APPLICATION OF MONODORA MYRISTICA LEAVES AS PHYTODISINFECTANT

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Abstract

This study was aimed to investigate the possibility of using monodora myristica leaf for the treatment of water. In rural area the main problems that are facing by people is the non-availability of clean water. Amongst the numerous techniques of pollutant removal, disinfection is an effective and useful process. In my work, the potential use of monodora myristica leaf for pollutants removal from water was attempted. The antimicrobial effect of monodora myristica leaves was also identified. Monodora myristica leaves treated with ethanol (phytodisinfectant) was used for the investigation. In this study various concentration of phytodisinfectant was used for various sources such as open well, canal water and river water. In this study also analyse the characteristics of water before treatment and after treatment with monodora myristica extract. With this cheap and eco-friendly disinfectant we can be substituted for expensive disinfectant.

Keywords: Monodora Myristica Leaf, phytodisinfectant

I. INTRODUCTION

Saving water to save the planet and to make the future of mankind safe is what we need now. With the growth of mankind, society, science, technology our world is reaching to new high horizons but the cost which we are paying or will pay in near future is surely going to be too high. Among the consequences of this rapid growth is environmental disorder with a big pollution problem. Anthropogenic activities have caused a great harm to the quality of our lifeline, i.e. water. Because of fast depletion of the freshwater resources, there seems to be a crisis of the same. Water pollution is a global concern and, it is the high time that we realize the gravity of the situation. Removing pollutants from water is the crying need of the hour and developing a cost effective and environmentally safe method to achieve the same.

There are many places where flood happens frequently, and clean water lacking is one of problems faced in such places due to its water sources contaminating. Since water is a human basic need, water procuring and managing became a very vital issue. For providing clean water, especially for places that had potential flood disaster in rainy season, various solutions always initiated by all related parties such as Government mitigation programs and survival actions of people around these unlucky places. These actions had their advantages and disadvantages respectively, or each strong points and constraints as well. One solution is easy to operate for instance, but didn't provide adequate capacity, whereas the other had ideal performance but could more costly.

For locations where no water source, clean water are supplied from other place using trucks for transportation and distribution, whereas in other areas that have improper water source condition people are treating their water sporadically in very small capacity level. For other cases, some companies program are arranged by social foundations to organize the projects, collect the branded standard water machines and operate the distribution. These actions are much appreciated and they are improved continuously for more efficient result from time to time. Using big trucks may constrained by disaster location infrastructure, whereas provide imported equipments that available in the market, although are capable to produce better quality output or guaranteed result but has the costly consequence with very specific or limited implementation, and usually initiated by project based approach.

For such case, new equipment should be re-
.
From the requirement side, clean water and drinking water quality standard are defined, such as Indian Standard for Drinking Water as per BIS specifications (IS 10500-1991) as a reference in treating surface and ground water. In some cases there are still obstacles to reach such quality standard either caused by high turbidity or iron/ manganese (Fe/ Mn) contamination, or organic/ ammonium/ undissolved compound content. Some conditions show that people are even pushed in using their water for sanitation only, whereas for cooking and drinking are unsolved.
Above mentioned problems are inspiring to develop water treatment that could be an alternative favor. It innovates a water treatment for appropriate needs that might use available raw water such as river or flood water at disaster location, in order to provide clean water demand easier for the people.
Monodoramyristica belong to the ananeceae family. According to the reports almost every part of the plant has economic importance. Although several studies have been carried out on the monodoramyristica antimicrobial effects. The present study demonstrates the effect of monodoramyristica leavesas natural adsorbents for removal of pollutants from water.

II. MATERIALS AND METHODS

1. Preparation of materials

Monodora myristica leaves were collected, Leaves were segregated and washed with tap water followed by distilled water and dried before use. 50 gms dried Monodora myristica leaf powder were taken in separate container. To this 25ml of ethanol was added and mixed well, and stored in air tight container.

Fig 1 Monodora Myristica leaves

procured for next event, as a new project.

