Biospectra : ISSN: 0973-7057, Vol. 17(2), September, 2022, pp. 67-78
An International Biannual Refereed Journal of Life Sciences

13(AS).
Animal Science

ISSN : 0973-7057

Int. Database Index: 616 www.mjl.clarivate.com

Efficacy of aqueous extract of some common medicinal plants for Ganges water

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Received : 16th May, 2022 ; Revised : 16th June, 2022

Abstract- Water is a valuable resource for the sustenance of life. No organism can exist without water. It is used in several purposes such as drinking, agriculture, bathing and recreation. The quality of water whether it is stagnant or free flowing should be maintained particularly for potable purpose. Since ancient times folk medicinal plants are used to treat impure water. To know the efficacy of the extract of some common medicinal plants, present investigation was undertaken using Ganges water for the experiment as its quality is degrading day to day. Total microbial count (CFU/ml), MPN of coliform bacteria, MPN of *E.coli* as well as some chemical properties of Ganges water were affected when aqueous extract of leaves of Neem (*Azadirachta indica*), Sahjan (*Moringa oleifera*) and Tulsi (*Ocimum sanctum*) were treated. Efficacy of aqueous extracts was observed in the manner of *Moringa oleifera* < *Ocimum sanctum* < *Azadirachta indica*. Scientific use of widely and easily available folk medicinal plants may be alternatives to purify water. Moreover, it may be cost effective.

Key words: Ganga river, Water, Bacteria, Tulsi, Sahjan, Neem, Efficacy

INTRODUCTION

All the organisms must have water to survive. Water is used for several purposes by human beings. A sufficient supply of safe and sound drinking water is universally identified as a basic human need. Yet millions of people in developing countries contaminate even a basic drinking water service. In that situation water occurring in ponds, lakes, rivers and streams are used for drinking and domestic uses.[1] These water bodies are usually polluted and contaminated with microorganisms that cause many diseases.

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The Ganga is the most sacred river to Hindus, it is not just a River, the mythological importance associated with this river is deep rooted in the Indian culture and is considered a "Holy River". It is also life line to millions of Indians who depend on it for their daily needs. But, today different types of disease-causing germs and chemicals are added to the Ganga river duo to different anthropogenic activities.[2-9] When this happens, the water becomes polluted or contaminated which is not safe for human life. The main reasons behind poor quality of Ganges River at Patna are sewage disposal, animal bathing, cloth washing, idol immersion and high silt discharge.[10] Consumption of contaminated fresh water is a major cause of waterborne

diseases and when it is used for the food preparation, cause food borne diseases. When surface water of river Ganga is used for various purposes, removal of undesirable chemical and biological contaminants become an important part of the water purification process.

Commonly used methods for water purification are UV- treatment, boiling, chlorination, coagulation, microfiltration etc. The conventional methods of water purification using aluminum sulphate, iron salts and chemicals in drinking water give rise to problem of deposition of sediment in distribution if not applied at correct dosage.[11] Many chemicals and devices used for treatment of water are high priced and beyond the reach of most people particularly in rural areas in developing world. So, there is an urgent need for the development and widespread promotion of simple disinfection technique of water purification for rural\tribal area.[12] Use of medicinal plants for water treatment are safe, economical, effective and easily available alternative.[13]

Plants are the important sources of medicine and a large number of drugs in use are derived from plants. The therapeutic uses of plant are safe, economical and effective as their ease of availability.[14] India has a very long, safe and continuous usage of many herbal drugs in the officially recognized alternative systems of health viz. Ayurveda, Yoga, Unani, Siddha, Homeopathy and Neuropathy. Millions of Indians use herbal drugs regularly, as spices, home-remedies, health foods as well as over –the counter as self-medication or also as drugs prescribed in the allopathic systems. Use of medicinal plants are suggested to remove impurities of water as well as to decrease the intensity of microbes in water.[15] Some medicinal plants also possess antimicrobial properties.[13,16-21]

Moringa oleifera (common name Sahjan) is native to India, but it is widely grown in South-Saharan Africa and South- America. Among the 13 species of *Moringa* of family Moringaceae, *M. oleifera* is the most widely cultivated species. Different parts of *M. oleifera* "the tree for purifying" are used as vegetables, spices (bark and roots) and cosmetic oil (seeds) all the parts of *Moringa* species are well known to be anti-inflammatory, anti-helminthic, anti-hypertensive, antibiotic, anti-bacterial, detoxifiers and immune builders.[13,22] *Ocimum sanctum* (common name Tulsi, family Lamiaceae), Queen of Herbs, the legendary "Incomparable One", is one of the holiest and most cherished of the many healing and health–giving herbs distributed mainly in the oriental region.[14,23] Genus *Ocimum* contains about 60 species in the tropical regions of Asia. It has antimicrobial, antimalarial, antiallergic and immunomodulator, antistressor, antifertility and antidiabetic effects. Tulsi (*Ocimum sanctum)* has also the capacity to purify water.[24] *Azadirachta indica* (common name Neem, family Meliaceae), one of two species are native to the Indian subcontinent i.e. India, Nepal, Pakistan, Bangladesh, Sri Lanka and Maldives. Neem is well known as anti-inflammatory, antiarthritic, antipyretic, hypoglycaemic, anti-gastric ulcer, antifungal, antibacterial and antitumor activities and a review summarizes the various therapeutic role of neem.[25-26]

The possibility of using medicinal plants that is cost effective, safe and easily available is increasing. In the present work, aqueous extracts of three medicinal plants *Moringa oleifera* (sahjan*), Ocimum sanctum* (tulsi*)*and *Azadirachta indica* (neem) were undertaken to study the ameliorating effects on impurities of water as well as on the intensity of microbes found in Ganga water taking as raw sample.

