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PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF *Moringa oleifera* TO TREAT DIFFERENT KINDS OF WATER SAMPLES

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ABSTRACT

Water quality and treatment are the most important issue in everywhere, especially in the developing countries, where safe and clean water is not continuously provided. *Moringa oleifera* is one of the best natural coagulants that has effectively used in water treatment. The aqueous extract of *Moringa oleifera* seeds was evaluated for their efficacy in reducing total bacterial load, coliform count and faecal coliform counts in the treatment of Cauvery river water. The preliminary phytochemical screening and antimicrobial assay were carried out using standard procedures. The results of the phytochemical analysis revealed differences in the presence of the phytochemicals among the extracts. The antibacterial assay results shows that *Moringa oleifera* seed aqueous extract exhibited broad spectrum activity against the test organisms with *Escherichia coli*.

Key words: *Moringa oleifera*, MPN analysis, Phytochemical screening, Antibacterial activity.

INTRODUCTION

Water quality is the most important things in human's life. Without water access, both in quality and safety, the human health will expose to risk due to low quality of safe drinking water, and human life can be threatened with many diseases such as diarrhoea, cholera, typhoid fever, and dysentery (Forsythe and Hayes, 1998). Most of the people in developing countries struggle to obtain access to safe water, especially in remote areas, where a treated drinking water supply is not available. They receive their drinking- water by some ways such as a small tanker driven by animal or carriage by themselves from public water sources (wells; lakes, rivers, streams and catchment areas) and most of this water is collected by women and children without hygienic practices and the sources itself may be poor in cleaning, unimproved, unprotected and normally located far away from their living area (Gundry *et al*., 2006). To overcome water contamination, aluminum salts have been used in treating of drinking water, but the excessive use of chemical coagulants increases the treatment cost and can be causing health and environment problems (Joshna and Vasu, 2013). *Moringa oleifera* Lam (*Moringaceae*) is highly valued plant. In addition to its compelling water purifying powers and high nutritional value. In the present study should concentrate the phytochemical screening and antibacterial activity of *Moringa oleifera*.

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MATERIALS AND METHODS

M.oleifera seed collection

The seeds were shelled to obtain the seeds coat and seed kernels separately and some were used without shelling as unshelled seeds. These plant parts were dried in the source of sunlight. The small pieces of dried plants were ground separately into powder form using an electric grinder.

Phytochemical tests (Brindha *et al*., 1981)

The analysis of phytochemicals from the solvent free extract of *Moringa oleifera* was individually performed using different quantitative tests for alkaloids,

flavonoids, saponins, carbohydrates, tannins, steroids, glycosides, fixed oils and fats, lignin, phenols, gum's and mucilage, protein and amino acid.

Extraction of phytochemicals

The individual phytochemical was extracted in the suitable solvent and stored in air-tight containers at 4ºC for further use.

Test for alkaloids

To a little of extract a few drops of Mayer's test reagent was added. Formation of precipitate indicates presence of alkaloids.

Test for flavonoids

1 ml extract was taken and a few drops of very dilute solution of ferric chloride were added. The colour changed to pale green or red brown colour which indicates the presence of flavonoids.

Test for saponins

One ml extract and one ml alcohol diluted with 20 ml distilled water and shaken well for 15 minutes. The formation of 1cm layer of foam indicated the presence of saponins.

Test for carbohydrates

Small amount of extract was dissolved separately in 5 ml distilled water and filtered. The filtrate was subjected to Molisch's test. Formation of reddish brown ring indicates the presence of carbohydrate.

Test for tannins

To 5ml of extract solution, 1ml of lead acetate solution was added. Flocculent brown precipitate indicates the presence of tannins.

Test for steroid

A small amount of aqueous extract of sample and a few crystal of sodium nitrate were taken in a dry test tube and heated gently for a minute. It was cooled and 0.5ml of concentrated H_2SO_4.

Test for glycosides

A small portion of extract was hydrolysed with hydrochloric acid for few hours on a water bath and the hydrolysate was subjected to legal's test to detect the presence of different glycosides.

