Background

Water is a vital natural resource for all life on earth which is not always suitable for all uses. Globally, surface waters are polluted with pathogens in most countries but are widely recorded in the developing world. Consumption of these polluted surface waters leads to waterborne disease outbreaks. Globally, about 663 million people depend on unimproved water sources and about 2.1 billion use contaminated water. According to World Health Organization (WHO) report, only 15% to 20% of the world’s population have access to safe drinking water. It was also reported that more than 700 million people, which are mainly living in developing countries, are without access to improved and adequate water. Each year, unsafe drinking water contributes to more than 25 million cases and 250,000 deaths from enteric fevers (typhoid and paratyphoid). In sub-Saharan African countries, only 24% of the population uses safely managed water sources and there is high morbidity in the age group below 5 years ranging from 10% to 35% which is mainly caused by a lack of safe water. In Ethiopia, communities are highly affected by waterborne diseases due to a lack of access to a potable water supply that obliges them to use unimproved and unsafe sources of water for domestic purposes. Providing safe, affordable and reliable water is an essential goal, yielding optimal health gains and contributing to the international targets for poverty reduction, nutrition, childhood survival, and environmental sustainability. Household water treatment methods like chlorine-based disinfectants, filtration, solar disinfection, and boiling are best for sub-Saharan African countries. However, only a small number of households are using them, and their consistency in use is dropping over time.
A millennium development goal (MDG) was aimed to halve the proportion of people without sustainable access to safe drinking water by 2015. However, Ethiopia achieved 57% of the goal which still needs more effort to provide safe drinking water. The country also launched a health extension program with water supply components in 2003 but was still unable to address what was planned.17

Chemical water treatment methods are in use, but behind their advantages, there are also disadvantages like expensive-ness, unavailability, detrimental effect on health and unskilled labor to handle chemicals.1,18-21 Aluminum sulfate (alum) has been indicated to be a causative agent in neurological diseases and the induction of Alzheimer’s disease. Chlorine and its compounds are also known to produce trichloromethane, a cancer precursor.1,20,22,23

Scientists are on the search for water treatment methods which are non-expensive and can be easily handled by the local community.24 Natural plants are the ones in the study and some were identified as effective in reducing agents causing waterborne diseases and turbidity of water. They are also stated as having good advantages like biodegradable sludge production, being virtually toxin-free, relatively cheap to obtain, and being locally available.19,22,25 These advantages currently attracted the attention of many researchers to study them for water treatment purposes as a coagulant, disinfectant or heavy metal removal.

This study was used to test the treatment efficiency of M. stenopetala and C. farinosa. A study conducted at Jimma University in Ethiopia reported that a 93% reduction in fecal coliform was recorded after treatment with 60 mg weight of C. farinosa root powder.24 It was also reported that crushed seeds of M. stenopetala were effective in reducing bacterial contamination by 90% to 99.9%.1

There are factors identified by different scientific researches like turbidity, pH, contact time, settling time, stirring time, temperature, and dosage/concentration that might affect the treatment efficiency of natural plants in reducing microbes or turbidity or heavy metals from water.1,22,24,26-28 The effect of turbidity, pH, settling time, temperature and dosage were considered in this study. Mixing/stirring time plus settling time is equal to contact time based on this study.

A study conducted in Ethiopia also stated that M. stenopetala leaf had 28.44% protein.29 Another study reported that M. stenopetala seed contains 42.6% of protein.30 C. farinosa seed contains 26.72% protein.31 Also, C. farinosa leaf contains 15.2% to 18.2% of protein, crude fiber and ash, respectively.32 Protein composition (especially water-soluble proteins) in plant parts has been predicted as removing microorganisms by serving as a coagulant or acting directly as growth inhibitors of the microorganisms.1 Because of this, C. farinosa (leaf and seed) and M. stenopetala (leaf and seed) with high protein composition were selected for this study. There is no previous study conducted on bacterial removal efficiency of C. farinosa (leaf and seed) and M. stenopetala leaf and little attention has also been given in research world to M. stenopetala in treating water. Thus, the aim of this study is to identify the most efficient plant part in removing E. coli from surface water between C. farinosa (leaf and seed) and M. stenopetala (leaf and seed).