MATERIALS & METHODS

The leaves of *Moringa* (Sahjan), *Ocimum* (Tulsi) and *Azadirachta* (Neem) were collected from Patna Science College campus of Patna University, Patna, Bihar in February- April, 2017.The water samples were collected from three ghats i.e. Kali ghat, Krishna ghat and Gandhi ghat (from upstream to downstream) of the river Ganga in Patna in pre-cleaned plastic and glass bottles and were used immediately for the experiments.Aqueous extracts of medicinal plants were prepared as per the method described with modifications in the quantity of powdered leaves.[12] Collected leaves were washed, air dried and grinded to a fine powdered and stored in an air tight container. The grinded leaves of the plants weighing 0.3 gm. 0.5 gm.0.7 gm and 0.9 gm were suspended in 300 ml of raw Ganga water sample each, to obtain the concentration of 0.1%, 0.16%, 0.23% and 0.3% of the extracts respectively. The suspension was mixed using magnetic stirrer for about 20 minutes. The suspensions were then left undisturbed for about 4 hours followed by centrifugation at 2800 rpm for 3 minutes. Finally, supernatants were filtered through muslin cloth, followed by Whatman filter paper no. 1. The raw Ganga water was considered as the control or normal sample for the comparison purpose. The filtrate and the

Ganga water were analyzed for some physicochemical parameters such as pH, conductivity, alkalinity, total dissolved solids (TDS), total hardness (TH), chloride using the standard procedures.[27]

The filtrate and the Ganga water were also analyzed for the colony forming units (CFUs) of microorganisms by serial dilution standard plate count method using nutrient agar media. The total coliform was determined by a statistical estimation called the Most Probable Number (MPN) test performed sequentially in three stages: presumptive, confirmation and complete test using lactose broth, brilliant green lactose and eosin –methylene blue agar medium following standard procedures.[27] Colonies of *Escherichia coli* can be differentiated on the basis of small size and presence of green metallic sheen. MPN of the total coliform bacteria was calculated by comparing the positive tubes from Mc Crady's statistical table and Thomas formula.[27]

RESULTS & DISCUSSION

pH

pH is defined as the intensity of the acidic or basic character of a solution at a given temperature expressed as negative logarithm of hydrogen concentration.[28] The permissible limit of pH in drinking water is 6.5 to 8.5.[29] The pH was observed in the range of 8.1 to 8.6 at sites 1 (Kali ghat), 8.2 to 8.4 at site 2 (Krishna ghat) and 8.0 to 8.3 at site 3 (Gandhi ghat). Decreasing trend of the pH value of each site was observed after using the different concentrations of aqueous extracts of the medicinal plants *Azadirachta*, *Ocimum* and *Moringa* (Figures 1, 2 &3). The present observation was similar to the findings of another investigators.[30,31] However, such type of finding was not seen in case of neem.[32] Higher concentration of the extracts also showed ability to lower down the pH of water sample in comparison to lower concentration of the extracts.

