kirby Bauer method (Kirby & Bauer, 1966).

Nutrient agar plates were prepared. A swab of the test culture *(Escherichia coli, Bacillus subtilis, Klebsiell sp, Staphylococcus sp*) was taken and inoculated on the surface of the agar plate completely so as to make a lawn. The agar surface was allowed to dry for 5 minutes before aseptically placing the discs. The whatmann No – 1 filter paper discs which were impregnated with known quantity of seed extract (10ml) from stock of (10gmper 10ml) was carefully taken and placed over the agar plate at least 15mm from the edge of the plate. The disc was gently pressed to give a better contact with the agar. Disc impregnated with sterile distilled water were maintained as control. The plates were incubated in an inverted position for 16 – 18 hrs at room temperature. The diameter of inhibitory zone was measured using a measuring scale.

Seed extracts used for extract included Antibacterial Assay

- Eugenia jambolana

- Moring oleifera
- Peltophorum ferrugineum
- Phyallnathus emblica

(a) Extract included Antibacterial Assay using solid medium

The pulverized seed at a concentration of 1% (W/V) was included in the nutrient agar medium before and after sterilization. Due to the uneven distribution of the powdered seed material in nutrient plated. Aqueous extract of the above mentioned seeds were prepared and included in the medium (10gm/10ml) for the further study. The discs loaded with known quantity of cultures were placed over the nutrient agar plates with (1%) and without (control) the seed extracts. The plates were incubated for 24hrs at room temperature. The diameter of bacterial growth zone was measured.

(b) Extract included Antibacterial Assay using Liquid medium

Nutrient broth was used for this experiment. Seed extract (2%) was included in the sterile nutrient broth. Bacterial cultures in mid – log phase (20µl) were inoculated into the nutrient broth without the seed extract served as control. After 48 hrs of incubation, growth in terms of turbidity at 540nm was measured using colorimeter.

II. C. EFFECT OF PH ON ANTIBACTERIAL ACTIVITY OF SELECTED SEED EXTRACTS

Following seeds were selected to determine the effects of pH on antibacterial activity.

- Eugenia jambolana
- Moring oleifera
- Peltophorum ferrugineum
- Phyallnathus emblica

The pH of seed extracts was measured using pH paper and recorded. The pH of the extracts was altered using 0.1N NaOH and 0.1N HCl and the pH of the seed extract used in this investigation was 1, 5, 8 and 12. Seed extracts showing various pH 1, 5, 7(natural pH), 8 and 12 are loaded in different filter paper discs and placed over the inoculated Nutrient agar plated. The plated were incubated at room temperature for 24 hrs. The diameter of inhibitory zone was measured.

II.D.EFFECT OF TEMPERATURE ON ANTI BACTERIAL ACTIVITY OF SELECTED SEED EXTRACTS

The given seeds were selected to determine the effect of temperature on antibacterial activity

- Eugenia jambolana
- Moringa oleifera
- Peltophorum ferrugineum
- Phyallnathus emblica

Aqueous seed extract were exposed to various temperatures such as 4^0C, 37^0C, 50^0C, 100^0C, 121^0C for 30 minutes. The seed extracts subjected to various temperatures were loaded on the different filter paper discs and placed over the inoculated plates. The plates were incubated at room temperature for 24 hrs. The diameter of inhibitory zone formed around the disc was measured.

II. E. ESTIMATION OF CLARITY OF WATER

Turbid water was collected from our college campus soon after the rain. 100ml of turbid water were dispensed in series of transparent disposable containers. Following pulverized seeds were used for water clarification, *Citrus aurantium, Eugenia jambolana, Moringa oleifera, Peltophorum ferrugineum, Phyllanthus emblica, Tamarindus indica, Terminalia chebula.* Each of the pulverized seed (100mg) was added to the container and vortex thoroughly. Then allowed to settle for 24 hrs, turbidity was measured at 540 nm using colorimeter. Turbid water without pulverized seed served as control.