Fig 2 MonodoraMyristica Leaves powder treated with ethanol

2. Collection of water

The water samples were collected from open wells, canal water and river water in sealed plastic cans, from Mulangue, Thottippal P O, Thrissur, which is a flood affected area. Water collected in a clean polythene bottles that had been pre-washed thoroughly rinsed with deionized water and then standard methods were used for analysis of water. The water sample stored at 4ºC during storage period to avoid any change in its characteristics. About 3 samples of each type of water, about 6 L of each samples were collected

Fig 3 water samples

3. Experimental work

The water samples from open well, canal water, river water are going to be treated with monodora myristica leaf powder treated with ethanol. Batch study is adopting for this method. Each sample of 1000 ml is treated with phytodisinfectant of 2g, 4g, 6g, 8g and 10g in Erlenmeyer flask. At the end of the desired contact time the mixtures will be filtrated using whatman no.42 filter paper,

samples bacteriological analysis will be done. Characteristics of collected water sample

Fig 4 Sample treating with phytodisinfectant

4. Determination of the phytochemical contents of the plant

(i) Test for presence of alkaloids

The presence of alkaloids in each sample was investigated using the method described by Harborne (1984).

An alcoholic extract was used and obtained by dispersing 2g of the powered sample in 10 ml of ethanol. The mixture was through shaken before filtering using Whatman No. 40 filter paper. 2 ml of the filtrate was added into a test tube and 3 drops of pirovic acid was mixed with it. The formation of light green colouration indicates presence of alkaloid.

(ii) Test for the presence of flavonoid

The determination of presence of flavonoid in the sample was carried out using the acid alkaline test described by Harborne (1984).

2 ml of the aqueous extract was added into a test tube and a few drops of Bench Concentrated ammonia (NH4) were also added. The formation of a yellow colouration shows presence of flavonoid. Confirmatory test was carried out by adding few drops of concentrated hydrochloric (HCL) into the yellow solution which turned colourless.

(iii)Test for the presence of phenols

The presence of phenols in the sample was carried out using the Harborne (1984) methods. The fat free sample was boiled with 50 ml of ether for 15 minutes. 5 ml of the extract was pipette into a 50 ml flask and 10 ml of distilled water added into it. 2 ml of ammonia hydroxide solution and 5 ml of concentrated amyl alcohol were also added. The mixture was allowed to react for 30 minutes for colour development.

(iv) Test for the presence of saponins

The presence of saponins in the samples was determined using Harborne (1984) method. before treating and after treating will also analyse.

Two tests were involved in the investigation, the froth test and emulsion test.

In the froth test, 2 ml of the aqueous extract was mixed with 5 ml of distilled water in a test tube. The mixture was shaken vigorously. A stable froth on standing indicates the presence of saponins.

In the emulsion test, 3 drops of groundnut oil, was added to the aqueous extract mixed with 5 ml of distilled water and shaken well. Formation of emulsion indicates the presence of saponins.

(v) Test for the presence of tannin

The presence of tannins in the samples was determined using the method described by Harborne (1984).

2 ml of the aqueous extract filtrate and 3 ml distilled water was put into a test tube. A few drops of 0.1 % ferric chloride was added to the mixture. The formation of a very dark precipitate indicated presence of tannin

5. Qualitative determination of the phytochemical constituents of the plant

i) Alkaloid determination

The determination of alkaloid concentration in the leaves of the plants was carried out using the alkaline precipitation gravimetric method described by Harborne (1984).