Previous studies were used as a base to select appropriate dosages and settling times that were used to treat surface water under this study and explained as follows. A study in Nigeria on the antimicrobial efficiency of M. oleifera seed in treating water used 3 dosages such as 50, 100, and 150 mg. It showed a total coliform reduction of 68.33%, 85%, and 97.5% at 50, 100, and 150 mg dosage per liter of water, respectively. The study also shows 96.3%, 96.6%, and 97.4% turbidity reduction at 50, 100, and 150 mg dosage per liter of water, respectively.24 A study conducted at Jimma University, Ethiopia on contaminant removal of water with C. farinosa root and leaf powder was used 60, 100, 300, 500, and 1000 mg dosages, and 15, 30, 45, 60, 75, 90, and 150 minutes of settling times. It showed that 60 mg dosage was efficient in removing fecal coliform by 93% with both Moringa oleifera (M. oleifera) seed and C. farinosa root powder. It also shows 98.6% and 99.4% total coliform reduction by C. farinosa root powder dosages of 60 and 100 mg, respectively. It also indicated that there was 90% and 99% turbidity reduction in 30 and 90 minutes of settling time, respectively, after treatment with 60 mg C. farinosa root powder.34 It was revealed that 100 mg/L M. stenopetala seed is equally effective in water clarification when compared with 200 mg/L of M. oleifera seed.1 So, based on these studies, 3 dosages such as 30, 60, and 100 mg, and 3 settling times such as 30, 60, and 90 minutes were selected for this study.

The water samples used for this study were collected from Lake Hawassa. It is the smallest Lake and the highest in altitude among the Ethiopian Rift valley closed basin lakes, located between 06°58′ to 07°14′ North latitudes and 38°22′ to 38°28′ East longitudes with an elevation of 1697 m above sea level. Its annual rainfall average is 969 mm.35 The lake is a shallow lake with an average depth of 13.3 m and a maximum depth of 23.4 m.36 It has a surface area of 90 km² and a drainage area of 1250 km² and a water storage capacity of the lake is 1.36 km³.38

**Methodology**

**Study area**

This study was conducted in the environmental health laboratory at Hawassa University, College of Medicine and Health Science in Hawassa City of Sidama region in Ethiopia.

M. stenopetala and C. farinosa are plants used for this study and were collected manually for free from farmers’ land around Holte Town of Derashe special Woreda in southern Ethiopia from June 17 to 21, 2021. The plants were identified for collection based on their botanical, biological, and ecological description.39-43 Each 50 g weight of 1 plastic bag of M. stenopetala leaf, C. farinosa leaf, and C. farinosa seed were collected.
separately. Also, 20 matured seed pods of *M. stenopetala* were collected and made dry. Matured seeds were collected from the 2 plants as shown in Figure 1. Each seedpod of *M. stenopetala* contains 12 to 35 round seeds and it was noted that 1 seed kernel of *Moringa* treats 1 L of water. So, a total of 10 pods of *M. stenopetala* containing the required seeds were used for this study. They were collected in the morning starting from 6:30 Am to 8:30 Am and taken to the laboratory immediately. Figure 1 shows *M. stenopetala* seed pod, *C. farinosa* seed fruits, *M. stenopetala* leaf, and *C. farinosa* leaf, respectively.

**Study design and period**

A cross-sectional study was conducted to assess the bacterial removal efficiency of *M. stenopetala* and *C. farinosa* (leaf and seed) from surface water from June 30 to July 06, 2021.

**Preparation of *M. stenopetala* and *C. farinosa* seed and leaf powder**

The *M. stenopetala* seeds with shells were removed from the pods and air-dried for 2 days. The shells were removed and the seed kernels were dried in a plastic bag at ambient temperatures (23°C-25°C) for 5 days before milling. Similarly, the collected leaves of *M. stenopetala* were also shaded to complete dryness at ambient temperatures (23°C-25°C) for 5 days before milling. The seed kernels and dried leaves were ground into a medium-fine powder with a local mill to achieve solubilization of active ingredients in the seed. Then, the powders of the leaf and seed were packed in a small plastic bag separately for safety (as seen in Figure 2) and kept in the laboratory under an ambient temperature of 22°C to 25°C and stored in a desiccator to create and maintain a dry environment until treatment and analysis.

