Research Article

Combined efficacy of *Azadirachta indica* and *Moringa oleifera* leaves extract as a potential coagulant in ground water treatment

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Abstract
Azadirachta indica and *Moringa oleifera* plants have been reported to have strong prospective in the improvement of physiological and chemical parameters of water however very few studies have been reported towards investigating the potential of combinatorial treatment of plant extracts in water treatment. Therefore in this study, we have tried to investigate the efficacy of combined treatment of *Azadirachta indica* and *Moringa oleifera* leaves extract in hexane on ground water. We have used Jar apparatus test (flocculator) to evaluate the purification potential of combined plant extracts treatment on physiological and chemical parameter of ground water in a dose dependent manner. Our experimental findings have strongly proven the better potential of combined extract on different water quality parameters including pH (8.4–7.1), total dissolved solids (525–201), total hardness (253–127), turbidity (15.70–5.10), fluoride content (2.80–1.00), and *Escherichia coli* count (325–30) in comparison to individual extracts of *Azadirachta indica* and *Moringa oleifera* leaves extract at the similar doses (0, 25, 50, and 100 mg/L). Therefore combinatorial treatment could be a strong alternate to the individual extracts in water purification.

Keywords Water treatment · *Azadirachta indica* · *Moringa oleifera* · Natural coagulant · Organic solvents · Ground water

1 Introduction

Nowadays, about 1.1 billion people are at risk due to unavailability of clean water and about 35% people in the developing countries die from water-related issues [1, 2]. Urbanization has been one of the major causes of environmental degradation and harmful human diseases in many developing countries [3]. Unavailability of clean water to the people residing in rural areas either due to the limited awareness or their reluctance in using chemical coagulants has been leading to several human diseases and morbidity [3, 4]. However according to a report of World Health Organization in 2012, clean water and proper sanitation can lessen death cases by about 94% [5]. Due to the expensive nature, limited unavailability and numerous side effects of chemical coagulants [6], there is a strong need to explore the potential of natural herbs for water purification in rural regions.

The possibility of use of plants that are inexpensive and easily available such as *Jatropha curcas*, *Moringa oleifera*, *Strychnos potatorum*, Guar gum, *Azadirachta indica*, *Clidemia angustifolia* and *Hibiscus sabdariffa* [7–15] to remove various contaminants from raw water will provide a inexpensive source of portable water. Amongst them, *Moringa oleifera*, (a tropical) has shown a wide potential in pharmaceutics, nutrition, cosmetics [16]. Several researchers have also validated its efficiency as good natural coagulant in water purification. *Moringa*

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oleifera seed powder has exhibited good coagulation efficiency in water purification [17–20] but with few limitations including limited availability, high sludge volume [21, 22]. Recently, we have investigated that treatment of *Moringa oleifera* leaves extract exhibited significant efficacy in ground water purification by improving various physiological and biological parameters [23]. *Azadirachta indica* has also shown strong biosorbent potential by removing several harmful toxic metals such as chromium and fluoride from waste water [24, 25]. Although both the plants have exhibited better coagulant potential but very less has been reported about the efficacy of combinatorial treatment of *Azadirachta indica* and *Moringa oleifera* leaves extract in improving the water quality parameters. Therefore, present study is designed to perform the comparative analysis between individual extract and combined extract of both *Azadirachta indica* and *Moringa oleifera* leaves extract using hexane solvent in water treatment.

2 Materials and methods

2.1 Materials

n-Hexane, and other chemicals were obtained from Himedia, India. Flocculator (Jar Apparatus) was used for evaluating the coagulation efficiency of plant leaves extract in water purification.

2.2 Ground water sample

In present study, raw ground water samples were collected from NIET (Noida Institute of Engineering and Technology) campus Greater Noida located in Gautam Buddha Nagar District of Uttar Pradesh, India. All the ground water samples were collected in sterile, polyethylene plastic bottles and used for experimental analysis. The treated samples were then stored in deep freezers till further analysis.

