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Study on the antibacterial activity of selected natural herbs and their application in water treatment

P. S. Harikumar and C. M. Manjusha

Water Quality Division, Centre for Water Resource Development and Management, Kozhikode-673571, Kerala, India

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Correspondence to: P. S. Harikumar (hps@cwrdm.org)

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Abstract

The microbial contamination of water is a world-wide environmental problem. Many traditional methods are being used in various parts of the world to purify the water. According to World Health Organization, 80 % of world's population living in rural areas relies on herbal traditional medicines as their primary health care. So the study on properties and uses of medicinal plants are subject to growing interests. An attempt was made to assess the antibacterial properties of certain selected herbs such as *Ocimum sanctum* (Krishna Tulsi), *Ocimum kilimandscharicum* (Karpoora Tulsi), *Ocimum tenuiflorum* (Ram Tulsi), *Azadirachta indica* (Neem), *Simarouba glauca* (bitterwood), *Caesalpinia sappan* (Pathimugam), *Cuminum cyminum* (Jeerakam), *Vetiveria zizanioides* (Ramacham), *Saraca indica* (Ashoka tree) and *Murraya koenigii* (curry leaves) against different bacteria such as total coliforms, faecal coliforms, *Escherichia coli*, *Bacillus* sp. and *Serratia* sp. The antibacterial activity of the plant extracts was determined by spread plate method, Kirby–Bauer disc diffusion method, most probable number (MPN) method and Petrifilm method. The shelf life of the herbal extract *Ocimum sanctum* (Krishna Tulsi) was also determined using a UV-visible spectrophotometer. A comparison study of the antibacterial efficiency of the three varieties of *Ocimum sanctum, Ocimum kilimandscharicum* and *Ocimum tenuiflorum* was also done. After the complete analysis of the antibacterial activity of different herbs, *Ocimum sanctum*, the most efficient herb, was selected and treatment methods based on the herb were developed so that it can be used conveniently in various households. Therefore *Ocimum sanctum* plant can be further subjected to isolation of therapeutic antimicrobial and pharmacological evaluation.

1 Introduction

It is a well known fact that most of the chemical disinfectants used for antibacterial activity generate various unwanted chemicals known as disinfections by products (DBPs)

in water. DBPs may cause harmful effects on humans such as hemolytic anemia, cancer risk, nervous system effect and liver effects. Chlorine, which is applied to water at various points in a water treatment for disinfection, combined with naturally occurring organic matter (NOM) to generate DBPs in general and halogenated DBPs is particular (Sunil et al., 2011). Medicinal plants are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth, and a large number of diverse types of plants grow in different parts of the country. Herbal medicine is still the mainstay of about 75–80 % of the whole population, and the major part of traditional therapy involves the use of plant extracts and their active constituents. Nowadays multiple-drug resistance has developed due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease. In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immunosuppression and allergic reactions. This situation forced scientists to search for new antimicrobial substances. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants (Bishnu Joshi et al., 2009).

2 Methodology

2.1 Preparation of different herbal extracts

The leaves (20 g) of the various herbs of *Ocimum sanctum, Ocimum kilimandscharicum, Ocimum tenuiflorum*, *Azadirachta indica*, *Simarouba glauca*, *Saraca indica*, *Caesalpinia sappan*, *Cuminum cyminum*, *Vetiveria zizanioides* and *Murraya koenigii* were soaked overnight in water, and then they were thoroughly ground using mortar and pestle. Extracts were then used for antimicrobial studies.

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2.2 Assessment of antibacterial activity of different herbal extracts

The antibacterial activity of different herbal extracts was determined separately using spread plate method (Lansing et al., 2002), Kirby–Bauer disc diffusion method (Harold, 2001), most probable number (MPN) method (APHA, 2012; Lansing et al., 2002), and Petrifilm method (Linton et al., 1997).

2.2.1 Spread plate method

Spread plate method was used to detect the initial and final bacterial count of the well water samples before and after the addition of different plant extracts. Then samples were checked for any reduction in the bacterial count; 0.1 mL pure cultures of microorganisms were uniformly spread on nutrient agar plates using an L-rod. The results were then observed after incubation of 37 °C for 24 h. Duplicates were also maintained for determining the accuracy of the results.

2.2.2 Kirby–Bauer disc diffusion method

In the disc diffusion method, each log phase bacterial inocula of *Escherichia coli*, *Serratia* sp., and *Bacillus* spp. (100 µL) were swabbed onto Petri plates containing 4 mm thick Müller–Hinton agar respectively and kept for 5 min. Pre-sterilized paper discs that were dipped into different herbal extracts of *Ocimum sanctum, Ocimum kilimandscharicum, Ocimum tenuiflorum*, *Azadirachta indica*, *Simarouba glauca*, *Saraca indica*, *Caesalpinia sappan*, *Cuminum cyminum*, *Vetiveria zizanioides* and *Murraya koenigii* were then placed in different microbe-inoculated plates with the help of sterile forceps and incubated at 37 °C for 24 h. A disc soaked in sterile distilled water acted as the control. Results were analysed by determining the clear zone of inhibition around the discs. Duplicates were also maintained for determining the accuracy of the results. A graph was also plotted by determining the different values measured, and it is given in Fig. 11.