Legal's test

To the hydrolysate 1ml of sodium nitroprusside solution was added and then it was made alkaline with sodium hydroxide solution. If the extracts produced pink to red colour, it indicates the presence of glycosides.

Test for fixed oils and fats

Few drops of 0.5N alcoholic potassium hydroxide were added to a small quantity of various extracts along with a drop of phenolphthalein. The mixture was heated on a water bath for 1-2 hrs. Formation of soaps or partial neutralization of alkali indicates the presence of fixed oils and fats.

Test for lignin

Few drops of the extract was added with alcoholic solution of the phloroglucinol and hydrochloric acid. The presence of lignin was indicated by the production of red colour.

Test for Phenols (Ferric chloride test)

A fraction of the extracts was treated with aqueous 5% ferric chloride and observed for formation of deep blue or black colour.

Test for gums and mucilage's

About 10ml of the extract was added to 25ml of absolute alcohol with stirring and filtered. The precipitate was dried in air and examined for its swelling properties and for the presence of carbohydrates.

Test for Amino acids and Proteins (1% Ninhydrin solution in acetone)

2ml of filtrate was treated with 2-5 drops of Ninhydrin solution placed in a boiling water bath for 1-2 minutes and observed for the formation of purple colour.

Isolation of bacteria

The cauvery river water samples were collected from different areas of Tiruchirapalli district. The collected water samples were analysed through MPN and biochemical test.

Inoculum preparation

The inoculum was prepared as follows; a loopfull of 3 to 4 isolated colonies was inoculated into 5 ml of suitable broth, incubated at 37^0 and 25^0 c for bacterial culture respectively. These actively growing bacterial suspensions were then adjusted with their respective suitable broth so as to obtain a turbidity visually comparable to that of 0.5 McFarland standard prepared by mixing 0.5 ml of 1.75%(w/v) barium chloride dehydrate with 99.5 ml of 1%(v/v) sulphuric acid. This turbidity to equivalent to approximately 1×10^8 CFU/ml.

Effect of antibacterial activity

The antibacterial effects of *M.oleifera* extract against gram negative bacteria have been studied. In the study, the antibacterial effects of the aqueous and ethanolic extracts from *M.oleifera* were examined against

E.coli. It was observed that *E.coli* where sensitive to the extract from the plant (Fernandes*et al*., 2002).

Antibacterial activity

In vitro antibacterial activities of selected bacteria were carried out using standard disc diffusion method.

Antibacterial activity index

Antibacterial index for individual chemical extracts of *Moringa oleifera* was calculated as the mean value of the zone of inhibition obtained against all individual bacteria.

Determination of minimum inhibitory concentration (MIC)

Each bacterial strain was diluted 1:10 with fresh sterile Mueller Hinton broth, streaked on the agar plates in a radial fashion and incubated at 37ºc aerobically for 24-48h. complete suppression of growth by specific concentration of an extract was regarded active. Each extract was examined at a concentration of 0.1, 0.5, 1.0, 5.0 and 10.0 mg/ml.

IR Spectrum Analysis

FTIR relies on the fact that the most molecules absorb light in the infra-red region of the electromagnetic spectrum. The absorption corresponds specifically to the bounds present in the molecule. The frequency ranges are measured as wave numbers typically over the range 4000 – 600 cm^{-1}.

Procedure

FTIR spectrum of the compound obtain from column chromatography was done using Shimadzu IR Affinity 1 instrument.

RESULTS

Table 1 showed that the presence of phytochemical components of *Moringa oleifera*. The seed of *M.oleifera* contained a number of phytochemical compounds such as alkaloids, flavonoids, saponins, carbohydrates, tannins, steroids, glycoside, fixed oils and fats, lignin, phenolic compound, gums and mucilage, proteins and amino acids.

The antibacterial activity of aqueous extract of *Moringa oleifera* was assayed in vitro by disc diffusion method against bacterial strain. Antibacterial activity was done at 64 μg concentration for the test organism. Table 2 showed that compound gave a maximum zone of inhibition on pathogenic organisms (Figure 1).

The chi-square values obtained respectively which was less than the calculated table value. $X^2(0.05) = 3.841$ at 5% level of significance. Above results lead to the conclusion that the data was consistent with the hypothesis, the diameter of inhibition zone obtained from the observed data showed the similarities with experimental data.