2.3 Extract preparation

The *Azadirachta indica* and *Moringa oleifera* leaves were collected from Noida Institute of Engineering & Technology Campus, Greater Noida. Fresh *Azadirachta indica* and *Moringa oleifera* leaves were washed with distilled water and were left for natural drying for 21 days. After natural drying, leaves were then dried in a hot air oven at 35 °C and crushed into fine powder. Fine powder was then used for Soxhlet extraction process.

2.4 Extraction process

Azadirachta indica and *Moringa oleifera* leaves extract were obtained by using soxhlet extraction method with *n*-hexane solvent (1:10 ratio where 1 is plant leaves and 10 is hexane solvent). About 20 g of *Azadirachta indica* and 20 g of *Moringa oleifera* leaves were transferred into a filter paper extraction thimble and extracted with 400 ml *n*-hexane for 9 h at a maximum temperature of 70 °C in a Soxhlet apparatus. The extraction process was continued until clear and transparent refluxing solvent was obtained [26, 27]. After the completion of Soxhlet extraction process, *n*-hexane was removed by drying the extract for solvent evaporation and finally the obtained extract was stored into an air tight container.

2.5 Coagulation process

Jar apparatus test (flocculator) was used to determine the efficacy of *Azadirachta indica* and *Moringa oleifera* leaves extract in water purification as described previously [23]. Flocculator comprises of four beakers (1000 ml capacity) and 4 paddles (20–200 rpm) for analyzing different samples simultaneously. Coagulants (combined and individual leaves extract) were added into each beaker with varying concentrations 25, 50 and 100 mg/L respectively. After treatment, the supernatant was filtered and various chemical, physiological, and biological parameters were analyzed.

2.6 Physiochemical analysis of untreated and treated ground water sample

Different physiochemical parameters of ground water prior and after treatment with a combination of *Azadirachta indica* and *Moringa oleifera* leaves extract were analyzed by using specific methods.

2.6.1 Determination of pH

As per the guidelines of BIS, optimum pH of drinking water should be in the range of 6.5–8.5 (BIS, 2012) [35]. Electronic method (pH meter) (Nihar Instruments, India) was used to measure pH variation in both treated and untreated ground water sample. pH meter was calibrated with standard buffer solution of base, neutral and acid before use [28, 29].

2.6.2 Determination of fluoride content

Ground water possesses more impurity of fluoride content in comparison to surface water due to industrial effluents and fluoride bearing rocks. High fluoride content (more than 2 mg/L) is associated with different ailments such as fluorosis [30]. Fluoride content was estimated by using Octa Aqua Test Kit (WT023, HiMedia) as per the manufacturer's instructions in both treated and untreated water sample.

2.6.3 Determination of total hardness

Hardness in water is mainly contributed by anions (HCO^{3-}, SO^{4-}, Cl^{-}, NO^{3-} and SiO^{3-}) and cations (Ca^{2+}, Fe^{2+}, Mg^{2+}, $Mn^{2+}$and Sr^{2+}) [31]. Hardness concentration more than 200 mg/L has been associated with scale deposition in ground water (WHO 2003). Total hardness in both treated and untreated ground water sample was evaluated by using Octa Aqua Test Kit (WT023, Hi Media) according to the manufacturer's protocol.

2.6.4 Determination of total dissolved solids or TDS

Several studies have reported that high concentration of total dissolved solids (i.e. more than 500 mg/L) leads to numerous gastrointestinal disorders and also decrease palatability [32]. TDS level in both treated and untreated water sample was measured by TDS Meter (Hanna Instruments).

2.6.5 Determination of turbidity

According to WHO, 2012 [33] the acceptable limit of turbidity in drinking water is 6 NTU. Turbidity in water increases due to the presence of particulate matter that results either due to inappropriate filtration or sediment re suspension. Turbidity in untreated and treated ground water sample was determined by Octa Aqua Test Kit (WT023, HiMedia).