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2.2.3 Most probable number (MPN) method

This method helped to detect the reduction in total coliform count, faecal coliform count and *Escherichia coli* count before and after the addition of varying concentration (2.5 mL, 5 mL, 7.5 mL, 10 mL, 12.5 mL and 15 mL) of different herbal extracts to 100 mL well water samples. Time duration of 4 h was maintained after the addition of different herbal extracts to the water samples and then subjected to the analysis using MPN method. In this method, a 10 mL sample was added to 3 tubes of double-strength MacConkey broth, whereas a 1 mL and 0.1 mL sample were added to the first 3 tubes and second 3 tubes of single-strength MacConkey broth respectively. Three loopfuls of positive MacConkey tubes were then used to inoculate onto brilliant green tubes for the detection of faecal coliforms. For the detection of *E. coli* (EC), broth was used as the nutrient medium. The total coliform, faecal coliform and *E. coli* count can be determined by referring to the MPN index table. Duplicates were also maintained for determining the accuracy of the results. A graph was plotted showing the percentage of removal of total coliforms, faecal coliforms and *Escherichia coli* in different herbal extracts, and it is given in Figs. 1–10. The percentage of reduction was calculated using the following formula:

$$\text{Percentage of reduction} = ((\text{Initial CFU} - \text{Final CFU})/\text{Initial CFU}) \cdot 100.$$

2.2.4 Petrifilm method

In this method the total coliform count and *Escherichia coli* count were analysed. A total of 1 mL of sample was added on the Petrifilm and was then spread using a spreader. The results were then observed after incubation of 37 °C for 24 h. Duplicates were also maintained for determining the accuracy of the results. A graph was also plotted showing the percentage of removal of total coliforms and *Escherichia coli*, and it is given in Figs. 1–10.

The mean, median and and standard deviation of percentage reduction values of total coliforms, faecal coliforms and *E. coli* with respect to different herbs were calculated and are shown in Tables 1–3.

2.3 Determination of the shelf life of different herbal extracts

Shelf life is the duration of time that foods, beverages, pharmaceutical drugs, chemicals, and many other perishable items are given before they are considered unsuitable for sale, use, or consumption. The shelf life of *Ocimum sanctum* was determined by checking their relatively stable value of absorbance in UV-VIS spectrophotometer at 650 nm. The day till it showed a difference in absorbance value indicated the shelf life of the plant extract. A graph was also plotted showing the absorbance at different days, and it is given in the Fig. 12.

2.4 Comparison study of the antibacterial efficiency of the three varieties of *Ocimum sanctum, Ocimum kilimandscharicum* and *Ocimum tenuiflorum*

The herb with highest antibacterial activity among the three varieties of *Ocimum sanctum*, *Ocimum kilimandscharicum* and *Ocimum tenuiflorum* was determined after the different antimicrobial detection tests such as spread plating, MPN method, Kirby–Bauer disc diffusion method and Petrifilm method.

2.5 Introduction of the herbal essence in various households

After the complete analysis of the antibacterial activity of different herbs, the most efficient herb identified was introduced in 22 local households to study the perception of the people and the effectiveness of the herbal extract on the removal of pathogenic bacteria. The total coliform count, faecal coliform count and *Escherichia coli* count before and after the addition of the herbal extract were determined. A quantity of 15 mL (3 teaspoons) of herbal extract was added to 100 mL of water samples and was then subjected to bacteriological analysis. The acceptance of herbal extract for treating the

drinking water source was ascertained through a survey. This monitoring in the selected households helped to check the efficiency of the improved water quality on the health especially reduction in morbidity due to water-borne diseases. The details of sampling stations are shown in Table 4, and the acceptance levels of different households are shown in Table 5. A graph was also plotted showing the percentage of removal of total coliforms, faecal coliforms and *Escherichia coli* in the different households and is depicted in Fig. 13.

3 Result and discussion

In this present investigation, all the extracts of natural herbs used for the study showed antibacterial activity. In the case of the extracts of *Azadirachta indica*, *Simarouba glauca*, *Saraca indica*, *Cuminum cyminum,* and *Murraya koenigii*, no antibacterial activity was shown against *Serratia* sp. and *Bacillus* sp. The results of MPN and Petrifilm methods gave similar results. Based on the MPN and spread plate methods, the percentage of removal of *E. coli* using 15 mL *Ocimum sanctum* extract in 100 mL well water sample was 100 %. The percentage reductions of total coliform in the extracts of *Ocimum sanctum, Ocimum kilimandscharicum, Ocimum tenuiflorum*, *Azadirachta indica*, *Simarouba glauca*, *Saraca indica*, *Caesalpinia sappan*, *Cuminum cyminum*, *Vetiveria zizanioides* and *Murraya koenigii* were found to be 75.12 %, 66.68 %, 62.6 %, 52.27 %, 42.63 %, 47.7 %, 54.98 %, 15.73 %, 60.22 % and 49.62 % respectively, whereas in the case of faecal coliform the percentage removal values were 76.61 %, 68.98 %, 65.12 %, 55.1 %, 50.02 %, 51.92 %, 59.43 %, 16.52 %, 62.38 % and 52.98 % respectively. In the case of *E. coli,* the percentage reductions were 79.47 %, 75.27 %, 67.43 %, 59.25 %, 53.23 %, 55.75 %, 61.77 %, 20.97 %, 64.18 % and 57.38 % respectively in *Ocimum sanctum, Ocimum kilimandscharicum, Ocimum tenuiflorum*, *Azadirachta indica*, *Simarouba glauca*, *Saraca indica*, *Caesalpinia sappan*, *Cuminum cyminum*, *Vetiveria zizanioides* and *Murraya koenigii* extracts. The shelf life of *Ocimum sanctum* herbal extract was detected as 16 days. This result showed that the *Ocimum sanctum* ex-

tract can be considered as a suitable material for use for 16 days, and after that its life ceases.

According to the Kirby–Bauer disc diffusion method, zone of inhibition was shown by all the herbal extracts for *E. coli*. But in the case of *Azadirachta indica*, *Simarouba glauca*, *Saraca indica*, *Cuminum cyminum,* and *Murraya koenigii*, no zone of inhibition was shown against *Serratia* sp. and *Bacillus* spp. This showed that there is no antibacterial activity for the extracts of *Azadirachta indica*, *Simarouba glauca*, *Saraca indica*, *Cuminum cyminum,* and *Murraya koenigii* against *Serratia* sp. and *Bacillus* spp. Highest zone of inhibition was shown by *E. coli* in *Ocimum sanctum* extract (2.2 cm). The variation in antibacterial activity of different herbal extracts against different microorganism may be due to the difference in activity of their chemical constituents against different microorganisms. The major chemical constituents of essential oil of fresh leaves of *Ocimum sanctum* are eugenol (57.94 %), β-caryophyllene (15.32 %), β-elemene (7.57 %) and germacrene D (9.10 %) (Mondal et al., 2007). These chemical components may be responsible for the efficiency of the *Ocimum sanctum* extract in showing the highest antibacterial activity by causing more cell damage in microbes compared to other herbs.

