

Study on the ameliorating effect of aqueous extract of *Moringa oleifera* on Ganga water

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Abstract

The quality of surface water is deteriorating day by day due to various anthropogenic activities. Ganga water is assumed polluted almost in the entire stretch of river. The medicinal plants may be useful for amelioration of pollution. The present work deals with the study of several chemical characteristics as well as microbial examination of water samples taken from few sites of Ganga river namely Krishna Ghat, Kali Ghat and N.I.T Ghat (Gandhi Ghat) at Patna. The study was focused on the evaluation of antimicrobial effect of *Moringa oleifera* (sahjan) leaves extract in the Ganga water. The aqueous leaves extract of different concentrations (0.1%, 0.16%, 0.23%, 0.3%) showed ameliorating results on pH and chloride content of water as well as microbial activity. Significant ameliorating results were observed on pH and chloride content of water. pH was reduced from 8.1 to 7.4 and chloride was reduced from 82.36 mg/l to 48.14 mg/l under plant extract treatment. Colony Forming Unit (CFU) was assessed by standard plate count (SPC) method and was found to be 468 CFU/ml for raw water, which was reduced to 143 CFU/ml when treated with plant extract. Similar decreasing trend was observed in case of MPN of coliform. So extract of *Moringa oleifera* may also be applied to purify drinking water. Moreover it will be cheaper for the people in comparison to other purification devices.

Keywords: Drinking water, Ganga river, *Moringa oleifera*, Plant extract, Water pollution

Article Info

DOI: [10.31018/jans.v10i4.1918](https://doi.org/10.31018/jans.v10i4.1918)

Received: October 10, 2018

Revised: November 16, 2018

Accepted: November 24, 2018

How to Cite

Paul, D. K. *et al.* (2018).

Study on the ameliorating effect of aqueous extract of *Moringa oleifera* on Ganga water. *Journal of Applied and Natural Science*, 10 (4): 1230 -1237

INTRODUCTION

A sufficient supply of safe and sound drinking water is universally identified as a basic human need. Yet millions of people in developing countries defect even a basic drinking water service. In that situation water occurring in ponds, lakes, rivers and streams are used for drinking and domestic purposes (Fetter, 2011 and Iscen *et al.* 2008). These water bodies are usually polluted and contaminated with microorganisms that cause many diseases (Sood *et al.* 2008). The reasons behind poor water quality of Ganges River at Patna are sewage disposal, animal bathing, cloth washing, idol immersion and high silt discharge (Jaiswal *et al.* 2017). 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 (Willey *et al.*, 2008). Most of the water borne diseases still remains one of the major reasons of morbidity and mortality of children in particular. When surface water of river Ganga is used for drinking purpose, 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 method of water purification using aluminium sulphate, iron salts add chemicals in drinking water and give rise to problem of deposition of sediment in distribution if not applied at correct dosage (WHO, 2004). Many chemicals and devices used for treatment of water are high-priced and beyond the reach of most people in developing world (Varshney, 1982). So, there is a need of safe, economical, effective and easily available alternative. Many workers are now suggesting using medicinal plants to remove impurities of water as well as to decrease the intensity of microbes found in water. The antimicrobial properties of medicinal plants have been reported by number of workers worldwide (Grosvenor *et al.* 1995, Adriana *et al.* 2007, Abu-Shanab 2008, Valle *et al.*, 2015, Gopalakrishnan *et al.*, 2016). The possibility of using medicinal plants that is cost-effective, safe and

easily available such as *M. oleifera* popularly known as “Sahjan” is increasing (Eilert *et al.*, 1981, Gassenschmidt *et al.*, 1995, Sotheeswaran *et al.* 2011, Onuoha and Alisa, 2013). *M. oleifera* is native to India, but it is widely grown in South-Saharan Africa and South-America (Grabow, 1985). Among the 13 species of *Moringa* in the family Moringaceae, *M. oleifera* is the most widely cultivated species (Price, 1985, Fahey, 2005). *M. oleifera* (vernacular name Alruway) “the tree for purifying”, used as a vegetable (leaves, fruits, immature pods, flowers and roasted seeds) spice (bark and roots), and cosmetic oil (seeds) (Njoku and Adikwu, 1997, Rebecca *et al.*, 2006, Anwar *et al.*, 2007). All parts of *Moringa* species show medicinal properties and they are well known to be anti-inflammatory, anti-helminthic, anti-hypertensive, antibiotic, anti-bacterial, detoxifiers, and immune builders (Thilza *et al.*, 2010). Hence the present study was undertaken to know the ameliorating effect of aqueous extract of *Moringa* species on the physicochemical and microbial characteristics of Ganga water so that it can be used as an alternative to expensive purification methods of water.

