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ISOLATION AND IDENTIFICATION OF MICRO ORGANISM FROM POND WATER AND ITS ANTIBACTERIAL EFFICACY AGAINST MORINGA OLIFERA

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Abstract:

The stagnant water reservoirs in urban area of India are severely contaminated for various purposes i.e. bathing, drinking and washing for humans and other animals. The contaminants due to surfactants, microbes, animals, nutrients, organic toxicants, etc. Some which can cause waterborne illness. Moringa olifera seeds extract was more effective than traditional antibiotics to combat the human pathogenic bacteria studied responsible for severe illness .The aim of the present study to isolate and identify the bacteria from pond water collected from Chidambaram , Annamalai Nagar .The microbial populations of the collected water sample were analyzed using standard plate count method .Based on the morphological and biochemical characterization the isolates were confirmed as Salmonella sp ,Pseudomonas sp ,Staphylococcus sp ,Escherichia coli ,Klebsiella sp ,Bacillus sp and Vibrio sp. The Moringa oleifera seed extracts were prepared using methanol, ethanol, acetone and aqueous extracts. The different crude extracts of Moringa oleifera were tested against all the isolated strains .Among the different extracts tested ,aqueous exhibited maximum zone of inhibition against Staphylococcus sp (27±0.2mm) followed by Salmonella sp (25±0.5) and Vibrio (25±0.1)

KEYWORDS: *Pond Water, Microorganism, Solvents, Moringa olifera seed, Crude extracts.*

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INTRODUCTION:

Moringa oleifera is native to the western and sub-Himalayan region, India, Pakistan, Asia minor, Africa and Arabia [1]. The *Moringa* tree is cultivated and used as a vegetable (leaves, pods flowers, roasted seeds), for spice (mainly roots), cooking and cosmetics oil (seeds) and as a medicinal plant (all plant organs) [2]. Important medicinal properties of the plant include antipyretic, antiepileptic, anti-inflammatory, anti-ulcerative [3] antihypertensive [4], cholesterol lowering [5], antioxidant [6], antidiabetic, hepatoprotective [7], antibacterial and antifungal activities [6]. In addition, *Moringa oleifera* seed possesses water purifying powers [8, 9]. They are known to be anti-helminthic, antibiotic, detoxifiers, immune builders and have been used to treat malaria [10] and it can also be used as a less expensive bio-absorbent for the removal of heavy metals [11]. *Moringa oleifera* is a highly valued plant, distributed in many countries of the tropics and subtropics. It has impressive range of medicinal uses with high nutritional value. Different parts of this plan contain a profile of important minerals, and a good source of protein, vitamin, â carotene, amino acids and various phenolics [12]. The *Moringa* plant provides a rich and rare combination of zeatin, quercetin, kaempferol and many other phytochemicals. However, a very important step in the screening of the sanitizing and preservative activity of a plant material is to evaluate its antimicrobial properties. It is important to evaluate the antimicrobial properties of *Moringa oleifera* leaves on some selected microorganisms.

Turbidity and color removal is one of the important steps in a water treatment process, which is generally achieved using coagulants. Many coagulants are widely used in conventional water treatment processes, based on their chemical characteristics. These coagulants are classified into inorganic, synthetic organic polymers and natural coagulants. The two most commonly used primary coagulants are aluminum and iron (III) salts (Okuda *et al*. 1999). However, recent studies have pointed out several drawbacks of using aluminum salts, such as Alzheimer's disease associated with residual aluminum in treated water and production and Narassiah 1998). There is also the problem of reaction of aluminum with natural alkalinity present in the water leading to a reduction of pH and low efficiency of coagulation in cold waters. To ease the problems associated with chemical coagulants, several studies have pointed out the introduction of natural coagulants produced or extracted from microorganisms, animals, or plants (Katayon *et al*. 2006). Wastewater treatment methods include precipitation, coagulation/flotation, sedimentation, filtration, membrane process, electrochemical techniques, ion exchange, biological process, and chemical reactions. Each method has its own merits and limitations in applications because of their cost. Presently, there is an increasing trend to evaluate some indigenous cheaper materials for the removal of these pollutants and pesticides from aqueous solutions. A large number of cheaper materials including industrial and agricultural wastes have been used to remove different pollutants from the industrial effluents for their safe disposal into the biosphere (Akhtar *et al*. 2009).