Spread plate method also helped to determine the antibacterial activity of different herbal extracts, and also the results were almost similar to the other methods used. The final colony-forming unit (CFU) tremendously declined compared to the initial CFU in the herbal extracts of *Ocimum sanctum, Ocimum kilimandscharicum, Ocimum tenuiflorum*, *Vetiveria zizanioides* and *Caesalpinia sappan*. Among these extracts *Ocimum sanctum* showed the highest antibacterial efficiency. In the other extracts also there was variation in CFU but was less compared to *Ocimum sanctum, Ocimum kilimandscharicum, Ocimum tenuiflorum*, *Vetiveria zizanioides* and *Caesalpinia sappan*.

From the comparative study between the extracts of *Ocimum sanctum, Ocimum kilimandscharicum* and *Ocimum tenuiflorum*, the *Ocimum sanctum* extract showed the best antibacterial activity followed by *Ocimum kilimandscharicum* and then *Ocimum tenuiflorum* extracts.

A study was conducted to determine the removal of bacteria in various households after the addition of herbal extract. Out of the 22 samples tested, 9 samples were found to be polluted with *E. coli*, and all of these 9 samples showed 100 % reduction after the treatment with *Ocimum sanctum* extract. In the case of faecal coliforms, 12 samples showed bacteriological pollution with faecal coliforms, and all these 12 samples showed 100 % reduction except in the case of Manakadavu (85.3 %), Madathil (79.1 %) and Cherinchal (64.3 %) sampling sites. But in the case of total coliforms, the highest percentage reduction was shown in the sampling site near Markaz School (100 %). This variation in percentage reduction can be attributed to the fact that same concentration (15 mL) of *Ocimum sanctum* extract was added to reduce bacterial load of different concentration from samples taken from different households. Therefore in the case of Manakadavu, Madathil and Cherinchal sampling sites, 15 mL of herbal extract may not be sufficient to reduce the faecal coliform count to 100 %. The initial bacterial load of these sampling sites was also higher compared to other sampling stations. This is similar in the case of total coliforms also.

4 Significance

The major significance of the study lies in the cost-effective treatment of faecally contaminated well water samples in various rural households. This can be achieved by using a natural herbal essence of *Ocimum sanctum*. The treatment is simple, eco-friendly, and reachable for all, and the components present in herbs have no side effects for humans compared to chemical treatments. Moreover, the water treated with these extracts serves both as germ-free and medicinal water. In rural areas the majority of the people are using the water without any treatment. They are also reluctant to use chemicals as disinfectants. Natural herbs used in this study can be effectively used as a disinfectant. Using these disinfectants, pathogenic bacteria from the water can be killed, and it can be made safe to use.

These findings will support the traditional knowledge of local users, and it will be a preliminary scientific validation for the use of these plants for antibacterial activity to promote proper conservation and sustainable use of such plant resources. Awareness of local community should be enhanced incorporating the traditional knowledge with scientific findings. The results of the present study support the folkloric usage of the studied plants and suggest that some of the plant extracts possess compounds with antimicrobial properties that can be further explored for antimicrobial activity. This antibacterial study of the plant extracts will demonstrate that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. The millenarian use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases.

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Table 1. Mean, median and standard deviation of percentage reduction values of total coliforms with respect to different herbs.

Site no.	Herbal extracts	Concentration (mL)	TC 1	TC 2	Mean	Median	TC value
1	*Ocimum sanctum*	2.5	55.1	55.3	55.2	55.2	55.2 ± 0.14
		5	63.3	63.5	63.4	63.4	63.4 ± 0.14
		7.5	70	70.2	70.1	70.1	70.1 ± 0.14
		10	85.3	85.3	85.3	85.3	85.3 ± 0
		12.5	87.3	87.5	87.4	87.4	87.4 ± 0.14
		15	89.2	89.4	89.3	89.3	89.3 ± 0.14
2	*Ocimum kilimandscharicum*	2.5	44.2	44.4	44.3	44.3	44.3 ± 0.14
		5	59.1	59.3	59.2	59.2	59.2 ± 0.14
		7.5	64.7	64.9	64.8	64.8	64.8 ± 0.14
		10	71.1	71.3	71.2	71.2	71.2 ± 0.14
		12.5	75	75.2	75.1	75.1	75.1 ± 0.14
		15	85.3	85.5	85.4	85.4	85.4 ± 0.14
3	*Ocimum tenuiflorum*	2.5	40.9	40.9	40.9	40.9	40.9 ± 0
		5	53.1	53.3	53.2	53.2	53.2 ± 0.14
		7.5	60.2	60.4	60.3	60.3	60.3 ± 0.14
		10	70	70	70	70	70 ± 0
		12.5	72.3	72.5	72.4	72.4	72.4 ± 0.14
		15	78.7	78.9	78.8	78.8	78.8 ± 0.14
4	*Azadirachta indica*	2.5	34.1	34.3	34.2	34.2	34.2 ± 0.14
		5	47.2	47.4	47.3	47.3	47.3 ± 0.14
		7.5	50	50	50	50	50 ± 0
		10	59.7	59.9	59.8	59.8	59.8 ± 0.14
		12.5	60	60.2	60.1	60.1	60.1 ± 0.14
		15	62.1	62.3	62.2	62.2	62.2 ± 0.14
5	*Simarouba glauca*	2.5	26.1	26.3	26.2	26.2	26.2 ± 0.14
		5	38.7	38.9	38.8	38.8	38.8 ± 0.14
		7.5	43.1	43.3	43.2	43.2	43.2 ± 0.14
		10	48	48.2	48.1	48.1	48.1 ± 0.14
		12.5	49.1	49.3	49.2	49.2	49.2 ± 0.14
		15	50.2	50.4	50.3	50.3	50.3 ± 0.14

Table 1. Continued.