II. F. MICROBIAL QUALITY OF WATER

After clarification using seed extract, the clarified water was serially diluted and used for enumeration of bacteria. Serial dilution were made for each sample, 0.1ml of 10-3 dilution were taken and inoculated on Nutrient agar plates and spread using 'L' rod. The plates wee incubated and numbers of colonies were counted for the analysis of bacterial count.

III. RESULTS AND DISCUSSION

Analysis of antibacterial activity of seed extract by disc diffusion method

Of the seed extracts tested, seven of them exhibited antibacterial activity namely *Eugenia jambolana, Moringa oleifera, Murraya koenjii, Peltophorum ferrugineum, Phyllanthus emblica, Terminalia chebula and Vitis vinifera. Moringa oleifera* showed highest antibacterial activity than other seed extracts. (Table:1). The order of inhibitory efficiency of

the tested seed extracts *Moringa oleifera* - Terminalia *chebula* - *Peltophorum ferrugineum* - *Eugenia jambolana* - *Vitis vinifera.* - *Murraya koenjii*- *Phyllanthus* emblica (Plate 1.1 – 1.7) showed inhibitory zone in decreasing order respectively. 5 of them showed no antibacterial activity namely *Annona squaamosa, Citrus aurantirum, Myristica fragrans, Pyrus malus and Tamarindus indica.*

Table 1: Analysis of antibacterial activity of seed extracts by disc diffusion method.

S. No	Botanical Name	Diameter of inhibitory zone (mm)			
		E.coli	*Bacillus sp*	*Klebsiella sp.*	*S.aureus*
1.	*Annona squamosa*	-	-	-	-
2.	*Citrus auarantium*	-	-	-	-
3.	*Eugenia jambolana*	13	11	10	14
4.	*Moringa oleifera*	25	25	26	25
5.	*Murraya koeniyii*	9	11	10	7
6.	*Myristica fragrans*	-	-	-	-
7.	*Peltophorum ferrugineum*	11	17	15	14
8.	*Phyllanthus emblica*	11	11	11	8
9.	*Pyrus malus*	-	-	-	-
10.	*Tamarindus indica*	-	-	-	-
11.	*Terminalia chebula*	18	17	19	14
12.	*Vitis vinifera*	9	13	7	8