MATERIALS AND METHODS

Sampling: *M. oleifera* leaves used in the study were collected from Patna Science College campus of Patna University, Patna, Bihar in February, March and April, 2017. Water samples were collected from three Ghats i.e. Kali Ghat (Site 1), Krishna Ghat (Site 2) and N.I.T (National Institute of Technology, Patna) Ghat (Site 3) of the river Ganga in Patna, Bihar in pre-cleaned plastics bottles and were immediately used for the experiments.

Preparation of aqueous extract of *M. oleifera*: Aqueous extract of *M. oleifera* was prepared as per the method of Sowmeyan *et al.* (2011) with modifications in the quantity of powdered leaves taken to treat poor water quality Ganga. Collected fresh leaves were washed, air dried and grinded to a fine powder and stored in an air tight container for further use. The ground leaves of the plant weighing 0.3gm, 0.5gm, 0.7gm and 0.9gm 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 extract respectively. The suspensions were mixed using magnetic stirrer for about 20 minutes. The suspension was then left undisturbed for about 4 hours followed by centrifugation at 2800 rpm (revolution per minute) for 3 minutes. Finally, supernatants were filtered through muslin cloth, followed by Whatman filter paper no.1. The raw Ganga water (without the ground leaves) was considered as the control for the comparison purpose.

Physicochemical analysis of water samples: The filtrate and the Ganga water were analyzed

for the physicochemical parameters such as pH, conductivity, total dissolved solids, total hardness, chloride, alkalinity; using the standard procedures as per APHA, AWWA and WEF (2005). pH was studied by digital pH meter (model, pH tester 10, Eutech instruments, oaklon). Conductivity was measured by digital conductance instrument (model, Eco tester, EC_{low}, Eutech instruments). Estimation of total dissolved solids was done by oven dried method. Total alkalinity was measured by titrating the sample with sulphuric acid taking phenolphthalein and methyl orange as indicator. Total hardness was measured by titrating it with standardized EDTA taking Erichrome Black-T as indicator. Chloride was estimated by argentometric titration method.

Microbiological analysis of water samples: The filtrate and the Ganga water were analyzed for the colony forming units (CFUs) of bacteria by serial dilution standard plant count method using Nutrient Agar Media. The total coliform in filtrate and Ganga water 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, Eosin-Methylene Blue (EMB) agar medium following standard procedures as per APHA, AWWA and WEF (2005). Colonies of *E. coli* can be differentiated on the basis of small size and the presence of green metallic sheen.

Computation of MPN: MPN of the total coliform bacteria was calculated by using comparing the positive tubes from MC Crady’s statistical table and Thomas formula (APHA, AWWA and WEF (2005)).

RESULTS AND DISCUSSION

Effect of extract of *M. oleifera* on 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 ion concentration ($\text{pH} = -\log [\text{H}^+]$) (Paul and Sanyal, 2017). Estimation of pH in Ganga water is one of the significant parameter in determining quality for portability. The permissible limit of pH in drinking water ranged from 6.5 to 8.5 (WHO, 2008). The pH observed at site 1 i.e. Kali Ghat was 8.4, whereas the pH of site 2 i.e. Krishna Ghat was 8.1 and the pH of site 3 i.e. NIT Ghat was 8.1; but after using of aqueous leaves extract of different doses, the pH of water in every site was decreased (Fig. 1). The decrease of pH in the present study is supported by the study of (Basra *et al.* 2014), who also observed rapid decrease in pH in treated sewage water of Faisalabad, Pakistan after 3-4 hour of treatment of aqueous extract of *M. oleifera*. Its extract contains cellulose, hemicellulose, lignin, and crude fiber. Its matrix network consists of fiber carbonaceous, carboxylic and amino func-