2.7 Total *Escherichia coli* analysis

In our study, *E. coli* count analysis was done according to the protocol described by previously [23]. In brief, ground water samples were collected from every individual jar (both treated and untreated sample) and diluted with 0.1% sterile peptone water (ranging from 10^{-1}, 10^{-2} and 10^{-3}). Then, both the treated and untreated ground water samples were filtered using Whatman membrane filters (0.45 µm) on petri dishes containing membrane lactose glucuronide agar (MLGA) followed by incubation at 37 °C for 24 h. Finally the *E. coli* were quantified as green colonies and were estimated by a count/100 ml [34].

2.8 Statistical analysis

All the experiments were performed in triplicates and data were represented as the mean ± SE of three individual experiments.

3 Results and discussion

3.1 Individual and combinatorial effect of leaves extract on pH

As per WHO guidelines, 2012, the acceptable value of pH in drinking water should be between 7 and 8 [33]. pH (negative logarithm of Hydrogen ion concentration) can be referred as the significant parameter for testing the quality of drinking water. Treatments with both individual as well as combined doses of *Azadirachta indica* and *Moringa oleifera* leaves extract have shown effective reduction in pH of water sample in a dose dependent manner in accordance with previous findings [35–40]. However combined leaves extract of both the plants has shown better pH reduction (8.4–7.1) at a lower dose in comparison to individual extracts of *Azadirachta indica* (8.4–7.5) and *Moringa oleifera* (8.4–7.4) (Fig. 1). Thus it can be clearly concluded that combined treatment increased the efficacy of plant extracts on pH reduction at a lower dose and therefore should be strongly preferred over individual plant extract.

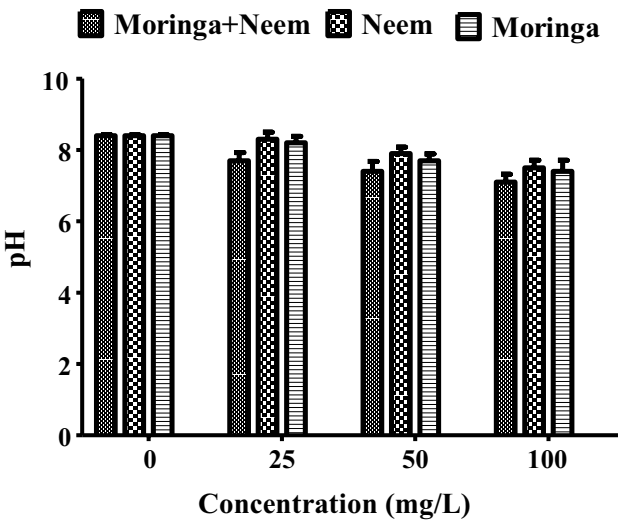

Fig. 1 Effect of individual and combinatorial treatment of leaves extract (Moringa + Neem, Neem and Moringa) on pH of water sample at a concentration of 25–100 mg/L. All the experiments were performed in triplicates and represented as mean ± SEM

3.2 Individual and combinatorial effect of leaves extract on TDS

Increased concentration of total dissolved solids is responsible for decreasing the availability of water (for different purposes including drinking, industrial and irrigation) by increasing the density of water. Dissolved solids also reduce the solubility of dissolved gases present in water thereby hampering the water quality. Further, the efficacies of both plants leaves extract were determined for the TDS removal. By definition, TDS (total dissolved solid) can be defined as the sum amount of both organic and inorganic substances found in numerous forms from molecular, ionized to micro-granular in water [28]. These organic or inorganic contaminants are present in water due to both artificial such as urban run-off, industrial wastewater, sewage, chemicals and natural resources. In Fig. 2, it can be seen that *Azadirachta indica* leaves extract have not shown significant effect on TDS removal at the dose of 25 mg/L whereas moringa leaves (525–279 mg/L) and combined leaves extract (525–201 mg/L) have shown significant reduction in a dose dependent manner (Fig. 2). Results strongly indicated that combinatorial treatment has shown better efficacy than both the individual extracts.