In terms of water treatment applications, MO seeds in diverse extracted and purified forms have proved to be effective in removing suspended material. MO extracts generate lower sludge volumes in comparison with aluminum, soften hard waters, and act as effective absorber of cadmium. If a physicochemical treatment applied during the first stage of the wastewater treatment is effective, then the organic load on any subsequent biological treatment phase will be considerably reduced (Bhuptawat *et al*. 2007).

The major concern in the use of seed extracts for water treatment applications is the residual organic seed powder that is dispersed in the finished water. MO is organic and biodegradable. If MO is proven to be active, safe, and inexpensive, it is possible to use it widely for drinking water and wastewater treatment. MO may become one of the cash products bringing more economic benefits for the producing countries (Okuda *et al*. 1999).

MATERIALS AND METHODS:

Sample collection

Water sample from a pond located at Annamalai University, Chidambaram, Tamil Nadu was collected. The samples for microbiological analysis and Physico-chemical analysis were collected in non-reactive borosilicate glass bottles of 500 ml capacity that had been cleansed and carefully given a final rinse with distilled water and sterilized. Water sample was taken from the pond by holding the bottle near its base in the hand and plunging it neck downward below the surface. Microbiological analysis and Physico-chemical activity of water sample was started as soon as possible after collection to avoid unpredictable changes in the microbial population (Gaudy, 1998).

Fig : Pond water

Physicochemical analysis of sample

Different parameters like odor, color, P^H, temperature, taste, total hardness, Turbidity, Dissolved Oxygen (DO), total dissolved solids (TDS), alkalinity were analyzed using standard procedures (APHA,1998).

Enumeration and isolation of bacteria

The collected pond water sample was serially diluted from 10^{-1} to 10^{-7}. Nutrient agar plates were prepared and 0.1 ml of samples were inoculated with different dilutions ranges from 10^{-4} to 10^{-7}. An uninoculated plate was set as control. The Plates were kept on incubator at 37° C for 24 to 48 hours. After incubation, the colonies were counted using colony counter and colony forming unit. Morphologically distinct colonies were picked and pure cultures were made by streak plate method. After repeating streak plate method several times, the isolated colonies were kept as slants for further identification and studies.

Biochemical characterization of isolates

The isolated pure cultures from pond water were further studied for biochemical characterization and identification.

Preparation of *Moringa oleifera* seed powder and Antibacterial activity test

Dry *Moringa oleifera* seeds were obtained from Government Agro agency, Nagapattinam, Tamil Nadu. Mature seeds showing no signs of discoloration, softening or extreme desiccation were used (John, 1988). The seed kernels were ground to a fine powder of approximate size 600 µm to achieve solubilization of active ingredients of the seed.

Fig 2: Moringa olifera seed powder

Preparation of *Moringa oleifera* seed crude extracts

10 g of *Moringa oleifera* seed powder was soaked in 100 ml of methanol, ethanol and acetone each in separate conical flasks, plugged with cotton and kept at room temperature for 3 days and filtered through Whatman No:1 filter paper. The filtrate was evaporated in petri dish at room temperature for 2-3 days till the volume was reduced to one-fourth of the original volume of the solvent prepared and stored at 4°C in air tight bottles (Horbone JB, 1973).

Preparation of aqueous extract

10 grams of powdered *Moringa oleifera* seed sample was dissolved in 100ml of distilled water and boiled for 2 hours. The residue was removed by filtering through Whatman N0:1 filter paper. The filtrates were further boiled till the volume was reduced to one fourth of original volume and stored at 4º C in air tight bottles (Harborne 1973).

Antibacterial activity assay by Agar well diffusion method

The isolated bacterial cultures from pond water were grown overnight in Nutrient Broth and were uniformly swabbed on the surface of the MHA plates using sterile cotton swabs. Four wells of 6mm size were made with sterile cork borer on the seeded plates. Around 100µl of different volumes of ethanolic, methanolic and acetone extracts of *Moringa oleifera* seed were added to each well aseptically. The plates were incubated without inverting for 24-48 hours at 37°C and the zone of inhibition was noted and recorded.