Site no.	Herbal extracts	Concentration (mL)	TC 1	TC 2	Mean	Median	TC value
6	*Caesalpinia sappan*	2.5	36	36	36	36	36 ± 0
		5	48.3	48.5	48.4	48.4	48.4 ± 0.14
		7.5	52.1	52.3	52.2	52.2	52.2 ± 0.14
		10	60.2	60.4	60.3	60.3	60.3 ± 0.14
		12.5	64.1	64.3	64.2	64.2	64.2 ± 0.14
		15	68.7	68.9	68.8	68.8	68.8 ± 0.14
7	*Cuminum cyminum*	2.5	13.8	14	13.9	13.9	13.9 ± 0.14
		5	14.3	14.5	14.4	14.4	14.4 ± 0.14
		7.5	15	15	15	15	15 ± 0
		10	15.8	16	15.9	15.9	15.9 ± 0.14
		12.5	16.9	17.1	17	17	17 ± 0.14
		15	18.1	18.3	18.2	18.2	18.2 ± 0.14
8	*Vetiveria zizanioides*	2.5	39.8	40	39.9	39.9	39.9 ± 0.14
		5	49	49.2	49.1	49.1	49.1 ± 0.14
		7.5	58.1	58.3	58.2	58.2	58.2 ± 0.14
		10	67.7	67.7	67.7	67.7	67.7 ± 0
		12.5	68	68.2	68.1	68.1	68.1 ± 0.14
		15	78.3	78.3	78.3	78.3	78.3 ± 0
9	*Saraca indica*	2.5	30	30.2	30.1	30.1	30.1 ± 0.14
		5	43	43.2	43.1	43.1	43.1 ± 0.14
		7.5	45.3	45.3	45.3	45.3	45.3 ± 0
		10	55.9	56.1	55	55	55 ± 0.14
		12.5	56	56.2	56.1	56.1	56.1 ± 0.14
		15	56.5	56.7	56.6	56.6	56.6 ± 0.14
10	*Murraya koenigii*	2.5	32.2	32.2	32.2	32.2	32.2 ± 0
		5	45.1	45.3	45.2	45.2	45.2 ± 0.14
		7.5	46.5	46.7	46.6	46.6	46.6 ± 0.14
		10	57.4	57.6	57.5	57.5	57.5 ± 0.14
		12.5	57.9	58.1	58	58	58 ± 0.14
		15	58.2	58.2	58.2	58.2	58.2 ± 0

Note: TC – total coliforms

Table 2. Mean, median and standard deviation of percentage reduction values of faecal coliforms with respect to different herbs.

Site no.	Herbal extracts	Concentration (mL)	FC 1	FC 2	Mean	Median	FC value
1	*Ocimum sanctum*	2.5	55.7	55.7	55.7	55.7	55.7 ± 0
		5	64.1	64.3	64.2	64.2	64.2 ± 0.14
		7.5	70.8	70.8	70.8	70.8	70.8 ± 0
		10	86.1	86.3	86.2	86.2	86.2 ± 0.14
		12.5	90.4	90.6	90.5	90.5	90.5 ± 0.14
		15	92.2	92.4	92.3	92.3	92.3 ± 0.14
2	*Ocimum kilimandscharicum*	2.5	45.2	45.4	45.3	45.3	45.3 ± 0.14
		5	60	60	60	60	60 ± 0
		7.5	65.2	65.4	65.3	65.3	65.3 ± 0.14
		10	72.3	72.5	72.4	72.4	72.4 ± 0.14
		12.5	82.8	83	82.9	82.9	82.9 ± 0.14
		15	87.9	88.1	88	88	88 ± 0.14
3	*Ocimum tenuiflorum*	2.5	44.2	44.4	44.3	44.3	44.3 ± 0.14
		5	54.6	54.8	54.7	54.7	54.7 ± 0.14
		7.5	63.1	63.3	63.2	63.2	63.2 ± 0.14
		10	70.4	70.6	70.5	70.5	70.5 ± 0.14
		12.5	78.7	78.9	78.8	78.8	78.8 ± 0.14
		15	79.1	79.3	79.2	79.2	79.2 ± 0.14
4	*Azadirachta indica*	2.5	37.2	37.2	37.2	37.2	37.2 ± 0
		5	48.5	48.7	48.6	48.6	48.6 ± 0.14
		7.5	50.2	50.4	50.3	50.3	50.3 ± 0.14
		10	62.1	62.3	62.2	62.2	62.2 ± 0.14
		12.5	63	63.2	63.1	63.1	63.1 ± 0.14
		15	69.1	69.3	69.2	69.2	69.2 ± 0.14
5	*Simarouba glauca*	2.5	29.1	29.3	29.2	29.2	29.2 ± 0.14
		5	40	40.2	40.1	40.1	40.1 ± 0.14
		7.5	46	46.2	46.1	46.1	46.1 ± 0.14
		10	55.2	55.4	55.3	55.3	55.3 ± 0.14
		12.5	64.1	64.3	64.2	64.2	64.2 ± 0.14
		15	65.1	65.3	65.2	65.2	65.2 ± 0.14

Table 2. Continued.