*C. farinosa* seeds with very thin coats/fruits were collected from the trees in a plastic bag. The coats were removed from the seeds. Then after, the seeds were dried in a plastic bag at ambient temperatures (23°C-25°C) for 5 days before milling. The collected leaves of *C. farinosa* were also shaded to complete dryness at ambient temperatures (23°C-25°C) for 5 days before milling. Then after, the same procedure was followed as used for *M. stenopetala* seed and leaves. Figure 2 shows powders prepared from *M. stenopetala* seed, *C. farinosa* seed, *M. stenopetala* leaf, and *C. farinosa* leaf, respectively.

**Water sample collection**

Water samples for all the tests were collected from Lake Hawassa which is found encircling one side of the city. The reason why water samples were collected from surface water (Lake Hawassa) is to take natural waters instead of preparing synthetic polluted water in the assumption that the rural communities on the other side of the lake are speculated to use the raw surface waters for domestic purposes. So, the treatment efficiency of the leaf and seed of the 2 plants was tested against the actual water used by the rural community.

The water samples were collected following surface water sampling procedures using high-density polyethylene (HDPE) bottles washed 3 times with sample water before collection. First, the water sample was collected into a disinfected 20 L container up to 17 L volume by using a sterilized 300 mL plastic sample bottle by holding the lower part of the bottle and submerging it to a depth of about 20 to 30 cm, with
the mouth facing slightly upwards. Then after, the water sample in the 20L plastic container was shaken/mixed and added to 48 sterilized 300mL volume sample bottles. Finally, the sample bottles with sample waters were capped and transported to the laboratory in an ice box within 15 minutes. After reaching the laboratory, the water samples were shaken and analyzed and placed in the refrigerator until treatment.

**Experimental phase**

Before starting treatment, the raw water sample *E. coli*, pH, temperature and turbidity were measured by using the membrane filtration method, digital pH meter, liquid-in-glass thermometer, and digital turbidity meter, respectively.

During treatment, each 1-L volume water sample was added to 3 beakers for treatment. Then, the beakers were labeled and placed on a magnetic stirrer at a speed of 60 revolutions per minute (rpm). The powders (30, 60, and 100 mg dosages) per each part of *M. stenopetala* and *C. farinosa* were measured on an analytical balance and manually added to the 3 beakers based on the respective dosages labeled on them. The water samples were then mixed with the aid of the magnetic stirrer for 5 minutes. After mixing, the water was allowed to settle for 30, 60, and 90 minutes on which analysis was done in each case. Finally, after treatment, the *E. coli*, turbidity, temperature and pH of treated water samples were measured to calculate the improvement from the reading before treatment.

This study followed the methods of a study conducted in Nigeria at Ahmadu Bello University on the antimicrobial efficiency of *Moringa oleifera* (*M. oleifera*) seed in the treatment of greywater.

**Bacteriological analysis**

A membrane filtration technique was used, both before and after the treatment of the water samples, to determine the amount of *E. coli* in the water. A total of 78 membrane filters with a pore size of 0.45 μm were used. Forty Petri dishes were sterilized and prepared for use on each day of the study, before starting water sample treatment and laboratory analysis. First, a 100 mL volume water sample was filtered on the filtration apparatus through a membrane filter. Then, the membrane filter was taken from the filtration apparatus by sterilized forceps and placed on top of an absorbent pad saturated with the Membrane Lauryl Sulfate Broth (MLSB) media on sterilized petri-dish. Then, the petri-dish lid was replaced and labeled with sample identification. The Petri dishes were then placed into the incubator (model DHP9052) and incubated for 24 hours at 44°C. Upon completion of the incubation period, the numbers of *E. coli* were enumerated using a digital colony counter based on the yellow color bacteria colonies formed on the membrane filter. Finally, *E. coli* concentrations were reported as colony-forming units per 100 mL of water (CFU/100 mL).