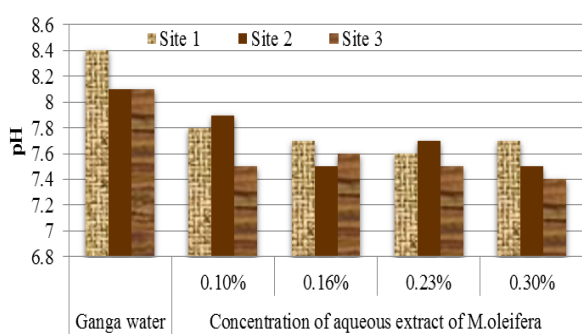


Fig. 1. Effect of different concentrations of aqueous extract of powder leaves of *M. oleifera* on pH of Ganga water.

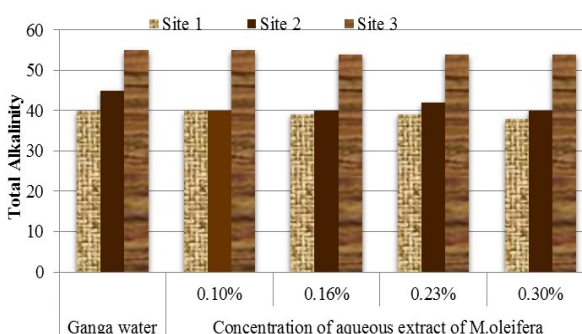


Fig. 2. Effect of different concentrations of aqueous extract of powder leaves of *M. oleifera* on total alkalinity of Ganga water.

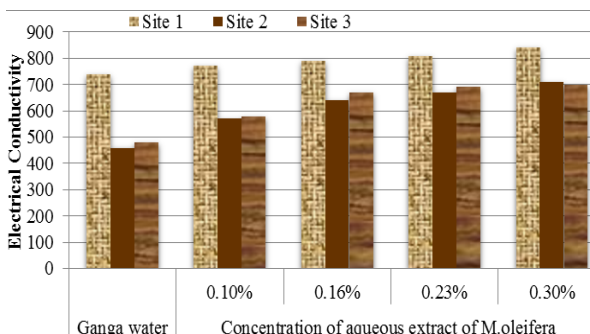


Fig. 3. Effect of different concentrations of aqueous extract of powder leaves of *M. oleifera* on electrical conductivity of Ganga water.

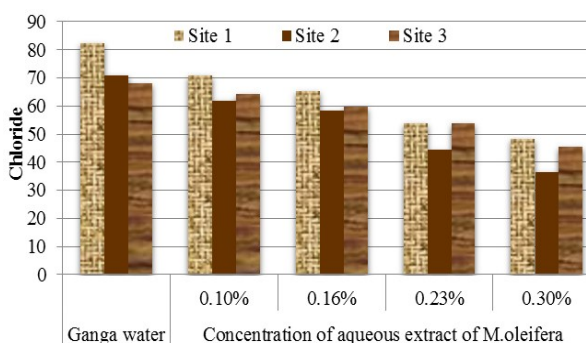


Fig. 4. Effect of different concentrations of aqueous extract of powder leaves of *M. oleifera* on chloride of Ganga water.

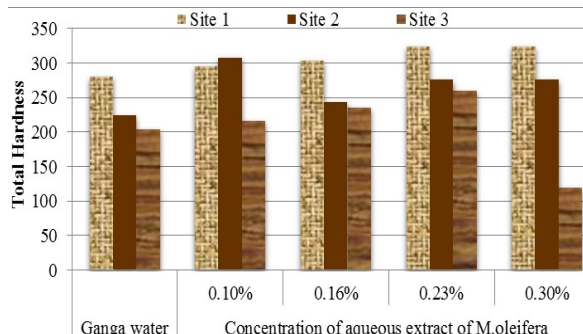


Fig. 5. Effect of different concentrations of aqueous extract of powder leaves of *M. oleifera* on total hardness of Ganga water.