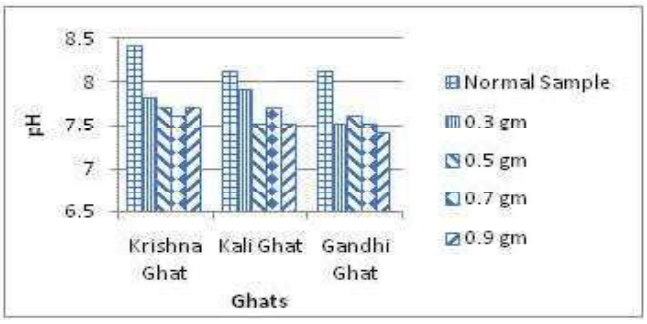

Fig.1-Effect of *Moringa oleifera* [sahjan] leaf extract on pH of Ganga Water

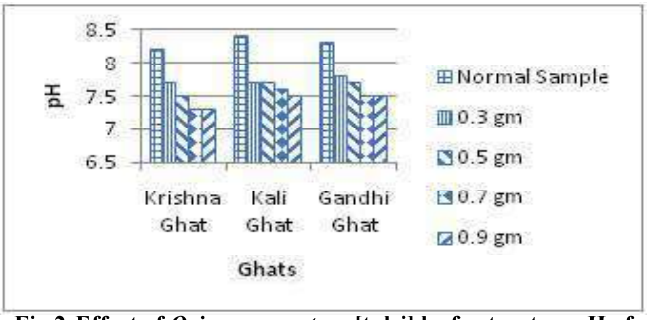

Fig.2-Effect of *Ocimum sanctum* [tulsi] leaf extract on pH of Ganga Water

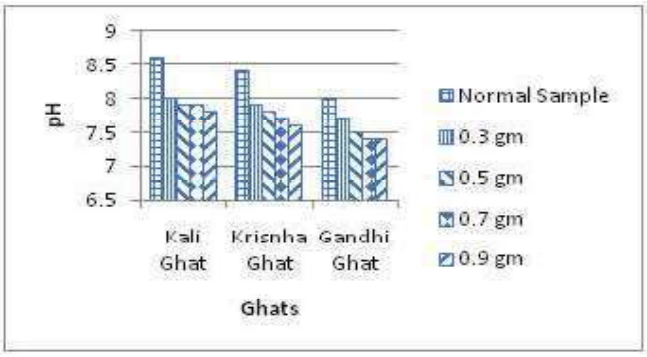

Fig.3-Effect of *Azadirachta indica* [neem] leaf extract on pH of Ganga Water

Conductivity

Electrical conductivity of the water sample depends on the presence of ions, mobility, concentration and temperature. It is a measure of the ability of an aqueous solution to carry electric current.[6] The ions concentration in the water is responsible for conductivity value.[33] The permissible limit in drinking water set on WHO is 0- 3000 (µS/cm). Range of conductivity observed was 320 to 480 µS/cm, 380 to 500 µS/cm and 360 to 485 µS/cm in Kali ghat, Krishna ghat and Gandhi ghat respectively. After using the different concentrations of extracts of tulsi, sahjan and neem, there was increasing trend of conductivity of water samples (Fig. 4,5 and 6). Thus, higher the concentration of extract in the solution, higher will be the conductivity. The reaction of the salt with the water is also one of the possible causes for the rise in the reading.[34]

Fig.4-Effect of *Moringa oleifera* [sahjan] leaf extract on conductivity of Ganga Water

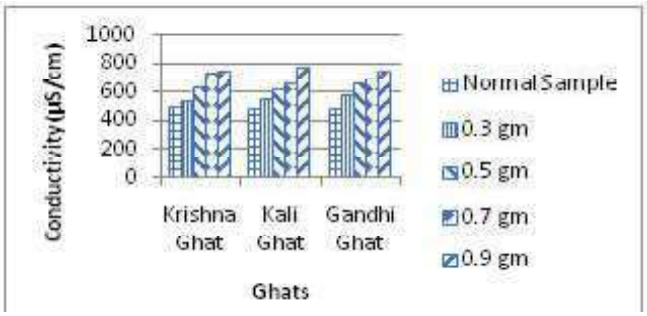

Fig.5-Effect of *Ocimum sanctum* [tulsi] leaf extract on conductivity of Ganga Water

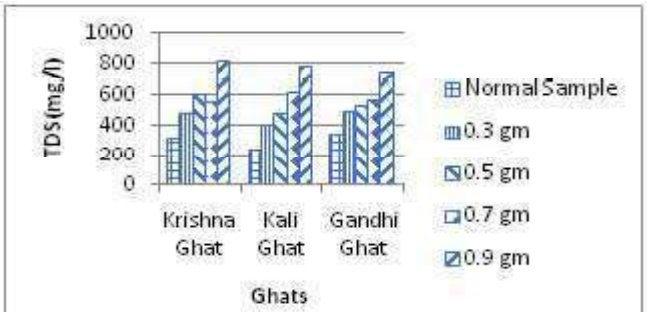

Fig.8. Effect of *Ocimum sanctum* [tulsi] leaf extract on total dissolved solids of Ganga Water

Fig.6-Effect of *Azadirachta indica* [neem] leaf extract on conductivity of Ganga Water

Fig.9. Effect of *Azadirachta indica* [neem] leaf extract on total dissolved solids of Ganga Water

Total Dissolved Solids (TDS)

Total dissolved solids (TDS) are a measure of the combined content of all inorganic and organic substances that are dissolved in a liquid in a molecular, ionized or micro-granular suspended form. A high content of TDS elevates the density of water, reduces solubility of gases and reduce utility of water for drinking. Total dissolved solids are determined as the residue left after evaporation of the filtered samples.[13] Beyond 500 mg\l, TDS my cause gastrointestinal irritation in human beings. 240 to 320 mg\l, 320 to 380 mg\l and 340 to 360 mg\l TDS were found in the three sites of sampling sites Kali ghat, Krishna ghat and Gandhi ghat respectively. After using aqueous leaves extracts of each concentration of plants were seen to increase (Fig. 7, 8 & 9) , possibly due to presence of fine particles of extract mixed with water sample, responsible for the increased TDS in water samples.[13]

Fig.7.Effect of *Moringa oleifera* [sahjan] leaf extract on total dissolved solids of Ganga Water