**Physicochemical parameters analysis**

**Temperature and pH of water.** Water samples temperature was measured by a liquid-in-glass thermometer and pH was measured by a digital pH meter (Model AD8000) in the laboratory both before and after treatment with the 2 plants’ leaf and seed powders. A buffer solution calibrated pH meter was used to measure the pH of the water sample in the laboratory. Accordingly, the results were read from the display.

**Turbidity.** A digital turbidity meter (MAX electronics (India)) was used to measure the turbidity of the water samples both before and after treatment with the 2 plants’ leaf and seed powders. First, the instrument was warmed-up for 2 to 3 minutes and calibrated. Then after, a test tube was filled with the sample water to the 10mL mark and placed in a tube holder, and a turbidity meter reading was taken directly from the instrument. The results were read directly from the digital turbidity meter display and reported as the nephelometric Turbidity Unit (NTU).

**Quality assurance.** To ensure data quality, a 1-day training was given for the 2 plants, leaf and seed collectors. The water samples were collected by the principal investigator. Before actual treatment and analysis, laboratory instruments were checked for proper functioning and some were calibrated. Before analysis was undertaken, all the reagents used were checked for expiration dates. All plastics and glassware utilized were disinfected with appropriate detergent and water solution, which was then rinsed thoroughly with distilled water. All containers for bacteriological analysis were sterilized in an autoclave (Model SA-300H) at 1210°C for 15 minutes. A flame was used to sterilize forceps and filtration apparatus in between each use. Triplicate tests were conducted on raw water samples before treatment by the plant parts.

**Data analysis**

Data from the result of this study were analyzed by using the statistical package for social sciences (SPSS) version 23. The significance of the difference in *E. coli* removal between *M. stenopetala* leaf, *M. stenopetala* seed, *C. farinosa* leaf, and *C. farinosa* seed was tested by using Friedman rank tests. In Friedman, the test is significant when *P*-value is less than .05 at a 95% confidence interval. And, the significance of the differences in *E. coli* removal within plant parts was tested by using the Wilcoxon–signed rank test, which is significant at a *P*-value less than .05. In addition to this, the factors that have an association with *E. coli* removal were determined by using the Kendall’s-Tau correlation, which shows a significant correlation/association at *P*-value less than .05.

**Ethical consideration**

Ethical clearance was obtained from Hawassa University, College of Medicine, and Health Science ethical board.
committee. During plant leaf and seed pod collection, the purpose of the study was clearly explained to farmers from which plant leaf and seed were collected. Informed verbal consent was obtained from participants for plant collection.

Result
Treating Lake Hawassa water sample by M. stenopetala and C. farinosa

Lake Hawassa water sample with an initial E. coli of 18 CFU per 100 mL of water, a pH of 7.91, a temperature of 22°C and turbidity of 12 NTU was treated with 30, 60, and 100 mg dosages of M. stenopetala and C. farinosa for a settling time of 30, 60, and 90 minutes. After treatment with M. stenopetala seed and leaf, there was 0 CFU per 100 mL of water. In reducing E. coli, the seed shows maximum efficiency at a minimum dosage of 30 mg after 90 minutes of settling time and the leaf shows maximum efficiency at a minimum dosage of 60 mg after 90 minutes of settling time. M. stenopetala leaf also shows the highest reduction in turbidity by 83.3% (from 12 NTU to 2 NTU) at 60 mg dosage after 90 minutes of settling time. Similarly, in water treatment with C. farinosa seed, there was 0 CFU per 100 mL of water. In reducing E. coli, the seed was more efficient at a minimum optimum dosage of 30 mg after 90 minutes of settling time compared to a similar amount of seed. C. farinosa seed showed 62% E. coli removal (from 18 CFU to 7 CFU per 100 mL of water) at a minimum optimum dosage of 30 mg after 30 minutes of settling time which shows its inability to reduce E. coli to 0 CFU per 100 mL of water.

The pH was recorded between 7.30 and 8.50 at a temperature of 20°C to 23.5°C. The details of the results are shown in Table 1.