Site no.	Herbal extracts	Concentration (mL)	FC 1	FC 2	Mean	Median	FC value
6	*Caesalpinia sappan*	2.5	38.3	38.5	38.4	38.4	38.4 ± 0.14
		5	49.5	49.7	49.6	49.6	49.6 ± 0.14
		7.5	56.1	56.3	56.2	56.2	56.2 ± 0.14
		10	68	68	68	68	68 ± 0
		12.5	70.1	70.3	70.2	70.2	70.2 ± 0.14
		15	74.1	74.3	74.2	74.2	74.2 ± 0.14
7	*Cuminum cyminum*	2.5	14.1	14.3	14.2	14.2	14.2 ± 0.14
		5	14.4	14.6	14.5	14.5	14.5 ± 0.14
		7.5	15.1	15.3	15.2	15.2	15.2 ± 0.14
		10	16.2	16.4	16.3	16.3	16.3 ± 0.14
		12.5	18.3	18.5	18.4	18.4	18.4 ± 0.14
		15	20.4	20.6	20.5	20.5	20.5 ± 0.14
8	*Vetiveria zizanioides*	2.5	42.9	43.1	43	43	43 ± 0.14
		5	50.3	50.3	50.3	50.3	50.3 ± 0
		7.5	60.3	60.5	60.4	60.4	60.4 ± 0.14
		10	68.1	68.3	68.2	68.2	68.2 ± 0.14
		12.5	72.2	72.4	72.3	72.3	72.3 ± 0.14
		15	80	80.2	80.1	80.1	80.1 ± 0.14
9	*Saraca indica*	2.5	32.7	32.9	32.8	32.8	32.8 ± 0.14
		5	44.2	44.2	44.2	44.2	44.2 ± 0
		7.5	47.2	47.4	47.3	47.3	47.3 ± 0.14
		10	52.2	52.4	52.3	52.3	52.3 ± 0.14
		12.5	65.7	65.9	65.8	65.8	65.8 ± 0.14
		15	69.1	69.1	69.1	69.1	69.1 ± 0
10	*Murraya koenigii*	2.5	33	33.2	33.1	33.1	33.1 ± 0.14
		5	46.3	46.3	46.3	46.3	46.3 ± 0
		7.5	49.1	49.3	49.2	49.2	49.2 ± 0.14
		10	60.2	60.4	60.3	60.3	60.3 ± 0.14
		12.5	60.7	60.9	60.8	60.8	60.8 ± 0.14
		15	68.1	68.3	68.2	68.2	68.2 ± 0.14

Note: FC – faecal coliforms

Table 3. Mean, median and standard deviation of percentage reduction values of *E. coli* with respect to different herbs.

Site no.	Herbal extracts	Concentration (mL)	*E. coli*1	*E. coli*2	Mean	Median	*E. coli* value
1	*Ocimum sanctum*	2.5	56	56.2	56.1	56.1	56.1 ± 0.14
		5	65.3	65.3	65.3	65.3	65.3 ± 0
		7.5	71.1	71.3	71.2	71.2	71.2 ± 0.14
		10	87.9	88.1	88	88	88 ± 0.14
		12.5	96.2	96.2	96.2	96.2	96.2 ± 0
		15	100	100	100	100	100 ± 0
2	*Ocimum kilimandscharicum*	2.5	49.4	49.4	49.4	49.4	49.4 ± 0
		5	60.1	60.3	60.2	60.2	60.2 ± 0.14
		7.5	70.5	70.7	70.6	70.6	70.6 ± 0.14
		10	86	86.2	86.1	86.1	86.1 ± 0.14
		12.5	91.9	92.1	92	92	92 ± 0.14
		15	93.3	93.3	93.3	93.3	93.3 ± 0
3	*Ocimum tenuiflorum*	2.5	45	45	45	45	45 ± 0
		5	55.2	55.4	55.3	55.3	55.3 ± 0.14
		7.5	64.1	64.3	64.2	64.2	64.2 ± 0.14
		10	73.1	73.3	73.2	73.2	73.2 ± 0.14
		12.5	81.7	81.9	81.8	81.8	81.8 ± 0.14
		15	85	85.2	85.1	85.1	85.1 ± 0.14
4	*Azadirachta indica*	2.5	38.7	38.9	38.8	38.8	38.8 ± 0.14
		5	49.4	49.6	49.5	49.5	49.5 ± 0.14
		7.5	54.2	54.4	54.3	54.3	54.3 ± 0.14
		10	63.3	63.5	63.4	63.4	63.4 ± 0.14
		12.5	74.2	74.4	74.3	74.3	74.3 ± 0.14
		15	75.1	75.3	75.2	75.2	75.2 ± 0.14
5	*Simarouba glauca*	2.5	30.8	31	30.9	30.9	30.9 ± 0.14
		5	42.7	42.9	42.8	42.8	42.8 ± 0.14
		7.5	50	50.2	50.1	50.1	50.1 ± 0.14
		10	58.3	58.3	58.3	58.3	58.3 ± 0.14
		12.5	68.2	68.4	68.3	68.3	68.3 ± 0.14
		15	68.9	69.1	69	69	69 ± 0.14

Table 3. Continued.