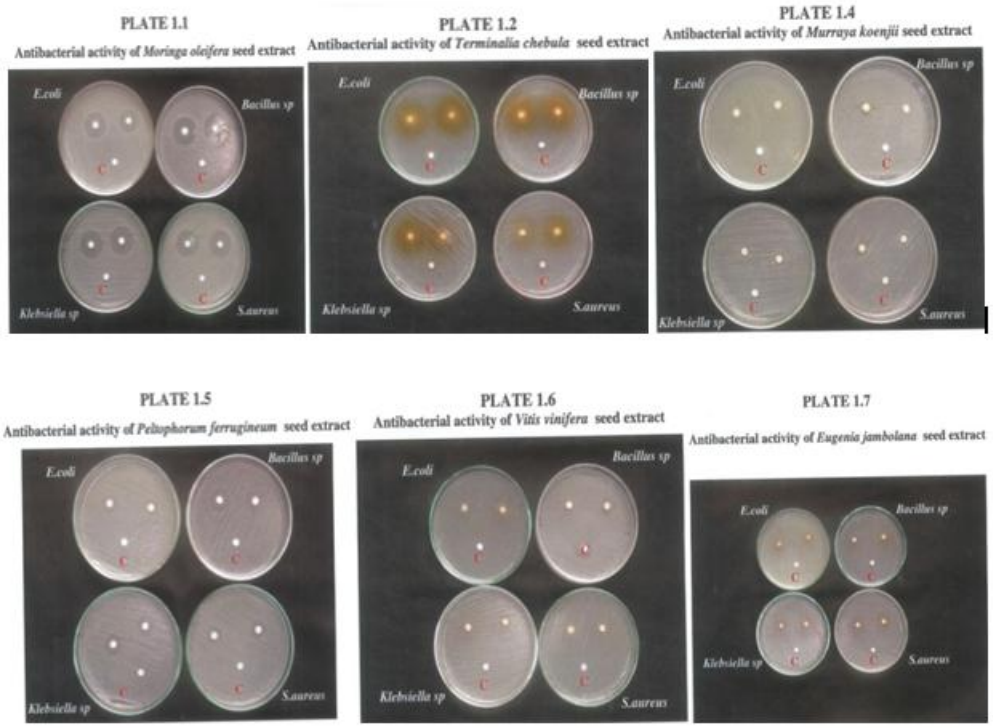
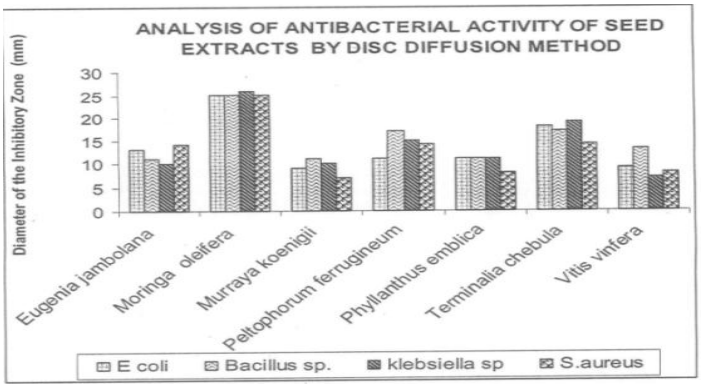
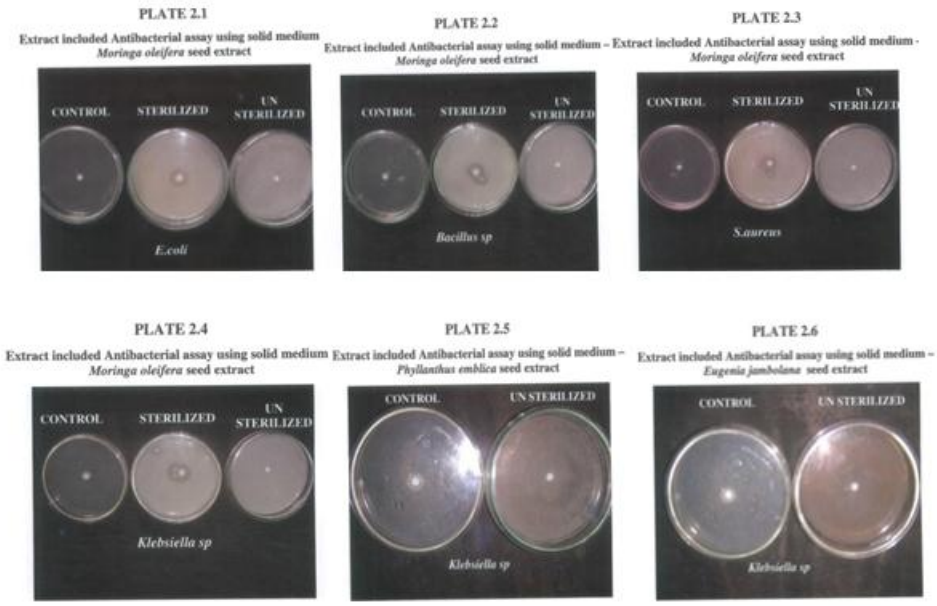


Fig. 1:



(a) Extract included Antibacterial Assay using solid medium

Sterilized and unsterilized seed extracts were included in the agar medium and plated respectively. After 24 hrs of incubation sterilized *M.oleifera* seed extract produced a clear zone of inhibition (plate - 2.1-2.4) where as plates containing seed extracts of *Eugenia jambolana* (Plate 2.5) and *Phyllanthus emblica* (plate 2.6) growth of bacteria was inhibited as compared to the control measured by diameter of the zone of inhibition. The plates without the seed extract served as control.