Chloride

Chlorides are available in all types of water, which is in natural fresh water generally low. It is discharged into water through domestic sewage. The chloride content of aquatic system is the indicator of pollution[5]. Chlorides are troublesome in irrigation water and also harmful to aquatic life. The permissible limit of chloride in drinking water set by WHO is 200 mg\l. Observed range of chloride in Ganga water in all the three sites Kali ghat, Krishna ghat and Gandhi ghat was 42.4 to 82.36 mg\l, 68.16 to 82.36 mg\l and 48.64 to 68.10 mg\l respectively. Aqueous extract of *Moringa* leaf was found to decrease the value of chloride content[13] whereas extracts of other two medicinal plants *Ocimum* and *Azadirachta* were found responsible to increase the values of chloride (Fig. 10, 11 & 12) as they are reported to have chloride compound.[35,36]

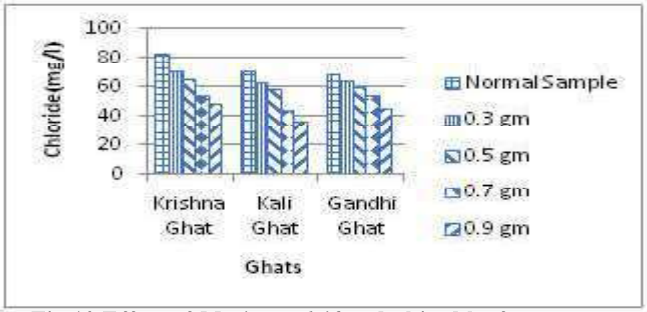

Fig.10.Effect of *Moringa oleifera* [sahjan] leaf extract on chloride of Ganga Water

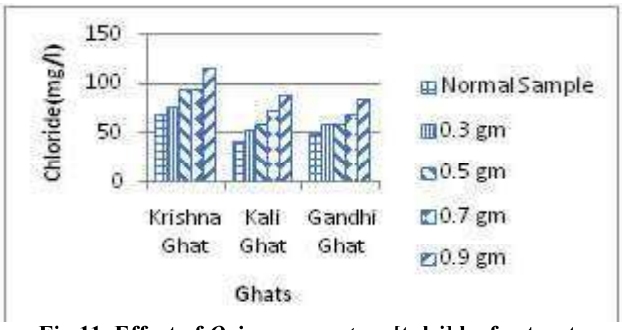

Fig.11. Effect of *Ocimum sanctum* [tulsi] leaf extract on chloride of Ganga Water

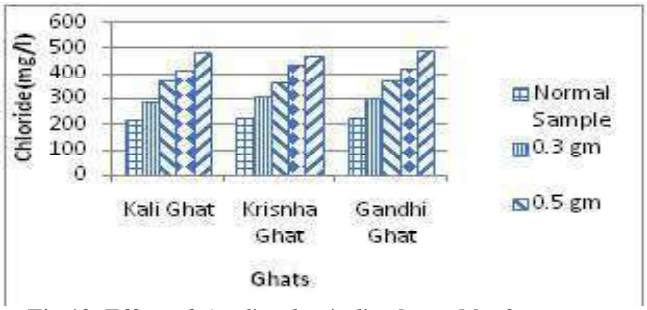

Fig.12. Effect of *Azadirachta indica* [neem] leaf extract on chloride of Ganga Water

Total Alkalinity (TA)

Total alkalinity(TA) refers to the capacity of water or any solution to neutralize acids. It is expression of buffering capacity. TA of water is mainly due to cations of calcium, magnesium, sodium and potassium. It is also due to carbonate and bicarbonate. Total alkalinity observed in all the three sites was in the range of 20 to 60 mg\l in the sites. Somewhat erratic trend of total alkalinity of water samples after using the extracts of selected plants (Fig. 13, 14 & 15) is enough to say that there were no obvious effects in the experiments. It is also reported by several workers.[13,37,38]

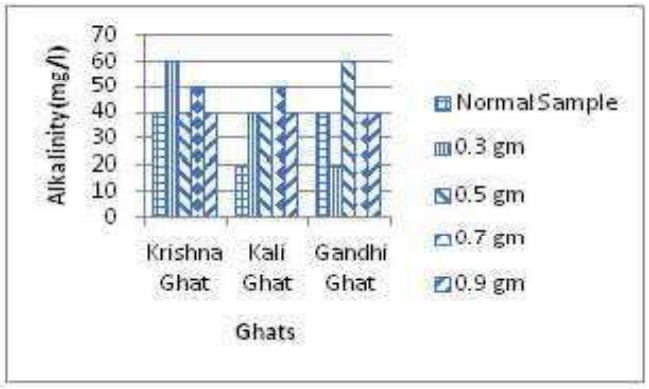

Fig.13. Effect of *Moringa oleifera* [sahjan] leaf extract on alkalinity of Ganga Water

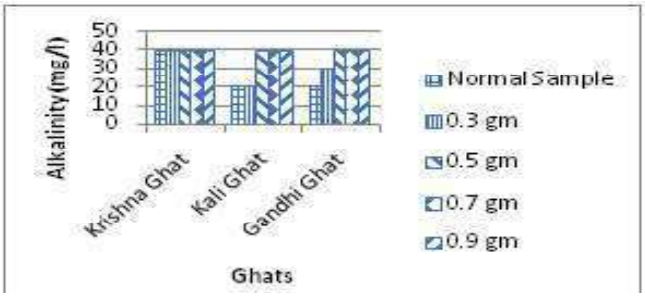

Fig.14. Effect of *Ocimum sanctum* [tulsi] leaf extract on alkalinity of Ganga Water