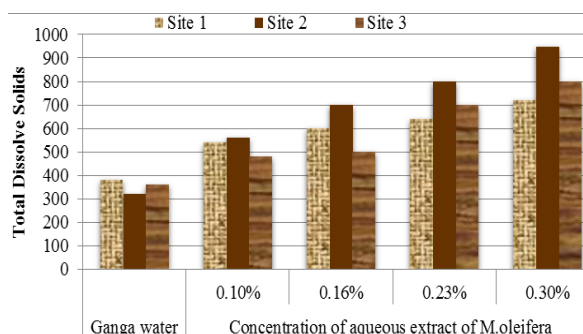


Fig. 6. Effect of different concentrations of aqueous extract of powder leaves of *M. oleifera* on total dissolved solids of Ganga water.

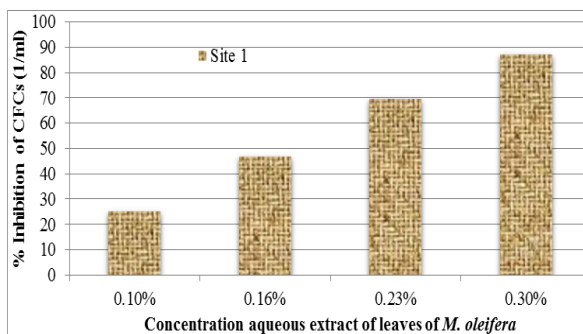


Fig. 7. Effect of different concentrations of aqueous extract of *M. oleifera* leaves on % inhibition of CFUs (1/ml) of Ganga water (Site 1).

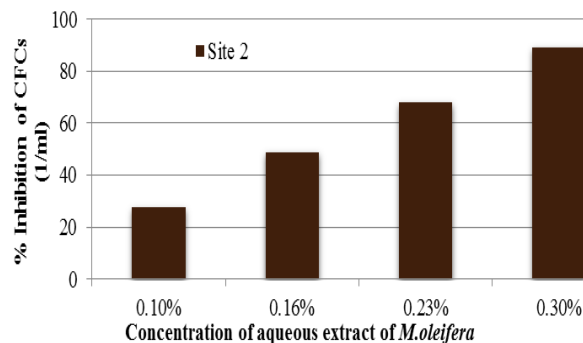


Fig. 8. Effect of different concentrations of aqueous extract of *M. oleifera* leaves on % inhibition of CFUs (1/ml) of Ganga water (Site 2).

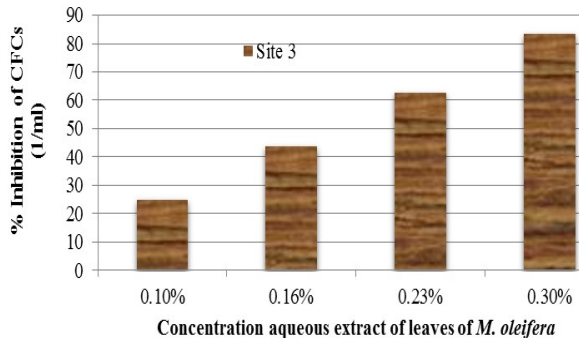


Fig. 9. Effect of different concentrations of aqueous extract of *M. oleifera* leaves on % inhibition of CFUs (1/ml) of Ganga water (Site 3).

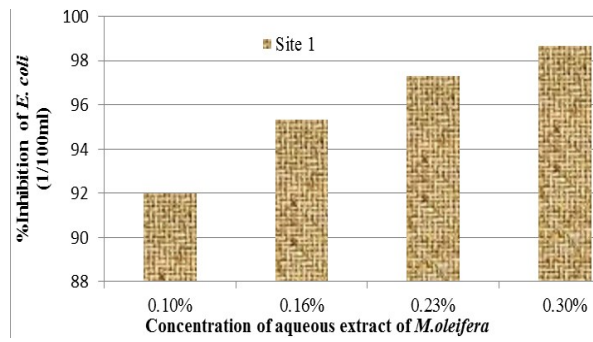


Fig. 13. Effect of different concentrations of aqueous extract of *M. oleifera* leaves on % inhibition of *Escherichia coli* (1/100ml) of Ganga water (Site 1).