Powdered sample of 5 g was soaked in 20 ml of 10 % ethanolic acetic acid. The mixture was stood for four hours at room temperature. Thereafter, the mixture was filtered through Whatman filter paper No 42. The filtrate was concentrated by evaporation over a steam bath to ¼ of its original volume. To precipitate the alkaloid, concentrated ammonia solution was added in the extract until it was in excess. The resulting alkaloid precipitate was recovered by filtration using previously weighed filter paper. After filtration, the precipitate was washed with 9 % ammonia solution and dried in the oven at 60oC for 30 minutes, cooled in a desiccator and reweighed. The process was repeated more than two times and the average was taken. The weight of alkaloid was determined by the differences and expressed as a percentage of weight of sample analyzed as shown below.

$$\% \text{ Alkanoid} = \frac{W2-W1}{\text{Weight of sample}} \times 100$$

Where

W1 = weight of filter paper

W2 = weight of filter paper + alkaloid precipitate

(ii) Flavonoid determination

The flavonoid content of the leaves of the plant was determined by the gravimetric method as was described by Harborne (1984). acetate extract which contained flavonoid was recovered, while the aqueous layer was discarded. A pre weighed Whatman filter paper was used to filter second, the residue was then placed in an oven to dry at 60oC. It was cooled in a desiccator and weighed. The quantity of flavonoid was determined using the formula.

$$\% \text{ Flavonoid} = \frac{W2-W1}{\text{Weight of sample}} \times 100$$

Where:

W1= Weight of empty filter paper

W2= Weight of paper + Flavonoid extract

(iii) Determination of phenols

The concentration of phenols in the leaves of the leaves of the plants was determined using the folincio Caltean colorimetric method described by Pearson (1976).

0.2 g of the powdered sample was added into a test tube and 10 ml of methanol was added to it and shaken thoroughly the mixture was left and to stand for 15 minutes before being filtered using Whatman No. 42 filter paper. 1 ml of the extract was placed in a test tube and 1 ml folincio Caltean reagent in 5ml of distilled water was added and color was allowed to develop for about 1 to 2 hours at room temperature. The absorbance of the developed colour was measured at 760 nm wave. The process was repeated two more times and an averaged taken. The phenol content was calculated thus,

$$\% \text{ Phenol} = \frac{100}{W} X \frac{AU}{AS} X \frac{C}{100} X \frac{VF}{VA} X D$$

Where,

W= weight of sample analyzed

AU= Absorbance of test sample

AS= Absorbance of standard solution

C= concentration of standard in mg/ml

UF= total filtrate volume

VA= Volume of filtrate analyzed

D= Dilution factor were applicable

(iv) Determination of saponins

The saponin content of the sample was determined by double extraction gravimetric method (Harborne, 1984).

5 g of the powdered sample was taken in a conical flask and 50 ml of water and 2 ml HCl solution was added. The solution was allowed to boil for 30 minutes. The boiled mixture was allowed to cool before it was filtered through Whatman filter paper No 42. 10 ml of ethyl

5 g of the powered sample was mixed with 50 ml of 20 % aqueous ethanol solution in a flask. The mixture was heated with periodic agitation in water bath for 90 minutes at 55o C; it was then filtered through what man filter paper No. 42. The residue was extracted with 50 ml of 20 % ethanol and both extracts were poured together and the combined extract was reduced to about 40 ml at 90oC and transferred to a separating funnel where 40 ml of diethyl ether was added and shaken vigorously. Separation was by partition during which the ether layer was discarded and the aqueous layer reserved. Re-extraction by partitioning was done repeatedly until the aqueous layer become clear in color. The saponins were extracted, with 60 ml of normal butanol. The combined extracts were washed with 5 % aqueous sodium chloride (NaCl) solution and evaporated to dryness in a pre-weighed evaporation dish. It was dried at 60o C in the oven and reweighed after cooling in a desiccator. The process was repeated two more times to get an average. Saponin content was determined by difference and calculated as a percentage of the original sample thus

$$\% \text{ Saponin} = \frac{W2-W1}{\text{Weight of sample}} \times 100$$

Where

W1 = weight of evaporating dish

W2 =weight of dish + sample

(v) Tannin determination

The tannin content of the leaves of the plants was determined using the Folin Dennis spectrophotometric method described by Pearson (1976).