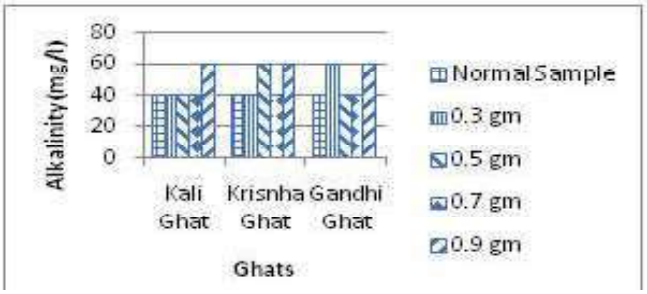

Fig.15. Effect of *Azadirachta indica* [neem] leaf extract on alkalinity of Ganga Water

Total Hardness (TH)

Total hardness (TH) is the measurement of mineral content in a water sample that is irreversible after boiling. It is caused due to the divalent metallic cations. The major anions associated with these cations are carbonates, bicarbonates sulphates and nitrates. Permissible limit of TH in drinking water is 300 mg\l. The range of total hardness of Ganga water in the sampling sites was 212 to 280 mg \l, 216 to 224mg \l and 204 to 226 mg\l in Kali ghat, Krishna ghat and Gandhi ghat respectively. Minimal increasing trend of total hardness in the experiments was observed after using the different concentration of extracts of leaves of medicinal plants undertaken for study (Fig. 16, 17 & 18). It is supported that aqueous extract of *Moringa oleifera* showed promising results in only one out of these samples with an optimum concentration of 0.3%.[13] However, it is suggested that hardness of water may be decreased through adsorption and interparticle bridging.[15,36,38]

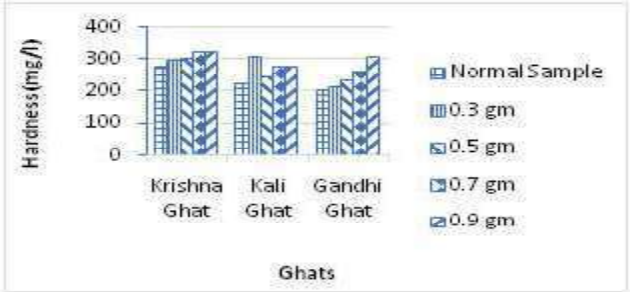

Fig.16. Effect of *Moringa oleifera* [sahjan] leaf extract on hardness of Ganga Water

Fig.17. Effect of *Ocimum sanctum* [tulsi] leaf extract on hardness of Ganga Water

Fig.18. Effect of *Azadirachta indica* [neem] leaf extract on hardness of Ganga Water

Colony Forming Units (CFUs) of total microorganisms

Microorganisms are vital components of all ecosystems whose diversity and abundance are greatly influenced by a wide range of factors.[39] Water, polluted due to domestic, food, dairy, agriculture and industrial wastes contain pathogens (disease causing micro organism). It is hazardous to human health if used as drinking water or food preparation. The microbes present in water provide an estimate of the presence of water borne pathogens. Microbial tests involve the determination of microorganism growing a colony (CFUs) on a nutrient medium by standard plant count (SPC) method.

Standard Plate Count (SPC)

SPC is used to measure the overall microbial quality of drinking water. It is useful in judging the efficiency in operation of various water treatment processes which determines density of aerobic and facultative anaerobic heterophilic bacteria in water. In the present investigation, average CFUs of microbes was in the range between 180 to 470\ml of water of the three sites of Ganga river. The trend of inhibition percentage on CFUs against different increasing concentrations (0.3 gm,0.5gm, 0.7gm and 0.9gm) of aqueous extracts of *Moringa oleifera*, *Ocimum sanctum* and *Azadirachta indica* were seen in all the three sites of Ganga (Table 1, Fig. 19, 20 & 21). The results showed reduction of colony forming units (1/ml of water) of microorganisms after using plant extracts. The *Moringa oleifera* caused average inhibition on CFU units(1\ml) as 25.95%, 46.42% and 68.34% and 86.62 %,*Ocimum sanctum* showed it as 27.37%,46.47%, 68.44% and 85.54%inhibition whereas it was 28.66%, 47.05%, 68.91% and 87.16% inhibition by *Azadirachta indica* due to the effects of different concentrations of extracts of the plants. The neem showed maximum inhibition in comparison to tulsi and sahjan. The effectiveness of selected plants in inhibiting the colony forming units was in accordance with the observations on water samples of Ganga river and Gomti river.[13,40]

Table 1- Effect of different concentration (gm) of aqueous extract of medicinal plants on % inhibition of CFUs (1/ml) of Ganga water at sampling sites Kali ghat (1), Krishna ghat (2) and Gandhi ghat (3)

Concentration of extracts → / Medicinal plants↓	0.3 gm	0.5 gm	0.7 gm	0.9 gm	Sampling sites
Moringa oleifera	25.21*	46.58	69.44	87.18	Site 1
	27.65	48.93	68.08	89.36	Site 2
	25.00	43.75	67.50	83.33	Site 3
Ocimum sanctum	27.23	46.46	68.45	86.42	Site 1
	28.65	47.35	68.24	84.63	Site 2
	26.25	45.61	68.44	85.57	Site 3
Azadirachta indica	29.36	47.42	68.56	86.34	Site 1
	28.73	47.36	69.44	88.67	Site 2
	27.89	46.38	68.76	86.49	Site 3

*All the data are presented in percentage.