Site no.	Herbal extracts	Concentration (mL)	*E. coli*1	*E. coli*2	Mean	Median	*E. coli* value
6	*Caesalpinia sappan*	2.5	40.1	40.3	40.2	40.2	40.2 ± 0.14
		5	51.2	51.4	51.3	51.3	51.3 ± 0.14
		7.5	57.1	57.3	57.2	57.2	57.2 ± 0.14
		10	68.5	68.5	68.5	68.5	68.5 ± 0.14
		12.5	76.4	76.6	76.5	76.5	76.5 ± 0.14
		15	76.8	77	76.9	76.9	76.9 ± 0.14
7	*Cuminum cyminum*	2.5	15.2	15.4	15.3	15.3	15.3 ± 0.14
		5	16.8	16.8	16.8	16.8	16.8 ± 0
		7.5	20.2	20.4	20.3	20.3	20.3 ± 0.14
		10	22.3	22.5	22.4	22.4	22.4 ± 0.14
		12.5	24.7	24.9	24.8	24.8	24.8 ± 0.14
		15	26.2	26.2	26.2	26.2	26.2 ± 0
8	*Vetiveria zizanioides*	2.5	43.2	43.4	43.3	43.3	43.3 ± 0.14
		5	53.1	53.1	53.1	53.1	53.1 ± 0
		7.5	60.5	60.7	60.6	60.6	60.6 ± 0.14
		10	70	70.2	70.1	70.1	70.1 ± 0.14
		12.5	77.7	77.7	77.7	77.7	77.7 ± 0
		15	80.2	80.4	80.3	80.3	80.3 ± 0.14
9	*Saraca indica*	2.5	33.3	33.5	33.4	33.4	33.4 ± 0.14
		5	45.5	45.7	45.6	45.6	45.6 ± 0.14
		7.5	51	51	51	51	51 ± 0
		10	60.1	60.3	60.2	60.2	60.2 ± 0.14
		12.5	71.8	72	71.9	71.9	71.9 ± 0.14
		15	72.3	72.5	72.4	72.4	72.4 ± 0.14
10	*Murraya koenigii*	2.5	34.1	34.3	34.2	34.2	34.2 ± 0.14
		5	47.3	47.3	47.3	47.3	47.3 ± 0
		7.5	52.1	52.3	52.2	52.2	52.2 ± 0.14
		10	62.6	62.8	62.7	62.7	62.7 ± 0.14
		12.5	73.7	73.9	73.8	73.8	73.8 ± 0.14
		15	74.1	74.1	74.1	74.1	74.1 ± 0

Table 4. Details of sampling stations.

Site no.	Sample code	Sampling station	Panchayath/Municipality/Corporation
1	T1	Eranjikkal	Kozhikode Corporation
2	T2	Vengeri	Kozhikode Corporation
3	T3	Cherinchal Staff Quarters	Kunnamangalam
4	T4	Chettikulam	Kozhikode Corporation
5	T5	Panthalayani	Koyilandy
6	T6	Villoonniyal	Thenjipalam
7	T7	Near IIM	Kunnamangalam
8	T8	Aakkoli	Kunnamangalam
9	T9	Near Markaz school	Kunnamangalam
10	T10	Manakadavu	Olavanna
11	T11	Pallipoyil	Chelannur
12	T12	Viruppil	Peruvayal
13	T13	Madathil	Peruvayal
14	T14	Kannamkulangara	Kozhikode Corporation
15	T15	Ashokapuram	Kozhikode Corporation
16	T16	Musliyarangadi	Nediyiruppu
17	T17	Makool Peedika	Vadakara
18	T18	Bilathikulam	Kozhikode Corporation
19	T19	Pathammile	Kunnamangalam
20	T20	Nadakkavu	Kozhikode Corporation
21	T21	Puliyanthodu	Kunnamangalam
22	T22	Cherinchal	Kunnamangalam

Table 5. Acceptance level of different households.

Site no.	Sample code	Acceptance level
1	T1	+
2	T2	+
3	T3	+
4	T4	+
5	T5	–
6	T6	+
7	T7	+
8	T8	+
9	T9	+
10	T10	+
11	T11	+
12	T12	+
13	T13	+
14	T14	+
15	T15	–
16	T16	+
17	T17	+
18	T18	+
19	T19	+
20	T20	+
21	T21	+
22	T22	–

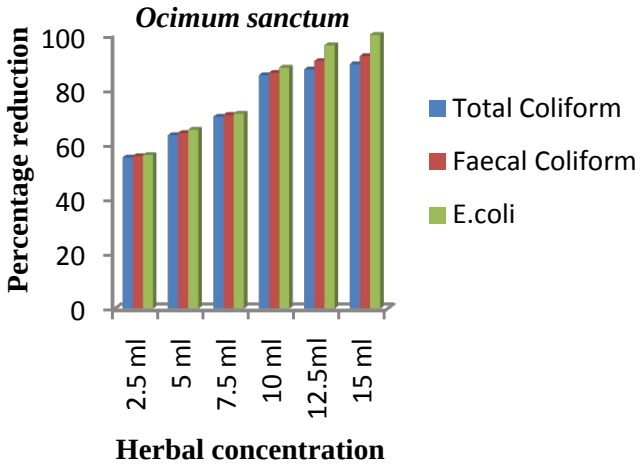

Fig. 1. Effect of *O. sanctum* on the reduction of bacteria.

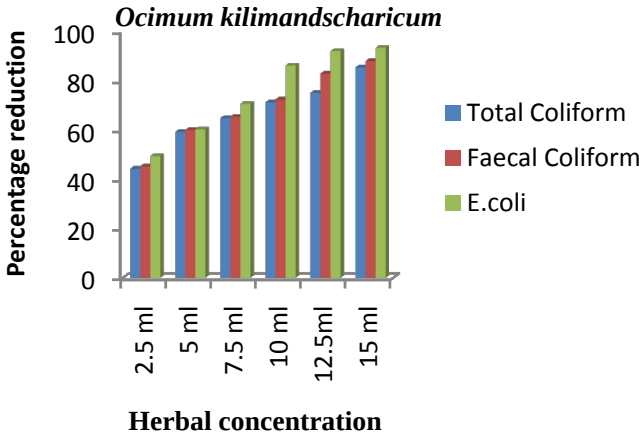

Fig. 2. Effect of *O. kilimandscharicum* on the reduction of bacteria.