The FT-IR spectrum is used to obtain the graph and band stretching was interpretated. (Table 3) (Figure 2).

DISCUSSION

In earlier studies *Moringa oliefera* phytochemical screening revealed presence of flavonoids and saponins, Tannins, Alkaloids, were reported by Napolean *et al.,* (2009) and Phytochemicals in fruits and vegetables may reduce the risk of a cancer possibly due to their dietary fibers, polyphenol antioxidants and anti-inflammatory effects (Brow and Arthur, 2001).

Table 1. Phytochemical analysis of *Moringa oleifera* aqueous extract (Sankar Narayan S *et al*., 2012)

Phytochemical constituents of *Moringa oleifera*		
S.NO	**Constituents**	**Aqueous extract**
1.	Alkaloids	++
2.	Flavonoids	+++
3.	Saponins	+++
4.	Carbohydrates	++
5.	Tannins	+
6.	Steroids	++
7.	Glycosides	+
8.	Fixed oils and fats	++
9.	Lignin	ND
10.	Phenols	+
11.	Gums and mucilage	++
12.	Amino acids and Proteins	++

Note: +++ =Relative abundance of compound; ++ = Moderate Abundance of compound; + = Relative low presence of compound; ND = Not Detected

Table 2. Zone of Inhibition formed by aqueous extract of *M.oleifera* seed against bacterial strain

S.No	Sample	Bacterial strain	µg	Zone of inhibition in diameter (mm)			$X^2=\Sigma[(o-E)^2]/E$
				Plant material	Standard value	Observed value	Observed crude
1.	*M.oleifera* Seed powder	*E.coli*	64µg	Seed powder (boiled)	20	15mm	1.20
2.				Seed powder (raw)	20	19mm	0.05

Table value x^2 (0.05) = 3.841 Chi-square value significance at 5% level

Table 3. Infrared spectrum analysis by *Moringa oleifera* seed powder (crude)

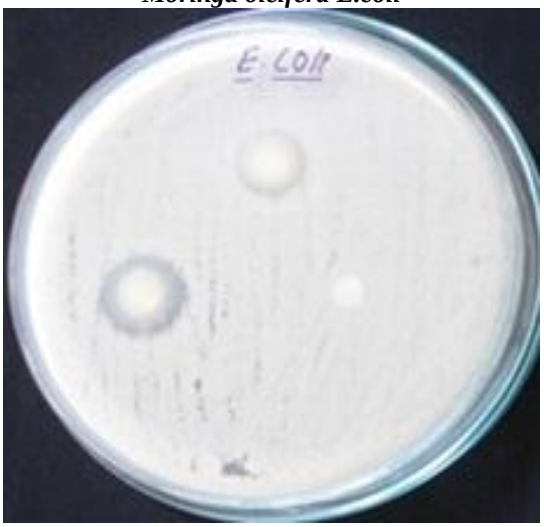
S.NO	Absorption of Peak Value	Stretching	Interpretation
1.	3696.64	O-H stretching	Alcohol group
2.	3369.64	O-H stretching	Alcohol group
3.	2924.09	C-H stretching	Methyl group
4.	2854.65	C-H stretching	Alkyl group
5.	2376.30	N-H stretching	⁄ NH2
6.	1901.81	c=c stretching	Allene c=c=c
7.	1876.74	C=0 stretching	Five membered ring(cyclic)
8.	1853.59	C=0 stretching	Five membered ring(cyclic)
9.	1811.16	C=0 stretching	Five membered A,B unsaturated
10.	1780.30	C=0 stretching	Saturated(acyclic compounds)
11.	1745.58	C=0 stretching	Aryl group
12.	1656.85	C=0 stretching	Tertiary amides
13.	1552.70	N=0 stretching	Ar-No2
14.	1527.62	N=0 stretching	Ar-No1
15.	1442.75	C-H def	-CH2-
16.	1406.11	C-0 stretching	Phenols
17.	1381.03	C-H def	C-H
18.	1271.09	C-0 stretching	Primary alcohols
19.	1238.30	c-o stretching	C=C-O-C
20.	1192.01	C-O stretching	Alcohol
21.	1141.86	C-0 stretching	Secondary alcohol
22.	1107.14	C-0 stretching	Secondary alcohol
23.	1060.85	C-0 stretching	C=C-O-C
24.	798.53	C-H def	Disubstituted(mefa)
25.	721.38	C-H def	-
26.	673.16	C-CL stretching	C-Cl
27.	651.94	-	Chloroalkenes
28.	603.72	-	Chloroalkenes