2 g of the powered sample was mixed with 50 ml of distilled water and shaken for 30 minutes in the shaker. The mixture was filtered and the filtrate used for the experiment. 5 ml of the filtrate was measured into 50 ml volume flask and diluted with 3 ml of distilled water. Similarly 5 ml of standard tanuric acid solution and 5 ml of distilled water was added separately. 1 ml of Folin-

Dennis reagent was added to each of the flask followed by 2.5 ml of saturated sodium carbonate solution. The content of each flask was made up to mark and incubated for 90 minutes at room temperature. The absorbance of the developed colour was measured at 760.nm wave length with the reagent blank at zero. The process was repeated two more times to get an average. The tannin content was calculated as shown below

$$\% \text{ Tannin} = 100/W \times AY/AS \times C/100 \times VF/VA \times D$$

Where,

W= Weight of sample analysed

AY=Absorbance of the standard solution

C=Concentration of standard in mg /ml.

VA= Volume of filtrate analysed

D= Dilution factor where applicable

using Disc diffusion method described by Ebi and Ofoefule (1997), 20 ml of the molten nutrient agar was seeded with 0.2 ml of broth culture of the test organisms in sterile Petri-dishes. To ensure a uniform distribution of the organisms the Petri dishes were rotated slowly. They were left to solidify and dish cups of 8.0 mm diameter were made in the agar. The Petri-dishes were allowed to stand for about 30 minutes at room temperature to allow for the proper diffusion of the extracts. The plates were then incubated at 37°C for 24 hours. The zones of inhibition in mm were measured and recorded.

7. Bacteriological analysis

Here each sample is treated with the phytodisinfectant made from the M. myristica. In such a way that each sample with 1000ml is treated with 2g, 4g, 6g, 8g, 10g of phyto disinfectant powder. And stir very well and kept for 24 hr. after that this will filtrate with whatman no. 42 filter paper. And resultant waters bacteriological analysis was carried out.

Bacteriological analysis was carried out for indicator organisms i.e. total and fecal coliform (E.coli) by most probable number (MPN) method. Ten tubes of MacConkeys broth arranged in two rows with a 100 ml blood culture bottle. First row containing 10 ml double strength MacConkeys broth was inoculated with 10 ml of water sample and 50 ml double strength MacConky broth was inoculated with 50ml of water sample. Second row containing 1 ml single strength MacConkeys broth medium was inoculated with 1 ml water sample respectively. Were incubated in an incubator at 44°C for 24 hr. After incubation, the number of bottles in which lactose fermentation with acid and gas production has occurred was counted. Finally,

6. Antimicrobial activity test

The sensitivity of the test organism to the phytodisinfectant powder was carried out

by referring to probability table, the MPN of coliform in 100 ml water sample was been estimated (Deepesh Kumar et al. 2013).

III. RESULT AND DISCUSSIONS

1. Phytochemical determination

The phytochemical screening of the leaves of M. myristica showed that the leaves of the plants contain alkaloids, flavonoids, phenols, saponins, and tannins (Table 1). Research reports showed that the presence of these phytochemicals in the leaves of these plants confer them for their medicinal value (Vadivu et al 2008). The pharmaceutical and therapeutic potentials of plants and their products are as a result of the presence of these phytochemicals in them (Edeoga et al., 2005; Bishnuet al., 2009). Alkanoids are plant derived compounds that are toxic or physiologically active and contain nitrogen in heterocyclic ring. They are basic and have a complex structure and are of limited distribution in the plant kindom (Okwu 2009). The flavonoids represent the most common and widely distributed group of plant phenolics, the presence of phenolic compounds indicates that the plants might be anti microbial agents. Phenolic compounds are toxic to living cells such as fungi and bacteria. It acts on cell by denaturing and coagulating the protein content of the cell. M. myristica contain compounds of 0.84% limonene, 0.83% α- pinene, 0.40% myrcene and 0.11% phellandrene (Okwu 2009). These phytoconstituents are monoterpenoids that not only posses anti-viral and anti-bacterial properties but also exhibit antifungal properties

Phenolics form a large group of naturally occurring diverse and wide spread

compounds. They are characterized by the presence of an aromatic ring with one or more hydroxyl groups. These phenolic compounds in M. myristica may be responsible for the anti-septic, antifungal or bactericides properties of the plant.