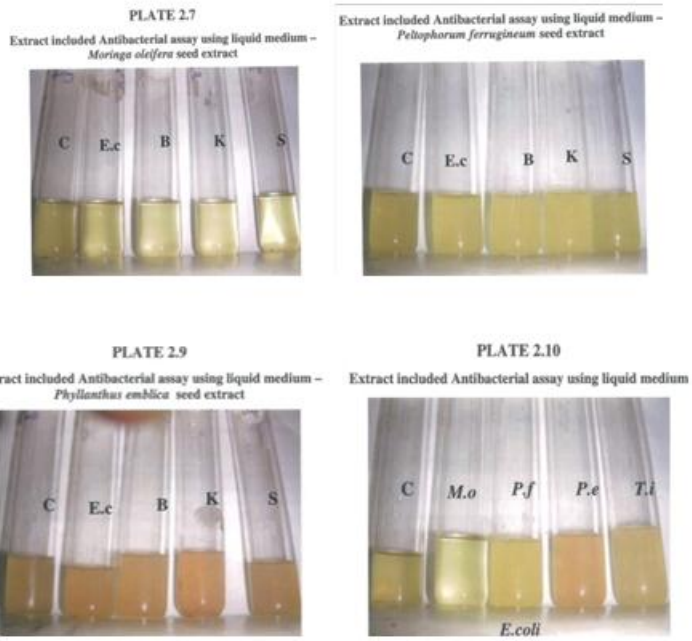
(b) Extract included Antibacterial Assay using Liquid medium

When target bacteria were inoculated in liquid medium containing seed extracts of *Moringa oleifera* (Plate 2.7, Table 3) *Peltophorum ferrugineum* (Plate 2.8) at a concentration allowed to grow growth in terms of optical density was lower than that of the control (Plate :2.10)

But the liquid medium containing *Phyllanthus emblica* (Plate 2.9) showed higher optical density.

Table 2: Extract included antibacterial assay using liquid medium.

Name of the Seed Extract	Turbidity at 540nm			
	E.coli	Bacillus sps	Klebsiella sps	S.aureus
Moringa oleifera	0.15	0.01	0.06	0.03
Peltophorum ferrugineum	0.06	0.22	0.21	0.63
Phyllanthus emblica	0.00	0.13	0.08	0.15
Tamarinduus indica	0.20	0.29	0.08	0.26
Control	0.09	0.17	0.26	0.09



II. C. EFFECT OF pH ON ANTIBACTERIAL ACTIVITY OF SELECTED SEED EXTRACTS

The pH of seed extract of *Moringa oleifera* was found to be neutral. Extract of *Moringa oleifera* (Plate: 3.2, Table 4) showed antibacterial activity even after the pH is altered. But the antibacterial activity was decreased in terms of the diameter of the inhibition zone formed around the disc, when the pH was raised to 12. Extracts of *Eugenia jambolana* (Table 3), *Phyllanthus emblica* (Table 6) *and Peltophorum ferrugineum* (Plate 3.1, Table 5) at natural pH exhibited antibacterial activity.

Table 3: Effect of pH on antibacterial activity of *Eugenia jambolana.*

Test Organism	Diameter of the inhibitory zone of seed extracts showing various pH(mm)				
	1	5	7	8	12
E.coli	17	-	13	-	-
Bacillus sps	9	-	11	-	-
Klebsiella sps	16	-	10	-	-
S.aureus	16	-	15	-	-

Table 4: Effect of pH on antibacterial activity of *Moringa oleifera.*

Test Organism	Diameter of the inhibitory zone of seed extracts showing various pH(mm)				
	1	5	7	8	12
E.coli	25	28	25	21	19
Bacillus sps	20	20	25	20	12
Klebsiella sps	20	21	26	20	12
S.aureus	21	22	25	14	13