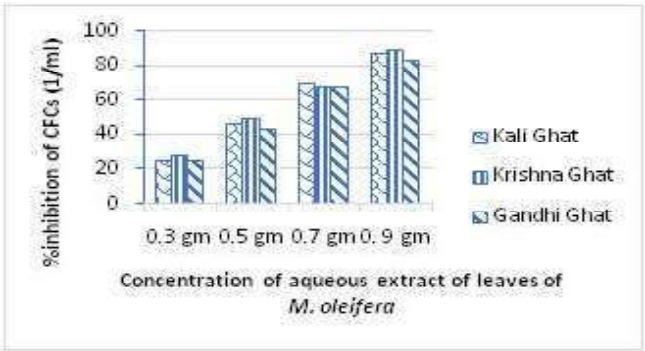

Fig. 19- Effect of different concentration of aqueous extract of *M. oleifera* leaves on % inhibition of CFUs (1/ml)of Ganga water

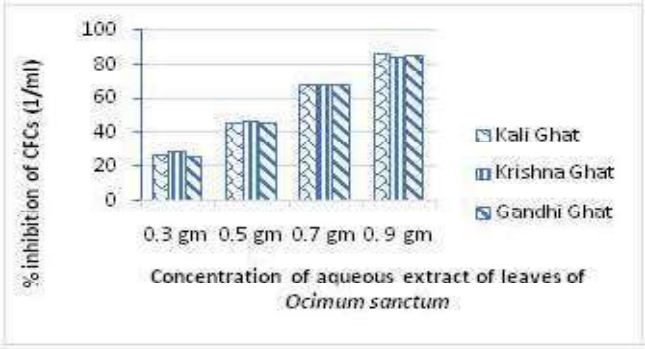

Fig. 20- Effect of different concentration of aqueous extract of *O. sanctum* leaves on % inhibition of CFUs (1/ml)of Ganga water

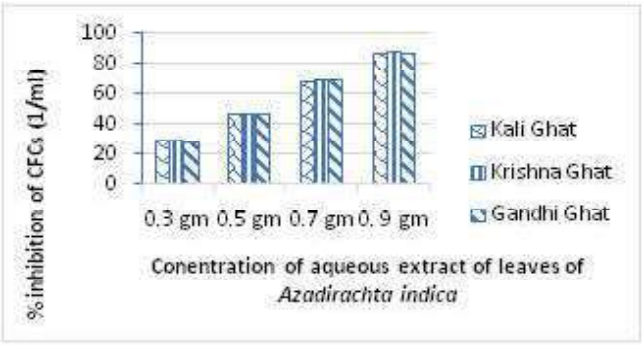

Fig. 21- Effect of different concentration of aqueous extract of *A. indica* leaves on % inhibition of CFUs (1/ml)of Ganga water

Maximum Probable Number (MPN) of Coliform Bacteria

The presence of total coliform in surface water comes from poor sanitation system. The MPN methods are commonly employed for enumerating coliform bacteria in water. The method is estimation of coliform bacteria which includes culture and enzyme -substrate techniques. Culture method relies on the detection of gas evolved when coliform bacteria ferment lactose (*E.coli*) or in the presence of turbidity (*Enterococci*). Average MPN of coliform bacteria found in the range of 1200 to 1600/ 100 ml of water in the river Ganga.

In case of MPN of coliform bacteria, the trend of percent inhibition in response to different increasing concentrations (0.3 gm,0.5gm, 0.7gm and 0.9gm) of aqueous extracts of all the three selected medicinal plants were seen in all the three sites Kalighat, Krishna ghat and Gandhi ghat of Ganga (Table 2, Fig. 22, 23 & 24). The positive results in the reduction of total coliform (1\ml of water) were found when plant extracts were used. The results were better than the results of CFUs.Average percent inhibition on total coliform bacteria (1/100 ml of water) was 90.06%,95.71%,96.46% in case of *Moringa oleifera* (Sahjan), 90.73%,95.71%,96.56% and 98.38% in case of *Ocimum sanctum* (tulsi), whereas it was 91.61%, 95.94%, 96.70% and 98.66% inhibition by *Azadirachta indica* (neem) due to the effects of different increasing concentrations of extracts of the plants. The neem showed maximum inhibition in comparison to tulsi and sahjan. It is reported that *Moringa oleifera* is a tool to slow down the intensity of coliform bacteria.[41-43] Neem has also been reported to have antibacterial properties of neem.[44] Antibacterial properties of extract of Sahjan, Tulsi and Neem are sensitive to *E.coli* as well as coliform bacteria.[40]