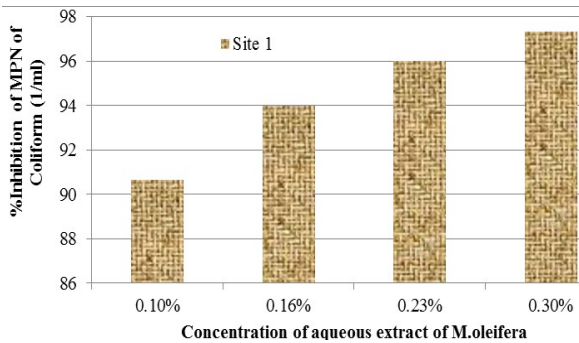


Fig. 10. Effect of different concentrations of aqueous extract of *M. oleifera* leaves on % inhibition of MPN of coliform (1/ml) of Ganga water (Site 1).

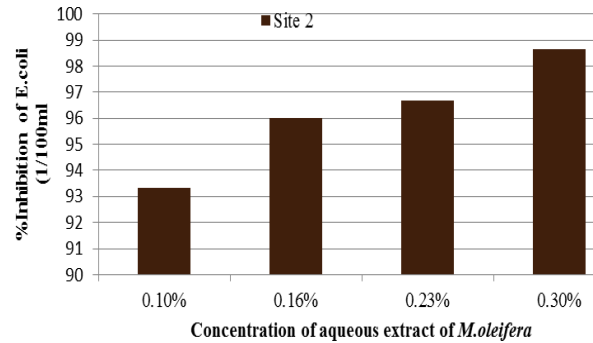


Fig. 14. Effect of different concentrations of aqueous extract of *M. oleifera* leaves on % inhibition of *Escherichia coli* (1/100ml) of Ganga water of (Site 2).

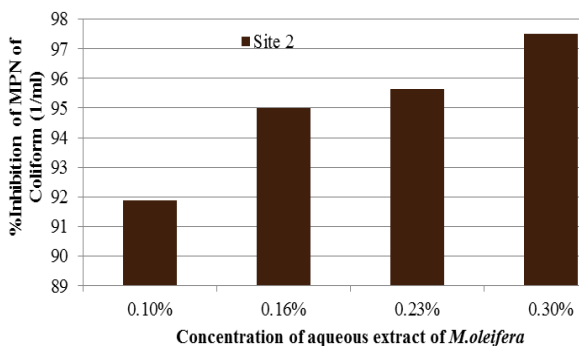


Fig. 11. Effect of different concentrations of aqueous extract of *M. oleifera* leaves on % inhibition of MPN of coliform (1/ml) of Ganga water (Site 2).

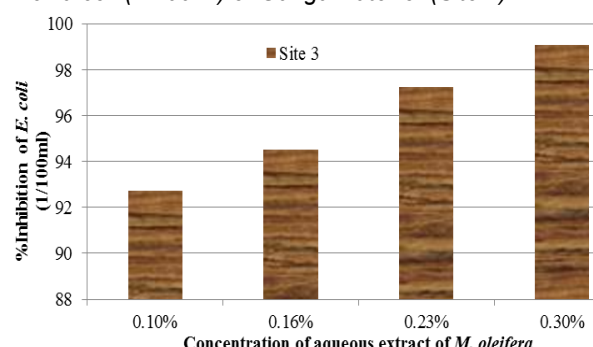


Fig. 15. Effect of different concentrations of aqueous extract of *M. oleifera* leaves on % inhibition of *Escherichia coli* (1/100ml) of Ganga water (Site 3).

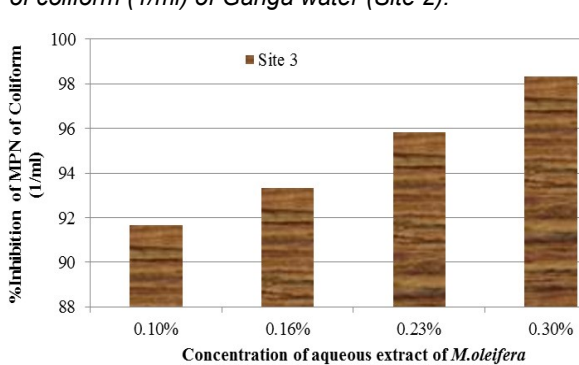


Fig. 12. Effect of different concentrations of aqueous extract of *M. oleifera* leaves on % inhibition of MPN of coliform (1/ml) of Ganga water (Site 3).