Table 5: Effect of pH on antibacterial activity of *Peltophorum ferrugineum.*

Test Organism	Diameter of the inhibitory zone of seed extracts showing various pH(mm)				
	1	5	7	8	12
E.coli	9	-	11	7	-
Bacillus sps	7	-	17	7	-
Klebsiella sps	9	-	15	-	-
S.aureus	9	-	14	7	-

Table 6: Effect of pH on antibacterial activity of *Phyllanthus emblica.*

Test Organism	Diameter of the inhibitory zone of seed extracts showing various pH (mm)				
	1	5	7	8	12
E.coli	11	-	11	-	-
Bacillus sps	9	-	11	-	-
Klebsiella sps	9	-	11	-	-
S.aureus	10	-	8	-	-

PLATE 3.1

Effect of pH on antibacterial activity of *Moringa oleifera* seed extract

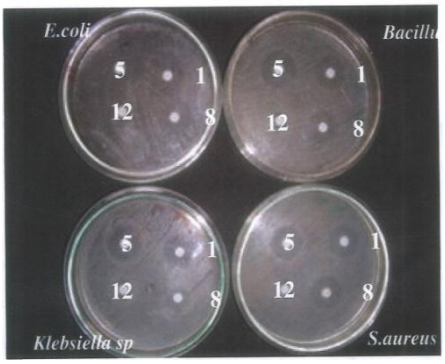
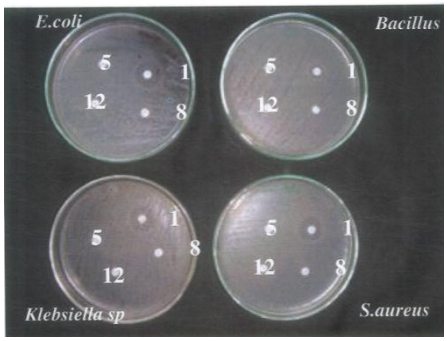


PLATE 3.2

Effect of pH on antibacterial activity of *Peltophorum ferrugineum* seed extract



II. D. EFFECT OF TEMPERATURE ON ANTI BACTERIAL ACTIVITY OF SELECTED SEED EXTRACTS

Seed extract of *Moringa oleifera* (Plate 4.1, Table 8), *Eugenia jambolana* (Plate 4.2, Table - 7) showed les antibacterial activity in terms of the diameter zone. When the temperature of the extract is changed the antibacterial activity is completely lost at 50^0C, 100^0C and 121^0C in *Peltophorum ferrugineum* (Table 9).

Table 7: Effect of temperature on antibacterial activity of *Eugenia jambolana.*

Test Organism	Diameter of the inhibitory zone of seed extracts showing various temperature in (mm)				
	4^0C	37^0C	50^0C	100^0C	121^0C
E.coli	7	13	7	9	8
Bacillus sps	7	11	-	9	7
Klebsiella sps	7	10	7	8	7
S.aureus	7	14	7	9	7

Table 8: Effect of temperature on antibacterial activity *of Moringa oleifera.*

Test Organism	Diameter of the inhibitory zone of seed extracts showing various temperature in (mm)				
	4^0C	37^0C	50^0C	100^0C	121^0C
E.coli	19	25	21	10	9
Bacillus sps	20	25	19	18	10
Klebsiella sps	17	26	19	14	12
S.aureus	23	25	21	22	16

Table 9: Effect of temperature on antibacterial activity *of Peltophorum ferrugineum.*

Test Organism	Diameter of the inhibitory zone of seed extracts showing various temperature in (mm)				
	4^0C	37^0C	50^0C	100^0C	121^0C
E.coli	9	11	-	-	-
Bacillus sps	8	17	-	-	-
Klebsiella sps	7	15	-	-	-
S.aureus	8	14	-	-	-

PLATE 4.1

Effect of temperature on antibacterial activity of *Moringa oleifera* seed extract