Table 2-Effect of different concentration (gm) of aqueous extract of medicinal plants on % inhibition of Coliform bacteria (1/100 ml) of Ganga water at sampling sites Kali ghat (1), Krishna ghat (2) and Gandhi ghat (3)

Concentration of extracts → / Medicinal plants↓	0.3 gm	0.5 gm	0.7 gm	0.9 gm	Sampling sites
Moringa oleifera	89.43*	94.80	95.00	97.60	Site 1
	90.45	95.60	97.40	98.40	Site 2
	90.30	96.70	97.00	98.99	Site 3
Ocimum sanctum	90.66	95.94	96.24	98.33	Site 1
	90.87	95.84	96.62	98.50	Site 2
	90.66	95.34	96.83	98.33	Site 3
Azadirachta indica	91.99	95.99	96.78	98.32	Site 1
	91.44	95.82	96.32	98.88	Site 2
	91.42	96.03	97.00	98.78	Site 3

*All the data are presented in percentage.

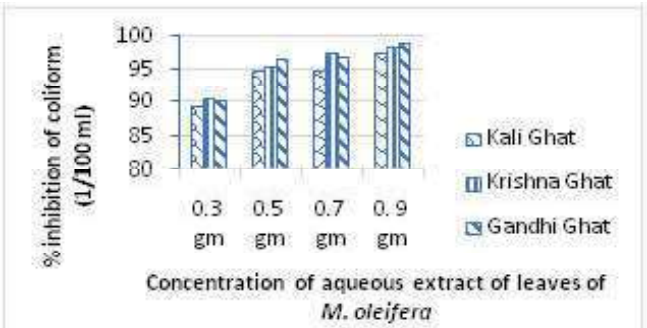

Fig. 22- Effect of different concentration of aqueous extract of *M. oleifera* leaves on % inhibition of MPN of Coliform (1/100 ml) of Ganga water

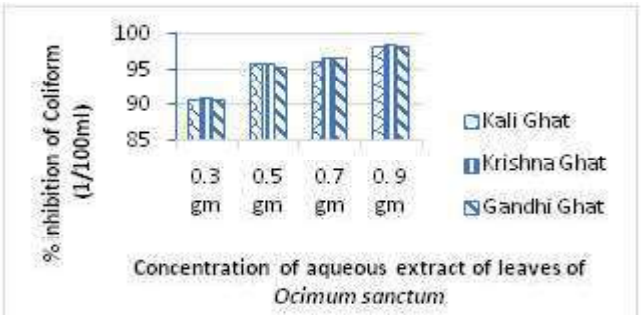

Fig. 23- Effect of different concentration of aqueous extract of *Ocimum sanctum* leaves on % inhibition of MPN of Coliform (1/100 ml) of Ganga water

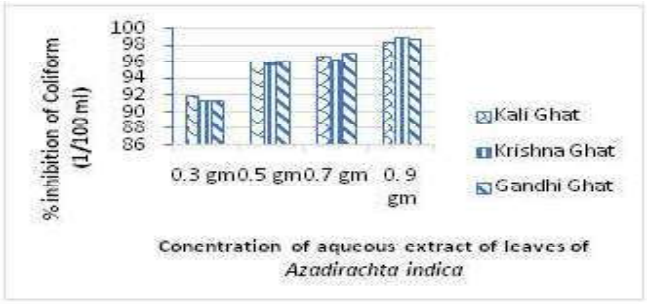

Fig. 24- Effect of different concentration of aqueous extract of *Azadirachta indica* leaves on % inhibition of MPN of Coliform (1/100 ml) of Ganga water

MPN of *Escherichia coli*

E. coli species are the subset of the coliform bacterial groups that are found in the intestinal tracts of humans and animals. Their presence is direct indicator of fecal contamination of water. Average *E.coli* in all the three sites were found as 756 MPN/100 ml of Ganga water.

Percent inhibition of MPN of *E. coli* (1/100 ml of water) was similar to the trend of coliform bacteria in response to different increasing concentrations (0.3 gm,0.5gm, 0.7gm and 0.9gm) of aqueous extracts of all the three selected medicinal plants were seen in all the three selected sites. (Table 3, Figures 25,26 & 27). After using plant extracts, positive results in the reduction of MPN of *E. coli* (1\ ml of water) were observed. The average percent inhibition on total coliform bacteria (1\ml of water) was 92.68%. 95.61%, 97.09% and 98.91% in case of *Moringa oleifera* (Sahjan), 92.69, 95.88%, 97.28% and 99.12% in case of *Ocimum sanctum* (tulsi) and 92.85%, 96.11%, 97.44% and 99.25% inhibition in case of *Azadirachta indica* (neem) when different concentrations (0.3 gm, 0.5gm, 0.7gm and 0.9gm) of aqueous extracts of medicinal plants were applied. All the three selected plants showed excellent positive efficiency against *E.coli*. However, neem was found better in comparison to tulsi and sahjan. The present trend was found similar to the observations of several workers.[45,46] It has been reported that *Ocimum sanctum* has effective antibacterial activity against *E. coli.*[24] *Azadirachta indica* was also found sensitive against *E. coli.*[44,47]