3.3 Individual and combinatorial effect of leaves extract on total hardness analysis

Combinatorial treatment of both plant leaves extract has depicted more significant reduction in total hardness of water sample (Fig. 3) as compared to individual extracts

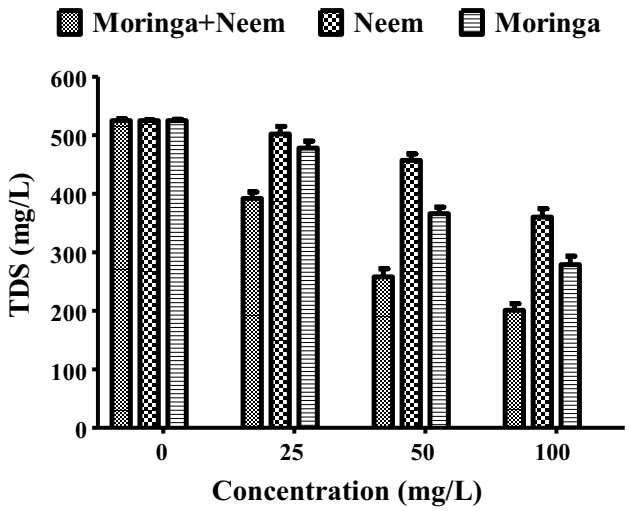

Fig. 2 Effect of individual and combinatorial treatment of leaves extract (Moringa + Neem, Neem and Moringa**)** on TDS (Total dissolved solids) of water sample at a concentration of 25–100 mg/L. All the experiments were performed in triplicates and represented as mean ± SEM

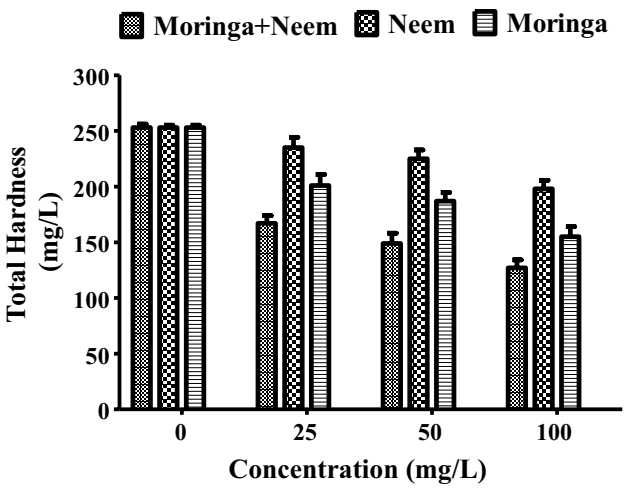

Fig. 3 Effect of individual and combinatorial treatment of leaves extract (Moringa + Neem, Neem and Moringa**)** on total Hardness of water sample at a concentration of 25–100 mg/L. All the experiments were performed in triplicates and represented as mean ± SEM

of *Azadirachta indica* and *Moringa oleifera* as depicted in earlier reports [41, 42]. Although both individual and combined extracts have shown significant decrease in total hardness of ground water in a dose dependent manner, combinatorial treatment (253–127 mg/L) has shown more reduction in the total hardness at the same dose in comparison to the individual extracts of Moringa (253–155 mg/L) and *Azadirachta indica* leaves (253–198 mg/L). Thus it can be concluded that combined extract treatment has improved the efficacy by combining the potential of both the individual extracts and therefore should be preferred over individual extract for showing better reduction efficiency in water treatment.

3.4 Individual and combinatorial effect of leaves extract on turbidity

After treatment with both individual and combined leaves extract of *Azadirachta indica* and *Moringa oleifera*, the turbidity was reduced significantly in dose dependent manner (Fig. 4). Moringa extract (15.7–5.10 NTU) has shown better efficacy in turbidity removal than *Azadirachta indica* leaves (15.7–6.8 NTU) However extracts in combination (15.7–5.10 NTU) has depicted better reduction in turbidity at the same dose of individual extracts of *Azadirachta indica* and *Moringa oleifera* thereby indicating the better potential of combined extract.