tional groups (Akhtar et al., 2007). These functional groups may be dissociated at various pH values and accordingly take part in the adsorption process (Sinha et al., 2016). Water soluble proteins of *M. oleifera* leaves are effective to maintain the pH in the range of 5 – 8 (Vieira et al. 2010a). **Effect of extract of *M. oleifera* on total alkalinity:** Alkalinity refers to the capacity of water or any solution to neutralize acids. It is expression of buffering capacity. Total alkalinity of water is mainly due to cations of calcium, magnesium, sodium and potassium. It is also due to combined carbonate or bicarbonate. The total alkalinity at site 1 i.e. Kali Ghat was 40 mg/l, alkalinity of site 2. i.e. Krishna Ghat is 45mg/l and the alkalinity of site 3

i.e. NIT Ghat was also 55 mg/l but after using of aqueous leaves extract of different concentration, the values of alkalinity changed slightly (Fig. 2). After treatment with 0.1% and 0.16% concentration, the values of alkalinity in Kali Ghat remain unchanged to 40 mg/l. The values of alkalinity slightly decreased when concentration of 0.16%, 0.23% and 0.3% is applied. In Krishna Ghat, after treatment values of alkalinity almost remain unchanged, only slight increase in the value was observed application of 0.23% concentration. While in NIT Ghat the concentration of alkalinity has almost remain unchanged after application of all the concentration of *M. oleifera*. According to the work of (Ndabigengesere and Narasiah, 1998) alkalinity remained almost constant with the use of *Moringa* seeds. In this study, it is revealed that leaves extract of *M. oleifera* did not significantly affect the alkalinity.

Effect of extract of *M. oleifera* on electrical conductivity: Electrical conductivity (EC) of the water sample ability depends on the presence of ions, mobility, concentration, and temperature. The permissible limit of EC in drinking water set by WHO is 0 – 3000 μ S. In this study, the conductivity observed at site 1 i.e. Kali Ghat was 740 μ S, whereas the conductivity of site 2 i.e. Krishna Ghat was 460 μ S and the conductivity of site 3 i.e. NIT Ghat was 480 μ S; but after using of aqueous *Moringa* leaves extract of different concentrations the values of conductivity were increased in the sample of every site (Fig. 3). The ions concentration in the water is responsible for conductivity value (Postolachi, 2015). 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 (Tunggolou and Payus, 2017 and Vieira *et al.* 2010b).

Effect of extract of *M. oleifera* on chloride: Chlorides are available in all types of waters. Amount of chloride in natural fresh water is generally very low. It is discharged into water through domestic sewage. The permissible limit of chloride in a drinking water set by WHO is 200mg/l. In this study, the chloride content at site 1 i.e. Kali Ghat was 82.36 mg/l, whereas the chloride content of site 2. i.e. Krishna Ghat was 71 mg/l and the chloride content of site 3. i.e. NIT Ghat was 68.16 mg/l but after using of aqueous leaves extract of different doses the chloride content of water sample were decreased (Fig. 4). Sowmeyan *et al.* 2011 observed that the herbal coagulants have less prospective on removal of chlorides but the present study showed that there is possibility of using *M. oleifera* to get desire value of chloride in water sample. It was observed that leaf extracts decreased the concentration of chloride in Ganga water because cations from the leaf extract attract negatively charged chloride ions present in un-

treated water and neutralize the chlorides (Mangale *et al.*, 2012, Patil *et al.*, 2017).

Effect of extract of *M. oleifera* on total hardness: Total hardness is the measurement of mineral content in a water sample that is irreversible by boiling. Hardness is caused due to the divalent metallic cations. The major anions associated with these cations are carbonates, bicarbonates, sulphates and nitrates. According to Indian Standard, 1991, the permissible limit of a total hardness in drinking water is 300 mg/l. In this study, the hardness at site 1 was 280 mg/l, whereas the hardness of site 2 was 224 mg/l and the hardness of site 3. i.e. NIT Ghat was 204 mg/l. After using aqueous leaves extract of different concentrations, the values of hardness of water sample was increased, but only with the 0.3% concentration of aqueous leaves extract, the total hardness of water at NIT Ghat was decreased to 120 mg/l (Fig. 5). (Muyibi and Evison, 1995 and Ndabigengesere and _Narasiah, 1998) observed that a significant hardness removal only obtained at relatively higher concentration. The present study support their conclusion as it exhibit that aqueous extract of *Moringa oleifera* showed promising results in only one out of three samples with an optimum concentration of 0.3%. According to Ali *et al.* (2010) and Sinha *et al.* (2016) as a polyelectrolyte it may therefore be suggested that *M. oleifera* decreased hardness in water through adsorption and inter-particle bridging.