Table 3- Effect of different concentration (gm) of aqueous extract of medicinal plants on % inhibition of *E.coli* (1/100 ml) of Ganga water at sampling sites Kali ghat (1), Krishna ghat (2) and Gandhi ghat (3)

Concentration of extracts → / Medicinal plants↓	0.3 gm	0.5 gm	0.7 gm	0.9 gm	Sampling sites
Moringa oleifera	93.33*	96.96	96.67	98.67	Site 1
	92.00	95.33	97.33	98.66	Site 2
	92.72	94.55	97.27	99.09	Site 3
Ocimum sanctum	92.80	96.63	96.80	99.64	Site 1
	92.60	94.90	97.60	98.75	Site 2
	92.69	96.10	97.45	98.77	Site 3
Azadirachta indica	92.74	95.30	97.43	99.84	Site 1
	92.84	96.67	97.54	98.96	Site 2
	92.98	96.36	97.37	98.97	Site 3

*All the data are presented in percentage.

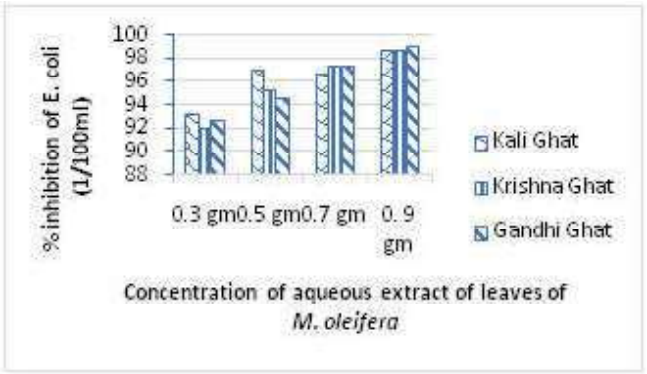

Fig. 25- Effect of different concentration of aqueous extract of *M. oleifera* leaves on % inhibition of *Escherichia coli* (1/ 100ml)of Ganga water

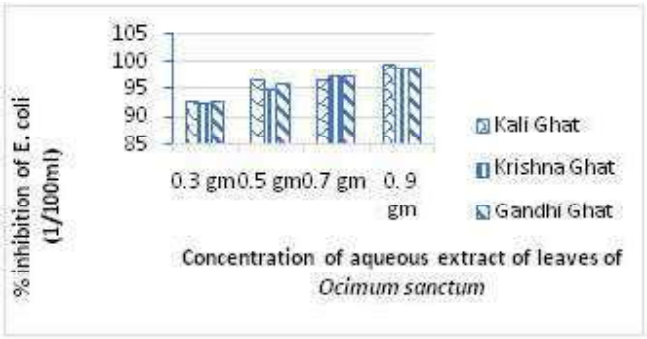

Fig. 26- Effect of different concentration of aqueous extract of *Ocimum sanctum* leaves on % inhibition of *Escherichia coli* (1/100ml)of Ganga water

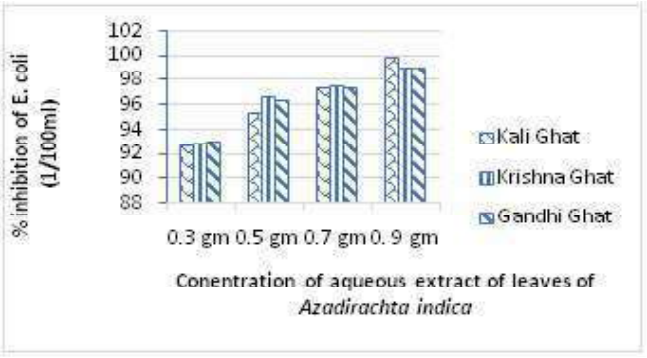

Fig. 27. Effect of different concentration of aqueous extract of *Azadirachta indica* leaves on % inhibition of *Escherichia coli* (1/100ml)of Ganga water

CONCLUSION

The present study indicated that aqueous extract of *Moringa oleifera*, *Ocimum sanctum* and *Azadirachta indica* leaves were very effective in reduction of pH and chloride. Efficient reduction (83.33% to 99.5%) of bacterial population from water sample by utilizing aqueous extract of *Moringa oleifera*, *Ocimum sanctum* and *Azadirachta indica* leaves manifest its use in treating diseases caused by bacteria.

The results also revealed that aqueous extracts of *Moringa oleifera*, *Ocimum sanctum* and *Azadirachta indica* leaves have antibacterial effects on the one species of coliform organisms tested i.e. *Escherichia coli*. Therefore, it could be concluded that aqueous extracts of *M. oleifera, Moringa oleifera*, *Ocimum sanctum* and *Azadirachta indica* are suitable for improving water quality and may be used instead of the conventional methods of water treatment. The leaves can be used in the areas where people live in extreme poverty and use contaminated drinking water having no facilities available for treatment. It is environmentally friendly and low-cost water treatment method without any toxic effect.

ACKNOWLEDGEMENT

Authors are thankful to The Director, Environmental Science & Management cum Head, Department of Zoology, Patna University, Patna (Bihar) for providing laboratory facilities.

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