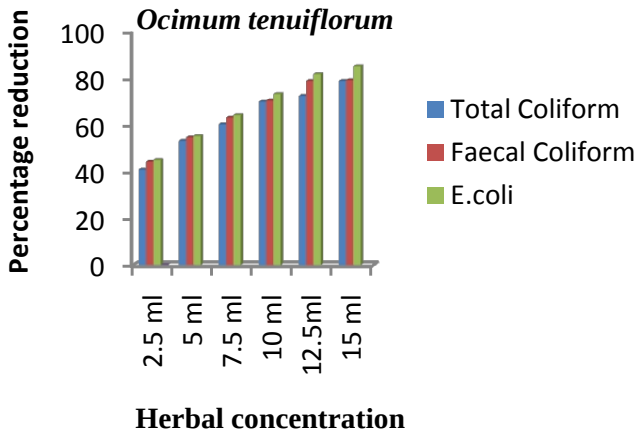

Fig. 3. Effect of *O. tenuiflorum* on the reduction of bacteria.

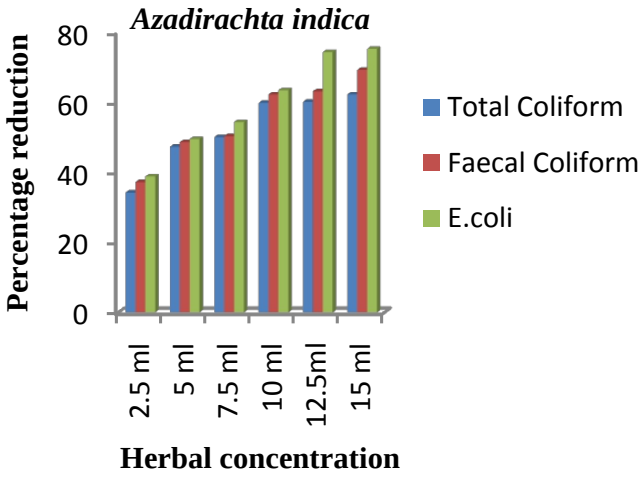

Fig. 4. Effect of *A. indica* on the reduction of bacteria.

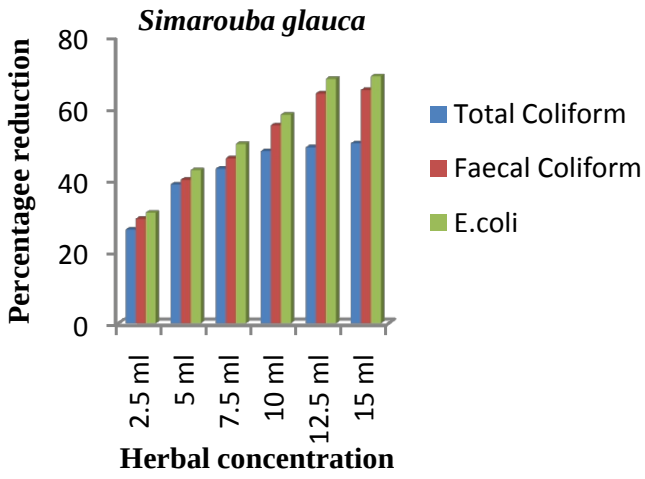

Fig. 5. Effect of *S. glauca* on the reduction of bacteria.

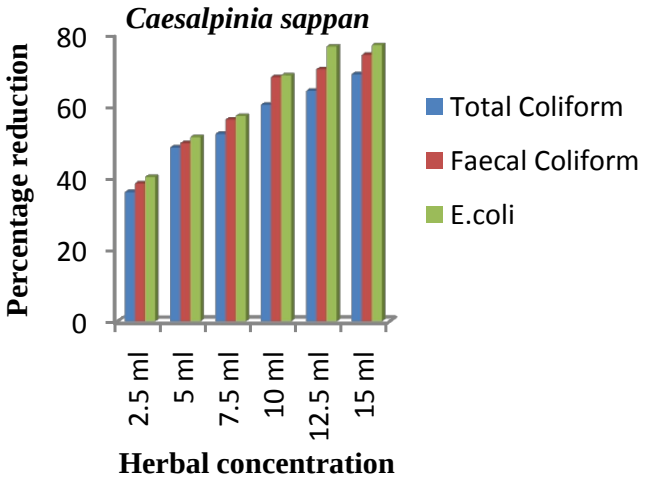

Fig. 6. Effect of *C. sappan* on the reduction of bacteria.

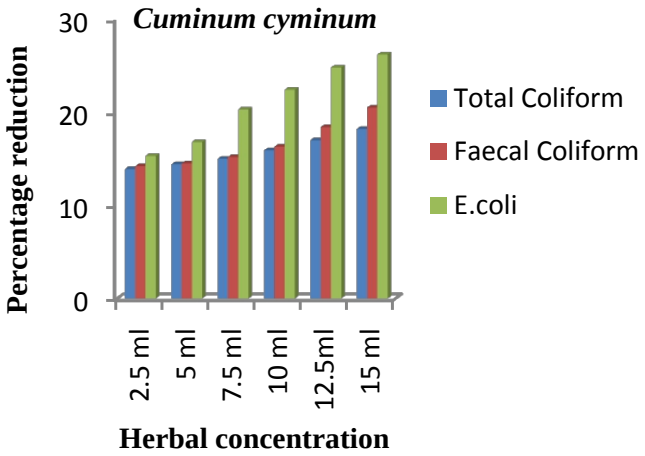

Fig. 7. Effect of *C. cyminum* on the reduction of bacteria.

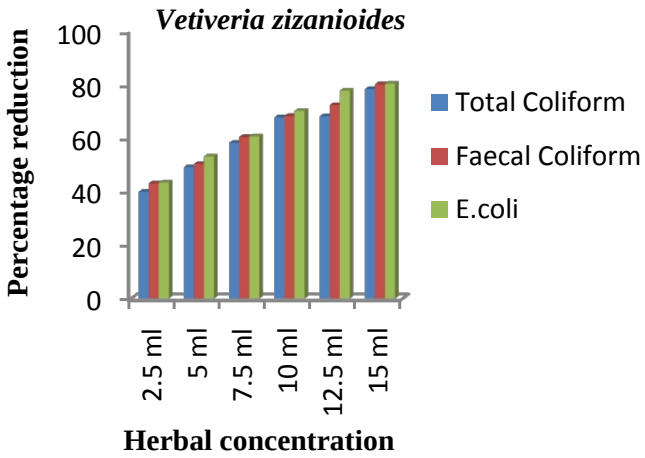

Fig. 8. Effect of *V. zizanioides* on the reduction of bacteria.