Table. 1 Presence of phytochemicals

Phytochemical compounds	Presence
Alkaloids	+
Flavonoid	+
Phenols	+
Saponins	+
Tannin	+

The investigation showed that the leaves of monodora myristica contains 4.64% of alkanoids, 3.88% of flavonoids, 2.25% of phenols, 2.85% of saponins and 0.23% of tannins. The flavanoid content is high in the leaf. The flavonoids represent the most common and widely distributed group of plant phenolics, the presence of phenolic compounds indicates that the plants might be anti microbial agents. Saponins natural tendency to ward off microbes make them good for treating fungal and yeast infections. Plant saponins help humans to fight fungal infections. Plants store these antifungal, antibacterial and antiviral chemicals for protection against microbial attack. Phytochemicals are stored in plants to protect the plant against the attack and inversion of microorganisms. So the leaves contain high phytochemical compounds and have antimicrobial activity on the human pathogens used in the test.

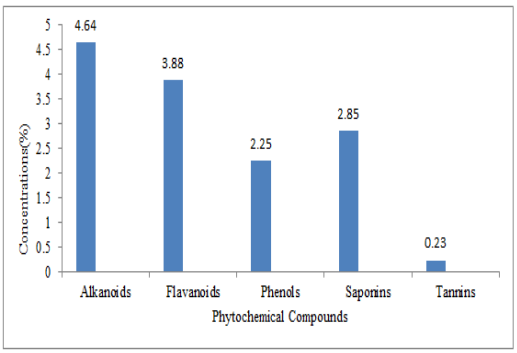

Fig 5. Percentage of phytochemical compounds

2. Bacteriological analysis

From the Fig.6 it is clear that the phytodisinfectant made by using monodora myristica leaves are effective in reducing the Coliform count MPN index/ 100 ml of all tested open well samples. Only sample 2 MPN value is found to be beyond the desirable limit of 10 MPN Index/100ml when treating 1000 ml of sample with 10gm of phytodisinfectant

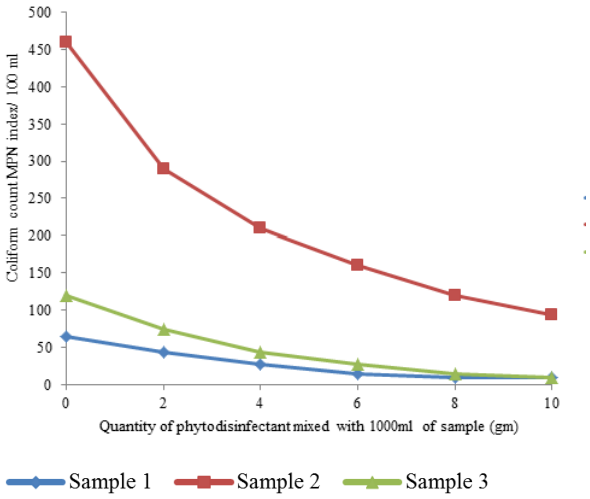

Fig. 6 Phytodisinfectant quantity v/s coliform count in open well samples

From the Fig.7 it is clear that the phytodisinfectant made by using monodora myristica leaves are effective in reducing the Coliform count MPN index/ 100 ml of all tested canal water samples. The entire sample found to be within the limit of 10 MPN Index/100ml when treating 1000 ml of sample within 10gm of phytodisinfectant