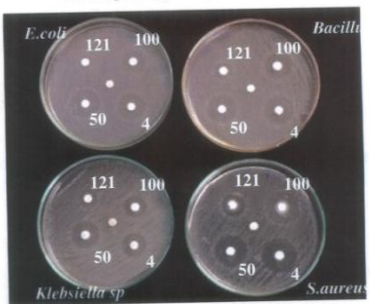


FIG.4

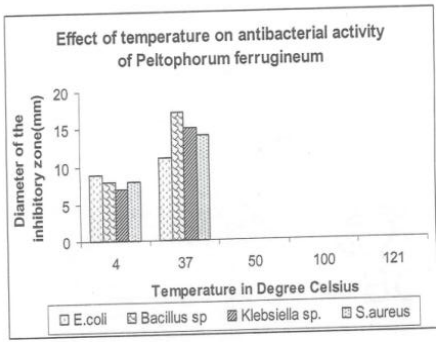
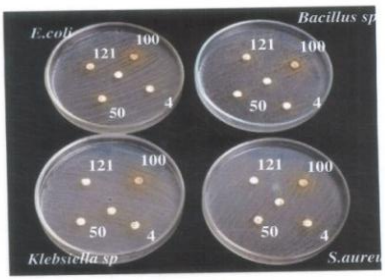


PLATE 4.2

Effect of temperature on antibacterial activity of *Eugenia jambolana* seed extract



II. E. ESTIMATION OF CLARITY OF WATER

Clarity of water was examined by using turbid water. When the seed extract was included the turbidity showed a drastic decrease in case of *M.oleifera* (Photo 5.1) within a day. Turbid water treated with *Peltophorum ferrugineum* and *Phyllanthus emblica* (Photo 5.2) took a week for clarification.

Table 10: Estimation of clarity of water after the addition of pulverized seeds.

Name of pulverized seed	Turbidity of water O.D at 540nm
Control	0.11
Moringa oleifera	0.01
Myristica fragrans	0.06
Peltophorum ferrugineum	0.01
Phyllanthus emblica	0.04
Tamarindus indica	0.23

PHOTO 5.2

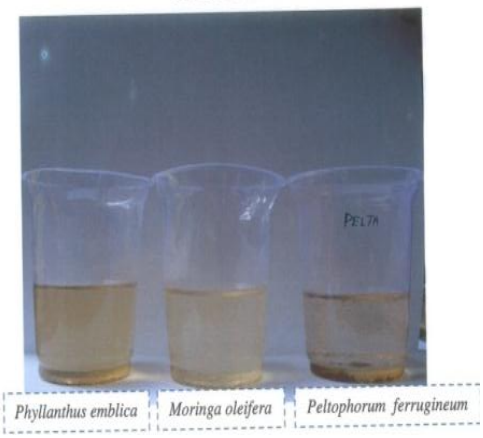
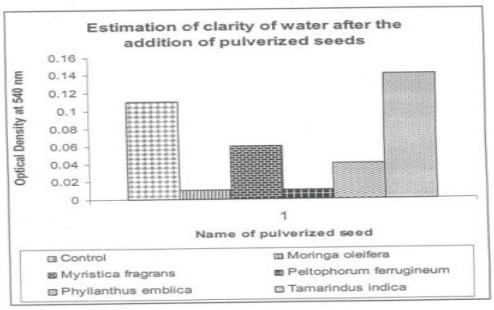


PHOTO 5.1

Estimation of clarity of water after the addition of pulverizised *Moringa oleifera* seeds

FIG.5



II. F. MICROBIAL QUALITY OF WATER

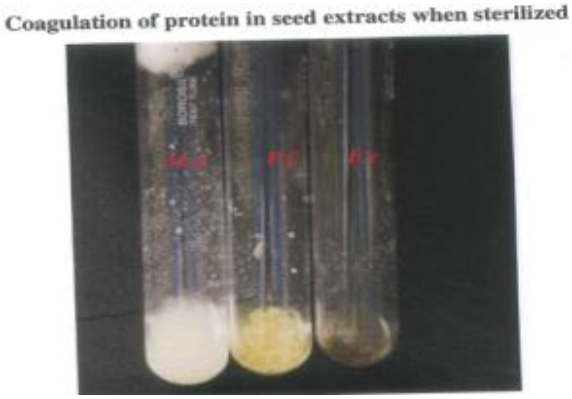
After clarification using seed powder the clarified water samples were collected and the bacteria were enumerated. Clarified water was serially diluted to 10^{-3}. Water treated with *Moringa oleifera* seed powder showed less number of bacteria (120 colonies) (Plate 6) as compared to the control (above 300 colonies).