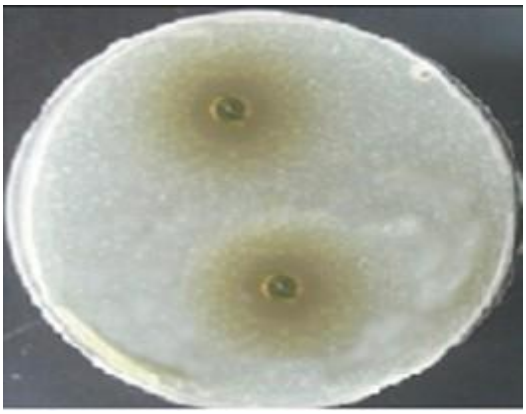

Fig 3: Antibacterial activity of aqueous extract of Moringa olifera against *Staphylococcus sp*

RESULTS:

Water sample from a pond located at Annamalai University, Chidambaram, Tamil Nadu was collected for the analysis and bacterial isolation. The physicochemical parameters were checked and noted.

Physicochemical analysis

Results of various physicochemical characteristics of pond water are given in table. 1

Table 1. Physicochemical characteristics of pond water

S. No	Parameters	Unit	Results	Standard
1	Odor	-	Odorless	-
2	Color	-	Light pale yellow	-
3	PH	-	7	6.5-8.5 (ISI acceptable limit)
4	Turbidity	NTU	0.26	5 (ISI acceptable limit)
5	TDS	Mg/Lt	3.00	500(ISI acceptable limit)
6	DO	Mg/Lt	9.2	4(ISI Totaante limit)
7	COD	Mg/Lt	0.32	10
8	BOD	Mg/Lt	3	5
9	Total alkalinity	Mg/Lt	18	200(ISI Desirable limit)
10	Chlorides	Mg/Lt	1171	250 (ISI Desirable limit)

Enumeration and Isolation of bacteria

Standard plate count

The bacterial colonies were isolated by the standard plate count method using colony counter. Bacterial colonies isolated from pond water using standard plate count method in the form of CFU/ml are shown in table. 2.

Table 2. Enumeration of bacterial colonies from Pond water sample using plate count method

Dilution	CFU/ml
10^{-4}	46+104
10^{-5}	35+99
10^{-6}	24+86
10^{-7}	19+80

Identification and characterization of bacteria

Water sample from pond water was examined for microbial analysis through various microscopic and macroscopic examinations such as staining, motility test, colony morphology and biochemical characteristics. Seven bacteria were isolated based on their morphological and structural characteristics. The results of which are tabulated in table 3.

Table 3. Colony morphology of bacterial colonies isolated from Pond water sample

Colony name/cultural characteristics	PSW-1	PSW-2	PSW-3	PSW-4	PSW-5	PSW-6	PSW-7
Colony color	Dull	Creamy yellow	Pale yellow	White	Grayish white	Dull	Yellow
Colony surface	Smooth	Smooth	Rough	Moist	Smooth	Rough	Smooth
Density	Transparent	Opaque	Opaque	Opaque	Opaque	Transparent	Opaque
Colony shape	Circular	Circular	Circular	Circular	Circular	Irregular	Irregular
Margin	Undulate	Even	Undulate	Entire	Even	Undulate	Entire
Elevation	Convex	Raised	Raised	Convex	Convex	Convex	convex
Gram reaction	Negative	Negative	Negative	Negative	Negative	Positive	Negative
Motility	Motile	Motile	Motile	Motile	Non Motile	Motile	Motile
Shape	Rod	Rod	Rod	Rod	Rod	Rod	Rod

PSW - Pond Water Sample

Biochemical characteristics of isolated organisms

The seven isolated colonies were further characterized by biochemical analysis. The biochemical parameters and the results observed had been interpreted in the table 4.