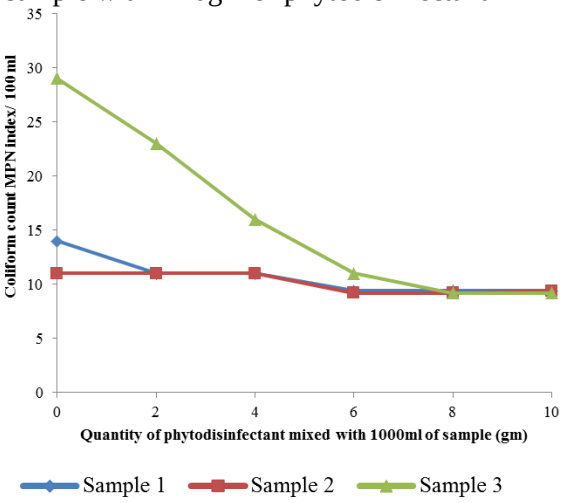

Fig. 7 Phytodisinfectant quantity v/s coliform count in canal water samples

From the Fig. 8 it is clear that the phytodisinfectant made by using monodora myristica leaves are effective in reducing the Coliform count MPN index/ 100 ml of all tested river water samples. The entire sample found to be within the limit of 10 MPN Index/100ml when treating 1000 ml of sample within 10gm of phytodisinfectant

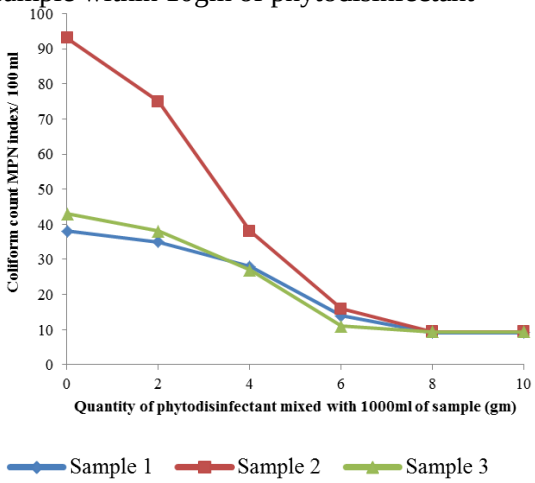

Fig. 8 Phytodisinfectant quantity v/s coliform count in river water samples

3. Sample analysis

After treatment open well samples pH value is decreased, but entire samples values are within the limit. The alkalinity of the samples are increased when treating with the phytodisinfectant. It is clearly shown that when 10g of disinfectant added to the sample 2 the TDS value reached the desirable limit. so we can not use the disinfectant more than 10g according with the TDS value. The hardness value is increased little, and entire samples are within the limit. The chloride values are increased when treated with the disinfectant. Sulphate value also increased when treating with the phytodisinfectant. Coliform count is decreased when treating with the phytodisinfectant made by using monodora myristica leaves.

After treatment canal water samples pH value is decreased, but entire samples values are within the limit. The alkalinity of the samples are increased when treating with the phytodisinfectant. TDS value increased when treating with phytodisinfectant. Three samples TDS value increased beyond the desirable limit. So according with IS:10500:1991 the samples can not be used for drinking purpose . The hardness value is increased little, and entire samples are within the limit. The chloride values are increased when treated with the disinfectant. Sulphate value also increased when treating with the phytodisinfectant. Coliform count is decreased when treating with the phytodisinfectant made by using monodora myristica leaves.

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IV CONCLUSION

The study shows that the phytodisinfectant powder which is made from the monodora myristica has an antimicrobial effect against humen pathogens of Escheichia coli, Staphylococcus aureus, Streptococcus pneumoniae and Psuedomonas aeruginosa. The phytochemical screening of the leaves of M. myristica showed that the leaves of the plants contain alkaloids, flavonoids, phenols, saponins and tannins. The antimicrobial activity of plant leaves powder is due to the effect of the phytochemical compounds presents in the plant leaves. The phytochemical compounds and its antimicrobial effect found in monodora myristica leaves give an eye opener for the discovery of new eco-friendly and economical phytodisinfectant.

From the present study of bacteriological analysis the monodora myristica has been found as an effective phytodisinfectant for water obtained from water sources having low degree of contamination or else water can be given prior filtration to reduce the contamination load. In rural area people use

the water from well or any other sources of water without any treatment. This water mostly contains E-coli as harmful microorganism. This Monodora Myristica leaves are easily available in the rural area and can be used for killing the harmful microorganism.

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