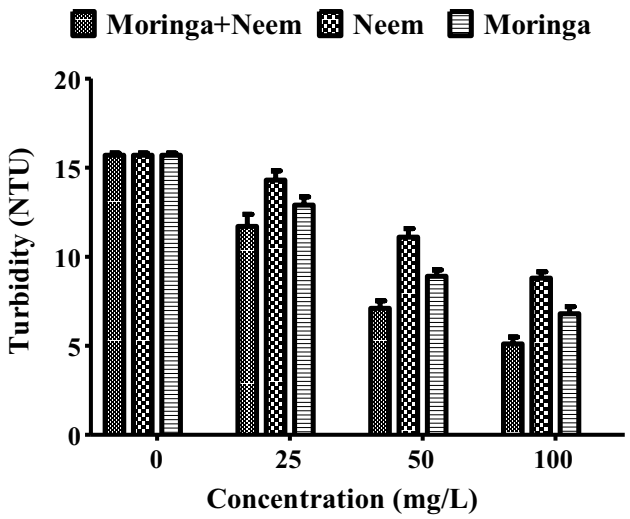

Fig. 4 Effect of individual and combinatorial treatment of leaves extract (Moringa + Neem, Neem and Moringa) on Turbidity of water sample at a concentration of 25–100 mg/L. All the experiments were performed in triplicates and represented as mean ± SEM

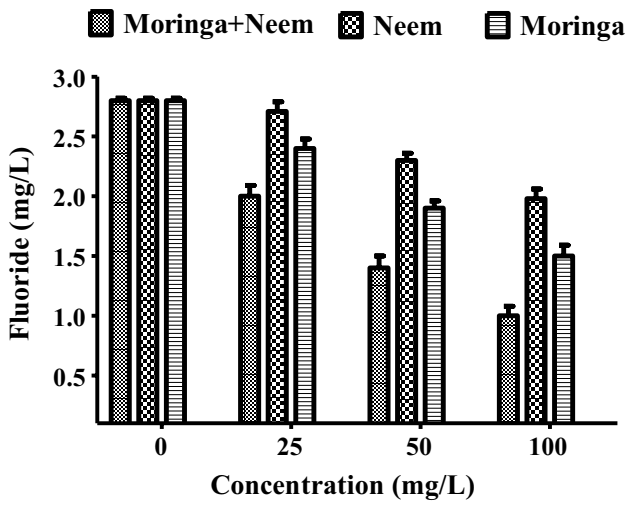

Fig. 5 Effect of individual and combinatorial treatment of leaves extract (Moringa + Neem, Neem and Moringa) on Fluoride content of water sample at a concentration of 25–100 mg/L. All the experiments were performed in triplicates and represented as mean ± SEM

3.5 Individual and combinatorial effect of leaves extract on fluoride

High fluoride content has been associated with several health related issues in humans such as skeletal or dental fluorosis and several studies have reported the presence of excess fluoride in groundwater of many developing countries [43]. Both the combined as well as individual extract treatment has shown significant reduction in fluoride content in a concentration dependent manner. *Azadirachta indica* leaves has shown significant reduction at the dose of 100 mg/L (1.98 mg/L) whereas *Moringa oleifera* extract has shown significant reduction at the dose of 50 mg/mL (2.40 mg/L) and 100 mg/L (1.50 mg/L). However, combinatorial treatment has shown the better reduction of fluoride content (2.8–1.00 mg/L) at the similar doses of individual extracts of both plants (Fig. 5). Thus it can be concluded that combinatorial treatment has enhanced potential in fluoride removal than the individual extracts in corroboration with our previous findings.

3.6 Individual and combinatorial effect of leaves extract on *E. coli*

In this study, effect of leaves extracts of *Azadirachta indica* and *Moringa oleifera* plants either in alone or in combination on biological contaminant i.e. *E. coli* in water sample was determined. *Azadirachta indica* and *Moringa oleifera* has been known for its anti bacterial and antifungal potential therefore we have evaluated the effect on *E. coli* count in treated water sample. It can be seen from the Fig. 6 that *Azadirachta indica* has shown better reduction in *E. coli* count than *Moringa oleifera* leaves extract. However after treatment of ground water sample with combined extract of *Azadirachta indica* and *Moringa oleifera*, there has been a more significant reduction in *E. coli* count than the individual extracts.