After treatment of water samples by both M. stenopetala seed and leaf, there were cell colony bursts on the membrane filter after 24-hour incubation, irrespective of the presence of E. coli colonies. The color of the membrane filters was also changed due to the color of seed and leaf powder in addition to the color of Membrane Lauryl Sulfate Broth (MLSB) media used as shown in Figure 3.

E. coli and turbidity removal efficiency between plants and their parts

The significance of differences in E. coli removal among C. farinosa and M. stenopetala after treatment of Lake Hawassa water samples was tested using Friedman rank test. The test is significant at a P-value < .05 at 95% confidence interval. And the significant difference in E. coli removal within plant parts was tested by using the Wilcoxon-signed rank test, which is significant at a P-value < .05.

The result of the statistical analysis revealed that, after treatment of Lake Hawassa water sample, the Friedman rank test shows a significant difference between C. farinosa leaf, C. farinosa seed, M. stenopetala leaf, and M. stenopetala seed in removing E. coli (P-value = .005, $X^2 = 13.000$, df = 3, N = 9). Comparing plant parts using Wilcoxon-signed rank test, there was no significant difference between M. stenopetala seed and leaf in E. coli removal (P-value = .062). But, there was a significant difference between C. farinosa seed and leaf in removing E. coli (P-value = .011). Based on the positive ranks from the value of the test statistics, C. farinosa seed had higher E. coli removal efficiency compared to its leaf. C. farinosa leaf and M. stenopetala leaf also show a significant difference in E. coli removal (P-value = .017). Based on the value of the test statistics, M. stenopetala leaf had higher E. coli removal efficiency compared to C. farinosa leaf. The test also shows no significant difference between C. farinosa seed and M. stenopetala seed in E. coli removal (P-value = .461).

Result from Kendall’s Tau correlation

The result of this study discovered that turbidity shows a strong positive significant correlation with E. coli removal during M. stenopetala seed (P-value = .022, correlation coefficient = .647). However, temperature and pH have no association with E. coli removal during M. stenopetala seed. Turbidity also shows a strong positive correlation with E. coli removal during M. stenopetala leaf (P-value = .009, correlation coefficient = .775). However, temperature and pH have no association with E. coli removal during M. stenopetala leaf. And similarly, turbidity, temperature and pH have no association with E. coli removal after treatment of Lake Hawassa water sample by C. farinosa seed and leaf.

E. coli removal at different doses and settling time

Figure 4 shows the effect of settling time on E. coli removal from Lake Hawassa water samples using M. stenopetala. As depicted in the result, there was a successive reduction in E. coli as settling time increased both for the leaf and seed. M. stenopetala seed was more efficient in removing E. coli at a smaller settling time compared to its leaf. The line moved to negative shows speed of removal efficiency.

The effect of dosage on E. coli removal from Lake Hawassa water sample by using M. stenopetala is displayed in Figure 5. The result found that as the dosage of M. stenopetala increased the E. coli removal also increased. Treated water with 0 CFU per 100 mL of water was achieved after treatment with M. stenopetala seed at a minimum optimum dosage of 30 mg/L after 90 minutes of settling time. M. stenopetala seed showed higher E. coli removal to 0 CFU per 100 mL of water at a smaller dosage when compared to M. stenopetala leaf. It is described in Figure 5.

The effect of settling time on E. coli removal from Lake Hawassa water sample by C. farinosa is shown in Figure 6. As depicted in the result, there was a reduction in E. coli as settling time increased with C. farinosa seed treatment. There is no relationship between C. farinosa leaf and settling time.
Table 1. Water quality result before and after treatment with *M. stenopetala* and *C. farinosa*, Hawassa city in Ethiopia, 2021.