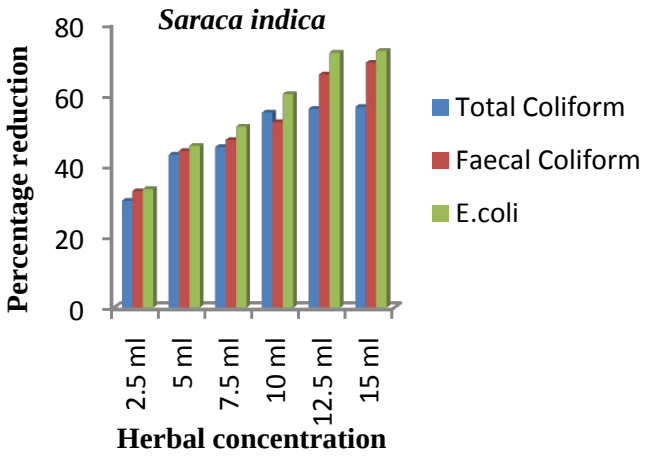

Fig. 9. Effect of *S. indica* on the reduction of bacteria.

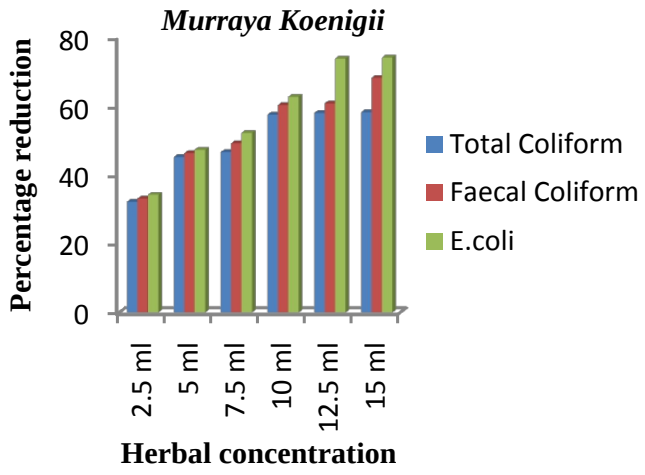

Fig. 10. Effect of *M. koenigii* on the reduction of bacteria.

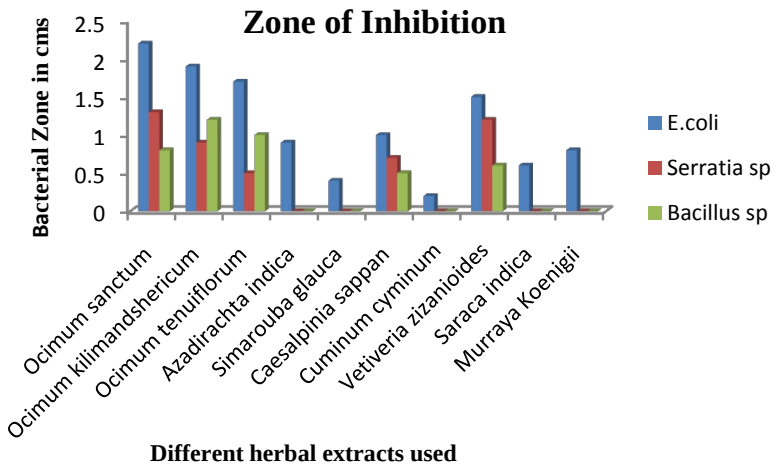

Fig. 11. Zone of Inhibition (cm) produced by different herbal extracts.

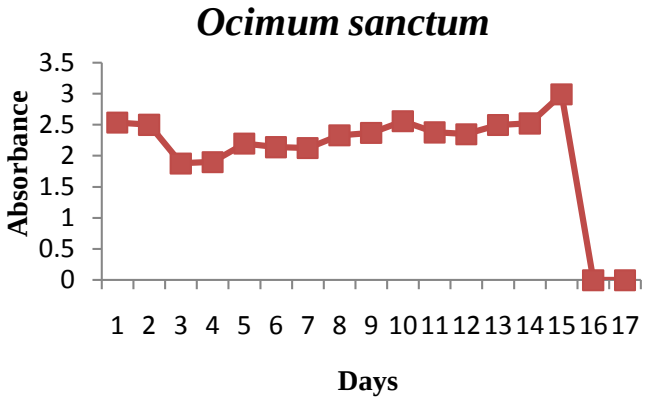

Fig. 12. Stability of *O. sanctum* at 650 nm during different days of observation.

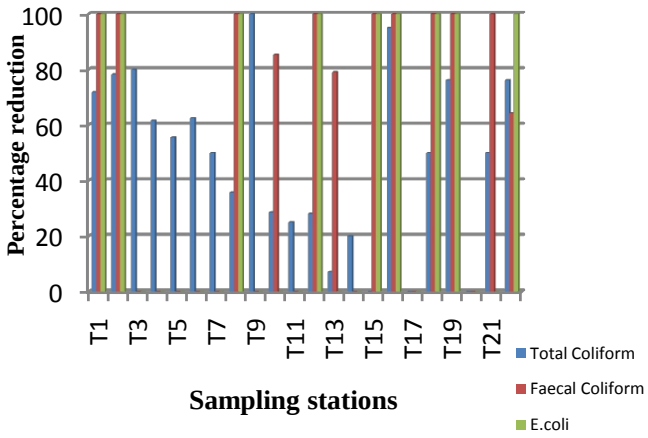

Fig. 13. Percentage reduction of coliforms during field study.