Several reports have proven the antibacterial potential of both *Azadirachta indica* and *Moringa oleifera* leaves against the *E. coli* strains [43–45]. Our previous study

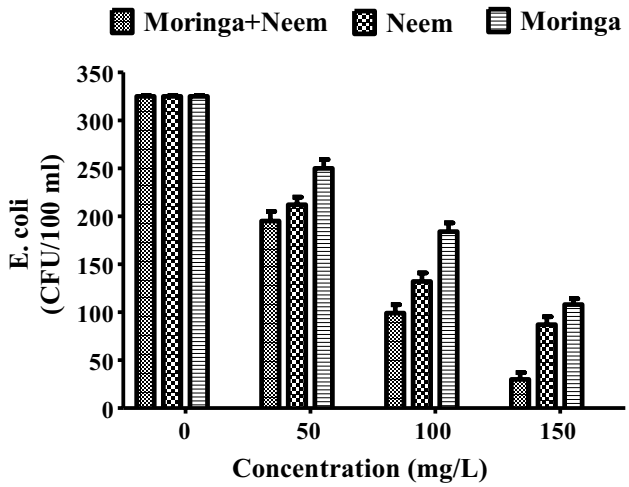

Fig. 6 Effect of individual and combinatorial treatment of leaves extract (Moringa + Neem, Neem and Moringa) on *E. coli* count of water sample at a concentration of 25–100 mg/L. All the experiments were performed in triplicates and represented as mean ± SEM

has also showed that *Moringa oleifera* leaf extract has shown significant antibacterial potential in water treatment process [23]. In addition, present study has revealed that combined extract of *Azadirachta indica* and *Moringa oleifera* leaves has depicted enhanced efficiency than the individual extracts. Through these results it can be hypothesized that the probable mechanism behind this antimicrobial elimination in treated ground water sample could be due to coagulation, precipitation and disruption of cell membrane in accordance with the previous findings [46–50]. Thus, after the treatment of ground water sample with natural coagulant, the cationic protein present in the *Azadirachta indica* and *Moringa oleifera* leaves extract interacted with negatively charged particle found in ground water sample. These interactions then resulted in precipitate development of particulate matter by the disturbance in the association of suspended particles. Thus it can be concluded that combined treatment of *Azadirachta indica* and *Moringa oleifera* leaves extract has better purification potential than the individual extracts in water treatment processes.

4 Conclusion

People residing in rural or tribal areas have been using the water from any sources without any treatment due to their limited awareness about the impurities present in water and their associated side effects. They are also reluctant in using any chemical coagulant for water purification due to their higher cost. Therefore there is a strong need to find a natural alternative for the people of rural areas. Plants with medicinal properties including *Azadirachta indica* and *Moringa oleifera* leaves used in this study can be effectively used as a strong natural coagulant for water purification. Using these extracts, not only harmful and pathogenic microbes from water can be removed but their quality parameters can also be improved with minimal or no side effects and thus could provide water safe for the people of rural areas. Coagulation potential of both individual and combined extract of *Azadirachta indica* and *Moringa oleifera* leaves has been exploited in our study to elucidate a cost effective method with minimal side effects. Experimental results showed that treatment with combined extract of *Azadirachta indica* and *Moringa oleifera* leaves extract can greatly improve both the physiological parameters (pH, turbidity, total dissolved solid) and chemical parameter (fluoride content) of ground water at the similar dose in comparison to individual extract. Furthermore combinatorial treatment has also exhibited significant antibacterial efficacy than the individual extract of *Azadirachta indica* and *Moringa oleifera* leaves extract. Therefore, this study strongly suggested that combined treatment of leaves extract obtained in hexane solvent could be a potent coagulant with less side effects and cost. Further studies are still needed to develop this concept into a cost efficient technique for providing safe water to the people residing in rural areas of developing countries.

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Data Availability The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Compliance with ethical standards

Conflict of interest All the authors associated with this manuscript declare that there is no conflict of interest.

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