| PLANT PART                          | DOSAGE | SETTLING TIME | PH  | TEMPERATURE (°C) | TURBIDITY (NTU) | E. Coli (CFU/100ML) | E. Coli REMOVAL EFFICIENCY IN % |
|-------------------------------------|--------|---------------|-----|-----------------|-----------------|---------------------|-------------------------------|
| Before treatment                    |        |               |     |                 |                 |                     |                               |
|                                     | 7.91   | 22            | 12  | 18              |                 |                     |                               |
| After treatment by *M. stenopetala* Leaf powder | 30 mg/L | 30 min | 8.10 | 23              | 8               | 15                  | 16.7                          |
|                                     | 60 min | 7.80 | 23 | 3 | 6 | 66.7 |                                 |
|                                     | 60 min | 7.24 | 23 | 4 | 6 | 66.7 |                                 |
|                                     | 90 min | 7.95 | 23.5 | 2 | 0 | 100 |                                 |
| After treatment by *M. stenopetala* Seed powder | 30 mg/L | 30 min | 7.30 | 22 | 12 | 14 | 22.2 |                                 |
|                                     | 60 min | 8.13 | 22 | 12 | 8 | 55.6 |                                 |
|                                     | 90 min | 7.82 | 22.5 | 8 | 0 | 100 |                                 |
| After treatment by *C. farinosa* Leaf powder | 30 mg/L | 30 min | 8.1 | 23 | 8 | 7 | 61.1 |                                 |
|                                     | 60 min | 8.0 | 22.5 | 5 | 16 | 11.1 |                                 |
|                                     | 90 min | 8.0 | 23 | 8 | 9 | 50 |                                 |
| After treatment by *C. farinosa* Seed powder | 30 mg/L | 30 min | 8.5 | 23 | 11 | 18 | 0 |                                 |
|                                     | 60 min | 7.8 | 22.5 | 8 | 15 | 16.7 |                                 |
|                                     | 90 min | 8.0 | 23 | 6 | 13 | 27.7 |                                 |
| After treatment by *C. farinosa* Seed powder | 30 mg/L | 30 min | 8.0 | 23 | 12 | 24 | −33.3 |                                 |
|                                     | 60 min | 7.8 | 23 | 9 | 17 | 5.5 |                                 |
|                                     | 90 min | 8.0 | 23 | 10 | 13 | 27.7 |                                 |
| After treatment by *C. farinosa* Seed powder | 30 mg/L | 30 min | 8.5 | 22.5 | 12 | 10 | 44.4 |                                 |
|                                     | 60 min | 8.4 | 22.5 | 11 | 5 | 72.2 |                                 |
|                                     | 90 min | 8.3 | 23 | 8 | 0 | 100 |                                 |
| After treatment by *C. farinosa* Seed powder | 30 mg/L | 30 min | 7.9 | 22 | 8 | 13 | 27.7 |                                 |
|                                     | 60 min | 8.3 | 22.5 | 8 | 2 | 88.9 |                                 |
|                                     | 90 min | 8.2 | 23 | 10 | 0 | 100 |                                 |
| After treatment by *C. farinosa* Seed powder | 30 mg/L | 30 min | 7.9 | 22.5 | 12 | 10 | 44.4 |                                 |
|                                     | 60 min | 7.9 | 22.5 | 9 | 3 | 83.3 |                                 |
|                                     | 90 min | 8.2 | 23 | 11 | 0 | 100 |                                 |
because it shows a slight increment in E. coli up to 60 minutes followed by decrement which is not associated with settling time.

Figure 7 illustrates the effect of C. farinosa dose on E. coli removal from Lake Hawassa water sample. It was found that there was a noticeable reduction in E. coli as C. farinosa seed dosage increased. The treated water using C. farinosa seed has 0 CFU per 100mL at a minimum optimum dosage of 30 mg after 90 minutes of settling time. On the contrary, C. farinosa leaf was not as effective in reducing E. coli to 0 CFU per 100mL of water and there was no relationship between the leaf dosage and E. coli removal.

**Discussion**

One of the good approaches used in the provision of safe water for rural communities of developing countries could be the use of natural plants. Some of such natural disinfectants could be M. stenopetala and C. farinosa. In this study, the efficiency of M. stenopetala and C. farinosa in removing E. coli from surface water was assessed.
The findings of this study show that there is no significant difference in *E. coli* removal between *M. stenopetala* leaf and *M. stenopetala* seed (*P*-value = .062). This might be due to the nature of both plant parts for having water-soluble proteins (active agents) which are stated as removing bacteria through coagulation or acting directly as growth inhibitors of the microorganisms.1,30,33 This study also shows that there is similar treatment efficiency between *C. farinosa* seed and *M. stenopetala* seed in *E. coli* removal (*P*-value = .461).