Table 4. Biochemical characteristics of isolated organisms from pond water

Colony name/Biochemical characteristics	PSW-1	PSW-2	PSW-3	PSW-4	PSW-5	PSW6	PSW7
Indole	Negative	Negative	Positive	Positive	Negative	Positive	Positive
Methyl Red	Negative	Negative	Positive	Positive	Negative	Positive	Negative
Voges Proskauer	Negative	Positive	Negative	Negative	Negative	Positive	Variable
Citrate Utilization	Negative	Positive	Negative	Negative	Positive	Positive	Positive
Amylase	Positive	Positive	Negative	Negative	Positive	Negative	Negative
Catalase	Positive	Positive	Positive	Positive	Positive	Positive	
H2S Production	Positive	Negative	Negative	Negative	Negative	Negative	Negative
Glucose Fermentation	A	AG	Negative	Positive	Positive	Positive	Positive
Sucrose Fermentation	A	AG	Negative	Variable	Positive	Positive	Positive
Lactose Fermentation	Negative	Negative	Negative	Positive	Positive	Variable	Variable

Antibacterial activity of *Moringa oleifera* seed extracts against isolated cultures

Antibacterial study of was determined against isolated cultures from collected pond water. The methanol, ethanol, acetone and aqueous extracts of *Moringa oleifera* were prepared and the antibacterial activity was determined using Agar well diffusion method. The results were shown in table 4.

Table 4. Antibacterial activity of *Moringa oleifera* seed extracts against isolated cultures

S. No	Microorganisms	Concentration of *Moringa oleifera* seed extracts (mg/ml) and Zone of inhibition (mm)								Positive control (Ampicillin)
		Methanol		Ethanol		Acetone		Aqueous		
		200	400	200	400	200	400	200	400	
1.	*Salmonella sp*	12±0.2	18±0.2	9±0.4	12±0.6	8±0.1	9±0.6	21±0.3	25±0.5	34 ± 0.5
2.	*Pseudomonas sp*	16±0.6	21±0.3	14±0.2	16±0.4	12±0.1	14±0.2	20±0.1	23±0.2	30 ± 0.4
3.	*Staphylococcus sp*	22±0.3	25±0.1	14±0.2	18±0.1	13±0.3	16±o.4	**22±0.4**	**27±0.2**	30 ± 0.2
4.	*Escherichia sp*	15±0.3	18±0.6	14±0.2	16±0.3	10±0.5	12±0.2	19±0.3	23±0.6	30 ± 0.6
5.	*Klebsiella sp*	13±0.2	15±0.2	11±0.4	13±0.1	10±0.8	12±0.1	19±0.2	23±0.3	32 ± 0.4
6.	*Bacillus sp*	15±0.3	17.±0.2	11±0.2	11±0.2	13±0.3	9±0.8	20±0.4	24±0.6	22 ± 0.3
7.	*Vibrio sp*	19±0.3	23±0.2	16±0.3	20±0.5	13±0.2	16±0.2	21±0.5	25±0.1	32 ± 0.1

DMSO – Negative Control

Mean ±SD

DISCUSSION:

Water sample from a pond located at Annamalai University, Chidambaram, Tamil Nadu was collected. The physico-chemical parameters of water samples have been interpreted. After analyzing the smelling sense of water samples were found to be odorless. The pH values of water sample were analyzed by pH meter was found to be pH 7, which proved to be slightly alkaline. The pH value was found within the permissible range of 6-8 by WHO. (Medera *et al)* reported that the pH of natural water ranges from 6.5 – 8.5 while deviation from the neutral 7.0 is caused due to CO2/ Bicarbonate/ Carbonate equilibrium.

Turbidity of the pond water is lower than the standard acceptable value of ICI i.e 5 NTU. (Ramesh *et al*)(9) reported the turbidity value of ground water at Erode from min to max range as 0-16 NTU with an average value of 0.86 NTU which was well within the acceptable limit given by ICI. The total dissolved solid (TDS) of pond water was found to be 200mg/lit which is unsuitable for drinking and irrigation.

Dissolved oxygen can be referred to as important indicator of healthy aquatic ecosystem as oxygen is necessary for aquatic animals. According to ICI (1982), 4mg/lt is the limit for DO for inland surface water subjected to pollution.

It was concluded that aquatic ecosystem of the ponds are distributed and does not ensures healthy aquatic life in water body. BOD values were 3mg/lit. So it was considered to be polluted. Similarly (Raman *et al)* reported that BOD values of canal water above the tolerance limits for inland surface water quality standards subjected to pollution.