Effect of extract of *M. oleifera* on total dissolved solids: Total dissolved solids (TDS) are a measure of the combined content of all inorganic (chiefly calcium, magnesium, potassium, sodium, bicarbonates) and organic substances that are dissolved in a liquid in a molecular, ionized or micro- granular suspended form. A high content of dissolved solid elevates the density of water, reduces solubility of gases (like oxygen) and reduce utility of water for drinking. TDS concentration beyond 500 mg/l, decreases portability and may cause gastrointestinal irritation. According to Indian Standard, 1991, the permissible limit of TDS in drinking water is 100 mg/l. In the present study, the observed value of TDS at site 1 i.e. Kali Ghat was 380 mg/l, at site 2 observed value of TDS was 320 mg/l and the TDS of site 3. i.e. NIT Ghat was 360 mg/l, but after using aqueous leaves extract of any concentration the TDS of water sample were started to increase, possibly due to presence of fine particles of extract mixed with water sample, which was responsible for the increasing of TDS in water sample (Fig. 6).

Colony forming units (CFUs) and total coliform bacteria: Microorganisms are vital components of all ecosystems whose diversity and abundance are greatly influenced by a wide range of factors (Kondratieff, 1985). For example, presence of "sewage fungus" community in water bodies indi-

cates high level of organic pollutants. Water pollution due to domestic, food, dairy, agriculture and industrial wastes contain pathogens (disease causing microorganism). They are hazardous to human health if used as drinking water or food preparation. The bacteria present in water provide an estimate of the presence of water borne pathogens. *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 faecal contamination of water. Microbiological tests involve the determination of bacteria growing a colony on a nutrient medium by standard plant count method and estimation of MPN (Most Probable Number) of coliform bacteria.

Standard plate count (SPC): SPC is used to measure the overall bacteriological quality of drinking water. SPC 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 this study, the trend of inhibition percentage as well as CFUs against different increasing concentration of powdered extract of *Moringa oleifera* were seen in all the sites of river Ganga (Fig. 7, 8 and 9). This result showed reduction of CFUs after using of plant extracts.

Among the three sites, maximum inhibition percent of CFUs was observed in Krishna Ghat which subsequently rose from 25.21% at 0.1% concentration to 89.36% at 0.3% concentration. CFUs of raw water was 468/ml which decreased to 250/ml, 60/l at concentration of 0.16% and 0.3% respectively. Kali Ghat (Site 1) exhibited maximum inhibitory property (87.17%) against CFUs at 0.3% concentration of *M. oleifera*, in comparison to Krishna Ghat (Site 2). At this site CFUs of raw water was 470/ml which decreased to 340/ml, 240/ml, 150/ml and 50/ml at concentration of 0.1%, 0.16%, 0.23% and 0.3% respectively. At site 3 (NIT Ghat), effectiveness of *M. oleifera* in inhibiting the CFUs was only about 25% at 0.1%. At 0.7% the effectiveness rose to 62.5%. At NIT Ghat, *M. oleifera* was most effective (83.33%) at concentration of 0.3%. The effectiveness of *M. oleifera* in inhibiting the CFUs was in accordance with the study on water sample collected from Gomti River by Kumar and Gopal, (2008).

Most probable number (MPN) of coliform bacteria: The most probable number (MPN) methods are commonly employed for enumerating coliform bacteria in water. The MPN method of estimation of coliform bacteria (estimation of concentration of target organisms) includes culture and enzyme – substrate techniques. Culture method relies on the detection of gas evolved when coliform bacteria (*E. coli*) ferment lactose or the presence of turbidity (*Enterococci*), colony formation per color to detect the target organisms.

MPN of the total coliform bacteria was calculated by comparing the positive tubes from MC Crady's statistical table and Thomas formula (APHA, AWWA and WEF, 2005). The aqueous extracts of *M. oleifera* leaves were assessed to observe antimicrobial activity to inhibit coliform. The increasing trend of inhibition percentage of microbes against different increasing dose of powdered extract of *M. oleifera* was seen in all the sites of river Ganga (Fig. 10, 11 and 12).