Treatment of water with 100 mg *C. farinosa* leaf, seems to slightly increase the *E. coli* from 18 to 24 CFU per 100 mL with a turbidity of 9 NTU. This might be because turbidity above 2 NTU affects the microbiological quality of water by complicating the detection of bacteria or by protecting/shielding pathogens from the action of disinfectants.54 This study revealed that after a certain reduction in turbidity, in a few of the samples, the turbidity starts increasing, especially during *C. farinosa* leaf and seed. This might be due to the “charge reversal” reaction which is stated as common in natural plant coagulants in which after subsequent turbidity reduction the formed flock becomes destabilized and result in a successive increase in turbidity.24,55

Treatment of the surface water with *C. farinosa* seed in this study resulted in 0 CFU per 100 mL (100% removal efficiency), which is slightly higher than the Jimma University study by *C. farinosa* root which showed 99.4% and 93% total coliform and fecal coliform removal, respectively.24 This higher efficiency in the present study might be due to the high protein composition in *C. farinosa* seed (26.72%) compared to the root (4.22%), which was stated as a plant component able to act as a disinfectant.1,31,56 *M. stenopetala* seed was more efficient in removing *E. coli* from surface water samples up to 0 CFU per 100 mL (100% removal efficiency), which is also confirmed by a study that reported bacterial load reduction of 90% to 99.9% by whole crushed seeds of *M. stenopetala*.1

The finding of this study also designated that 0 CFU per 100 mL (100% removal efficiency) was achieved at 60 mg/L dosage of *M. stenopetala* seed powder. The findings of this study are higher than a study conducted in Nigeria using *M. oleifera* seed which showed 85% antimicrobial efficiency at 100 mg/L dosage.34 A study conducted in Ethiopia also reported that *M. oleifera* seed powder showed the highest bacterial load reduction of 97.5% at 80 mg/L dosage which is slightly lower than the result of this study.28 This might be due to the high watersoluble protein content in *M. stenopetala* seed (42.6%) when compared to *M. oleifera* seed (23%-27%), which is a component able to act as a disinfectant.1,30,57

It was confirmed that 100% *E. coli* removal efficiency can be attained using *M. stenopetala* seed and *C. farinosa* seed at a minimum optimum dosage of 30 mg at 95 minutes of contact time, which is equal in treatment efficiency at 1.5 g per 1 L of water by free chlorine concentration after 30 minutes of contact time. This shows that free chlorine concentration is effective at low concentrations and shortest contact time when compared to

![Figure 6](image-url) Effect of settling time on *E. coli* removal, Hawassa city in Ethiopia, 2021.

![Figure 7](image-url) Effect of *C. farinosa* dosages on *E. coli* removal, Hawassa city in Ethiopia, 2021.
the 2 plants. The result also has similar treatment efficiency with Dolichos lablab (Hyacinth Bean) extract exposed to sunlight after treatment, which showed 100% E. coli removal within 2 hours.

The turbidity reduction from 12 NTU to 4 NTU (66.7%) was reached using a 100 mg/L dosage of M. stenopetala seed. This result is slightly lower than a study conducted on M. stenopetala seed that reported 98.5% turbidity removal by 100 mg/L powder dosage after 60 minutes of settling time. The difference might be due to the variance in the type of raw water used since the present study used surface water while the study in comparison used tannery wastewater. Likewise, a turbidity reduction of 59% was achieved using C. farinosa leaf, which is lower than a study conducted at Jimma University which showed a turbidity reduction of 90% by C. farinosa leaf. This might be due to differences in the initial turbidity of the raw water used since the initial turbidity of this study was 12 NTU which is lower than the initial turbidity of 938 NTU obtained under the Jimma University study.

The WHO guidelines and Ethiopian water quality standards recommend drinking water must have a maximum permissible turbidity limit of 5 NTU, 6.5 to 8.5 pH and no detectable E. coli per 100 mL of water. After treatment of the surface water sample, only M. stenopetala seed and leaf dosages showed E. coli, pH and turbidity within the recommended standards. For instance, M. stenopetala leaf showed E. coli of 0 CFU per 100 mL of water, pH of 7.95 and turbidity of 2 NTU after treatment by 60 mg dosage.