Alkalinity of water represents presents of hydroxyl ions (-OH) in water. Hence it has the capacity of water to neutralize to strong acid. Total alkalinity was found to be 8-0 mg/lit.

According to Cholanky high value of chloride is not good for irrigation and also harmful to aquatic life. It means the pond water is highly contaminated with human and animal waste.

The collected pond water was serially diluted and plated in nutrient agar for the isolation and identification of bacteria. The isolated pure cultures from pond water were further studied for biochemical characterization and identification on the basis of staining, motility test, colony morphology, colony characteristics and after performing biochemical activities. Specific boatel populations were identified in the pond water. *Salmonella sp, Pseudomonas sp, Staphylococcus sp, E. coli, Klebsiella sp, Bacillus sp, and Vibrio sp* were identified.

In 2012, Panneerselvam and Arumugam reported that the collected water samples were processed for bacterial isolation using the nutrient agar, Mac Conkey agar, blood agar and EMB agar. The conventional methods of swabbing and streaking were used. Pure colonies of isolates organisms were identified and characterized using standard microbiological technique. The bacteria were isolated from water samples yielded 16 isolates representing 6 different types of bacterial species *viz., E. coli, K. pneumoniae, Vibrio cholerae Proteus Sp, Pseudomonas aeruginosa* and *S. aureus*. From this study it was concluded that the water is commonly contaminated with microbes and this contamination may be playing a role in the transmission of potentially harmful organisms.

Antibacterial activity of Moringa oleifera seed extracts (Methanol, Ethanol, Acetone and Aqueous) were interpreted against the isolated bacterial cultures. Ampicillin (10µg) was used as positive control, where as DMSO was used as negative control. The aqueous extract showed potential activity against all the tested bacterial cultures followed by Methanol extract. The aqueous extract of *M. oleifera* in the concentration of 400 mg/ml showed highest inhibitory activity (27±0.2mm) against *Staphylococcus aureus* followed by *Salmonella sp* (25±0.5) and *Vibrio sp* (25±0.1).

It has been reported that crushed seed extract of Moringa oleifera had bactericidal activity against *Staphylococcus pyogenes* and *Pseudomonas aeruginosa* (Suarez *et al*., 2005). Philip *et al*., (2013) reported that *Moringa oleifera* seed extract was resistant to *Pseudomonas aeruginosa* at 50mg/ml. Philip *et al*., (2013) reported that ethanolic extract of defatted seed. *M. oleifera* was sensitive to *Proteus mirabilis* (5mm) and *Salmonella Typhi* (1.7mm).

(John *et al* 1986) identified the bacterial substances in Moringa seeds as Pterygosperin, Moringine and the glycosides 4 (α-L-rhamhylozyl)–benzlisiothiocyanate and 4 (α-L-rhamhylozyl) phenyl acetonitrile. These substances have been shown to inhibit mainly *Bacillus subtilis, Mycobacterium phlei, Serratia, E. coli, pseudomonas, Shigella and Streptococcus.* The antibacterial activity of *Moringa olifera* seed has been highlighted by many authors (Olsen *et al*, 1987; Madren *et al, Kawo* 2007)

The antimicrobial activity of *Moringa olifera* seed is due to the presence of an array of phytochemicals, but most importantly due to the activity of short polypeptide named 4(α-L-rhamhylozyl) benzyl-isothiocyanate. The peptide may act directly on microorganism the result in growth inhibition by disrupting cell membrane synthesis (or) synthesis of essential enzyme (Silvetre *et al*, 2000; Suarez *et al* 2003).

CONCLUSION:

Microbial analysis confirmed that B*acillus, Proteus, Pseudomonas aeruginosa, Staphylococcus, Streptococcus and E. coli* was identified. Hence, on the basis of overall physico-chemical and microbial analysis we can say the water is unsafe for drinking and domestic purpose. On the observation of antibacterial analysis against the pathogenic bacteria, seed extracts of *Moringa oleifera* showed potential antibacterial activity against *Staphylococcus sp*. From this present study, we conclude that the seed residues of *Moringa oleifera* can be mixed with water for safe consumption and drinking purposes.

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