PLATE - 6

MICROBIAL QUALITY OF WATER

OBSERVATION OF COAGULANT PROTEIN

When the seed extracts were subjected to sterilization (121^0C at 15 psi for 20 min) coagulation was observed only in *M. oleifera and Peltophorum ferrugineum* (Plate 7). This may be due to the presence of coagulant proteins and clarification of water may be due to the settlement of particles present in the turbid water because of such proteins.



III. CONCLUSION

In the present investigation, analysis of antibacterial activity of selected seed extract was carried out. Both the seed extract and seed powders were used for the clarification of water. Before examining the clarification and quality of water, twelve seeds were screened for their antibacterial activity against the pathogenic bacteria such as *E.coli, Bacillus sp, Klebsiella sp., S.aureus*. Of the tested seed extract, seven of these showed antibacterial activity and inhibitory efficiency would be given as *M. oleifera > T.chebula> P.ferrugineum> E. jambolana> Vitis vinifera>M.koenijii>Phyllanthus emblica.* In order to find the stability of the seed extracts, to various pH and temperature treatment, pH and temperature of the seed extract were altered and when this extracts used for antibacterial analysis, the extracts showed antibacterial only at their natural pH and temperature except in the case of *M.oleifera,* antibacterial activity was observed in all pH and temperature tested. When the seed powder and the extracts were used for the clarification of water, *Moringa oleifera, Peltophorum ferrugineum, Phyllanthus emblica* possess high clarification property and increase the quality of water than other seed powders. It could be concluded that seeds of *Moringa oleifera Lam, Peltophorum ferrugineum and Phyllanthus emblica* as a potential water clarifying agent which could replace the harmful chemical coagulants.

REFERENCE

1. Agarwal H, Shee. C, Sharma. A Isolation of a 66kDa protein with coagulation activity from seeds of *Moringa oleifera*, Journal of Agriculture and Biological sciences, 2007; 3(5): 418 -421.
2. Bhatai S, Othman Z & Latif Ahmad – A Pretreatment of palm oil Mill effluent using *Moringa oleifera* seeds as natural coagulant, Journal of Hazardous materials, 2006; 145(2): 120 – 126.
3. Crapper, Dr. Krishnan SS and Dalton, AJ Brain aluminium distribution in Alzheimer's disease and experimental Neurofibillary degeneration, Science 1973; 180(4085): 511– 513.
4. Folkard G.K., Sutherland J.P., & Shaw: R. Water clarification using *Moringa oleifera* seed coagulant. In; Shaw, R.(ed), Running Water. Intermediate Technology Publications, London, ISBN 1999; 109 – 112.
5. Ghebremichael KA, Gunaratnak R, Henriksson H, A simple purification and activity assay of the coagulant protein from *Moringa oleifera* seed. Water Research, 39(11): 2338 – 44.
6. Kirby U, Bauer A, Sherris, Turch M, Antibiotic susceptibility testing by a standardized single disk method, American Journal of clinical pathology 1996; 45: 493.
7. Miller R.G., Kopfler F.C The occurrence of aluminium in drinking water, JAWWA, 1984; 76: 84 – 91.
8. Ndabigengessere A, Narasiah KS, Active agents and mechanism of coagulation of turbid waters using *Moringa oleifera*, Water Research, 1995; 29: 703 -710.
9. Postnote (2002) Access to water in developing countries No. 178. www.parliament, UK/Past/Pn178.pdf.
10. WHO Guidelines for Drinking Water quality Surveillance and control of community supplier 3(2) World Health Organization, Geneva, 1997.