Turbidity was identified by this study as a factor that negatively affects E. coli removal during M. stenopetala seed (P-value = .022, correlation coefficient = .647) and leaf (P-value = .009, correlation coefficient = .775). As turbidity decreases, there is a subsequent decrease in the number of E. coli using M. stenopetala seed and leaf. The finding of this study is also supported by the studies done in Nigeria on M. oleifera seed that reported a subsequent decrease in total coliform bacteria as turbidity decreases. Also, a study conducted at Gondar University in Ethiopia on M. oleifera seed reported that there was a decrease in total coliform as turbidity decreased. This might be due to the low turbidity in waters increasing the effectiveness of disinfection and/or it might also be because microorganisms from water can be removed by the action of coagulation.

As confirmed from the result, the dosage has an association with E. coli removal using M. stenopetala seed, M. stenopetala leaf, and C. farinosa seed. This means that as dosage increases there was a decrease in the number of E. coli. This finding is also supported by studies conducted at Ebonyi State University and elsewhere in Nigeria which reported that as dosage increases there was a decrease in total coliform bacteria.

A study conducted at Jimma University in Ethiopia also supports the finding as M. oleifera seed and C. farinosa root powder dosages increased, there was a subsequent decrease in total coliform. This might be because an increase in power increases the number of active agents in plant parts that act as a disinfectant which in turn results in higher bacterial removal efficiency.

This study exhibited that settling time has a relationship with E. coli removal using M. stenopetala seed, M. stenopetala leaf, and C. farinosa seed. As settling time increases there was a consequent decrease in the number of E. coli under this study. A study conducted at Jimma University in Ethiopia reported in agreement with the finding of this study that stated an increment in settling time results in a succeeding decrease in fecal coliform during C. farinosa root and M. oleifera seed. This might be due to an increase in the contact time will provide sufficient opportunity so that the E. coli will be accessed by the active agents in the water which made it effective.

Conclusion
This study concludes that M. stenopetala and C. farinosa seeds have the highest E. coli removal efficiency from an initial 18 CFU per 100 mL to 0 CFU per 100 mL of water at a minimum optimum dosage of 30 mg at 90 minutes of settling time. M. stenopetala leaf was also the second in reducing E. coli to 0 CFU per 100 mL of water at 60 mg optimum dosage at 90 minutes of settling time. However, C. farinosa leaf was unable to reduce E. coli to 0 CFU per 100 mL of water. M. stenopetala seed and leaf showed pH, turbidity and E. coli values to the recommended standards of WHO guidelines. Turbidity, settling time and dosage are also found to be the parameter that affects E. coli removal using M. stenopetala seed and leaf whereas settling time and dosage were also identified as parameters that have a relationship with E. coli removal during M. stenopetala (leaf and seed) and C. farinosa seed.

The pollution status of surface waters varies across seasons. So, consideration of water samples of all seasons of a year should be considered by researchers’ study on surface water treatment.

Abbreviations
E. coli: Escherichia coli; CFU: Colony-forming Unit; NTU: Nephelometric Turbidity Unit; WHO: World Health Organization; Moringa stenopetala: M. stenopetala; Cadaba farinosa: C. farinosa; Moringa oleifera: M. oleifera.

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Authors Contributions
DT and AE conceived the idea of the study. DT carried out water sample collection, treatment and analysis. EM and AE helped and guided during water sample collection, treatment
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and analysis. DT and AE carried out the data analysis and statistical portion of the study. DT, EM, and AE are participated and reviewed in data analysis, interpretation of results and manuscript writing. DT, EM, and AE reviewed and finalized the manuscript. The authors read and approved the final manuscript.

Availability of Data and Materials
All available data are found in the research article.

Ethics Approval and Consent to Participate
Ethical approval has been obtained from Hawassa University College of Medicine and Health Science Ethics Committee on Human Research.

Consent for Publication
Not applicable.

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