The presence of total coliform in surface water comes from poor sanitation system. For total coliform, it is perceived that the maximum number of total coliform (1600/100ml) was found in water samples procured from Krishna Ghat (Site 2). Among the treatments, *M. oleifera* was most effective in inhibition of the total coliform at Site 1 (98.33% at 0.3% concentration). The results obtained is in accordance with the findings of (Lea 2010) that the use of *Moringa* extracts achieves up to 99% bacterial removal from untreated water. MPN of total bacterial population of untreated water at this site was 1200/100ml which showed decreasing trend to 80/100ml (inhibition percentage was 93.33%) and 50/100ml (inhibition percentage was 95.83%) at concentration of 0.16% and 0.23% respectively. Krishna Ghat also exhibit inhibitory property (97.5%) against total coliform at 0.3% concentration. At this site total coliform of untreated water was 1600/100ml which decreased to 40/100ml at 0.3% concentration. At Kali Ghat (Site1), effectiveness of *M. oleifera* in inhibiting the total coliform was 90.66% at 0.1% concentration. At 0.7% concentration the effectiveness rose to 96%. At this site total coliform of untreated water was 1500/100ml which decreased to 90/100ml at dose of 0.16% concentration. Abdullah et al. (2014) treated drinking water with powdered extract of *M. oleifera* seeds in the region of Faisalabad district of Pakistan while Hendrawati et al. (2016) treated groundwater, sampled from a well in Pamulang, Jakarta as well as wastewater, sampled from a textile industry in Karawang, Jakarta. Both the studies revealed that *M. oleifera* can be used as a remedy to slow down the growth of coliform populations of untreated water. Present study showed that the powdered extract of *M. oleifera* have a bactericidal property. The bacterial species which were culture, stop growing back after aqueous extract of *M. oleifera* were added.

MPN of *Escherichia coli*: It is deduced from the present study that *Moringa oleifera* has an antibacterial property. Fig. 13, 14 and 15 shows the effect of different doses of aqueous extract of leaves of *M. oleifera* in inhibition of *Escherichia coli* (1/100ml) of untreated water of three study sites. Among the treatments, extracts of *M. oleifera* was found to be most effective against controlling the *E. coli* at Site 3 (99% at 0.3% concentration). After Site 3, Site 2 exhibit maximum inhibito-

ry property (98.6%) against *E. coli* at 0.3% concentration. At this site total coliform of raw water was 1600/100ml which decreased to 40/100ml at dose 0.3% concentration. At Kali Ghat, effectiveness of *M. oleifera* in inhibiting the *E. coli* was 92%, 95.33% and 97.33% at 0.3, 0.5 and 0.7gm respectively. The increasing trend of inhibition percentage of *E. coli* against different increasing dose of powdered extract of *Moringa oleifera* was observed in all the sites of river Ganga Nancy and Ezekiel, (2014) treated tap water, stream water and dam water with powdered extract of *M. oleifera* seeds in Kitale town, Kenya while Vieira et. al, (2010b) treated water sample collected from a lake and river of Sobral, Ceará, Brazil. The studies showed that extracts of *M. oleifera* has antimicrobial effects on the species of coliform organism tested, *E. coli*. Both the studies revealed that *M. oleifera* can be used as a remedy to slow down the coliform populations in untreated water showing bactericidal property. Aqueous extract of *M. oleifera* showed remarkable results in water treatment and can be successfully applied in water treatment instead of conventional method of water treatment which are costly and are in beyond the reach of poor people.

Conclusion

The present study indicated that aqueous extract of *M. oleifera* 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 *M. oleifera* leaves manifest its use in treating diseases caused by bacteria. The results also revealed that aqueous extract of *M. oleifera* leaves has antibacterial effects on the one species of coliform organisms tested i.e. *Escherichia coli*. Therefore, it could be concluded that aqueous extract of *M. oleifera* is 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 are living in extreme poverty and are drinking contaminated surface water as no facilities are available for the treatment. It is environmental friendly and low-cost water treatment method without exhibiting any toxic effect.

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