Phytochemicals such as carbohydrates, reducing sugars, steroids and alkaloids were found to be moderate in concentration. Steroids are used in the stimulation of bone marrow and growth. It stimulation lean body mass and also play vital roles in the prevention of bone loss in elderly men (De-piccolli *et al*., 1991).

In previous studies, the natural alternative methods have been applied in water treatment, especially in the rural area (Folkard *et al*., 1993; Doer, 2005; and Onwuliri and Dawang, 2006) by using natural compounds found in plants such as Okra (Agarwal *et al*, 2001), Rice, fenugreek and psyllium as coagulant materials to remove the contaminants from drinking water.

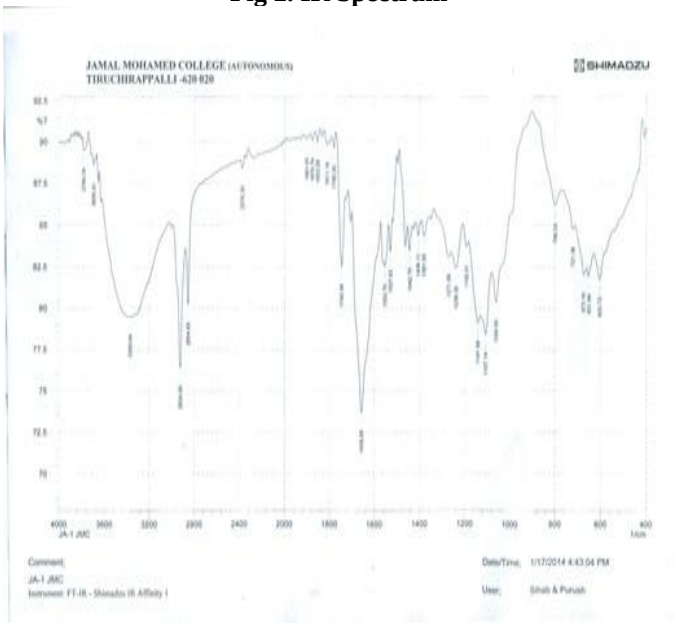
Reports have also described the Moringa seeds powder is most effectively used to treat, purify and contaminant removal from drinking water (Olsen, 1987; Daniyan *et al.*, 2011; Prabhu *et al.*, 2011). In Sudan, dried seeds of *Moringa oleifera* have been used by women in rural areas as a natural alternative to replace the alum (aluminum sulphate) in the removal of turbidity from water (John and Dirar, 1979; Muyibi and Evison, 1994).

Fig 1. Zone formation formed by aqueous extract of *Moringa oleifera E.coli*



1. Seed powder (boiled) 2. Seed powder(raw) 3. Control

Fig 2. IR Spectrum



In present study *Moringaoliefera* seed extract contained a number of phytochemicals such as alkaloids, flavonoids, steroids, carbohydrate, glycosides, lignin, saponins, tannins, Fats and oils, phenols, Amino acids and proteins, gums and mucilage. These data corroborated the findings of other authors where these compounds exhibited antimicrobial activities. This would indicate that the antibacterial activities observed in the present study could attributed to such compounds.

The present study showed that different compounds were separated from *Moringa oleifera* seed extract by using IR-Spectroscopy analysis.

CONCLUSION

Moringa oleifera is one of the most widely used (seeds) in water treatment as the natural coagulants because of low cost, highly biodegradable, short shelf life, safe to human health and the environment when compared to synthetic coagulants either organic or inorganic. The present works conclude that it contains valuable biochemical compounds and antibacterial efficacy. So the *M.oleifera* seeds treated water is very safe to drink.

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