Study on the Antibacterial activity of selected Natural Herbs and its application in Water Treatment

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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. 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 Baeur 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 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* was selected as the most efficient herb with 100% reduction *of E.coli* in 15 ml *Ocimum sanctum* added sample and also treatment methods based on the herb was 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.

Keywords: Microbial contamination, Antibacterial activity, *Ocimum sanctum.*

1. INTRODUCTION

It is a well known fact that, most of the chemical disinfectants used for antibacterial activity generate various unwanted chemicals known as disinfection by products (DBPs) in water. 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). The introduction of natural herbs serve as an alternate to chemical treatment to prevent such DBPs formation. 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 extract 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, immune-suppression 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. (Bishnu Joshi et al., 2009). Therefore, there is a need to study on the antibacterial properties of natural herbs and their application in water treatment.

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* was soaked overnight in distilled water and then it was thoroughly ground using motor and pestle along with 100 ml distilled water and filtered. Extracts was then used for antimicrobial studies.

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 Baeur Disc Diffusion method (Harold, 2001), Most Probable Number (MPN) method (APHA, 2012 and 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 and then was checked for any reduction in the bacterial count. 0.1 ml pure cultures of microorganisms were uniformly spreaded on nutrient agar plates using an L-rod. The results were then observed after incubation of 37^0C for 24 hours. Duplicates were also maintained for determining the accuracy of the results.

2.2.2. KIRBY BAEUR DISC DIFFUSION METHOD

In the disc diffusion method, each log phase bacterial inoculums of *Escherichia coli*, *Serratia sp.* and *Bacillus sp.* (100 µl) was swabbed on to petriplates containing 4 mm thick Muller Hinton agar respectively and kept for five minutes. Pre-sterilized paper discs that was dipped in to 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* was then placed in different microbe inoculated plates with the help of sterile forceps and incubated at 37^0C for 24 hours. A disc soaked in sterile distilled water acted as the control. Results was 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 was given in the fig: 11.

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 hrs was maintained after the addition of different herbal extracts to the water samples and was then subjected to the analysis using MPN method. In this method, 10 ml sample was added to 3 tubes of double strength Mac conkey broth whereas 1 ml and 0.1 ml sample was added to first 3 tubes and second 3 tubes of single strength Mac conkey broth respectively. 3 loopful of positive Mac conkey tubes were then used to inoculate on to 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 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 was given in the fig:1-10 The Percentage of reduction was calculated using the formula,

Percentage of reduction = ((Initial CFU-Final CFU) / Initial CFU)*100

2.2.4. PETRIFILM METHOD

In this method the Total Coliform count and *Escherichia coli* count was analysed. 1 ml of sample was added on the petrifilm and was then spread using a spreader. The results were then observed after incubation of 37^0C for 24 hours. 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 was given in the fig: 1-10. The percentage reduction values of Total Coliforms, Fecal Coliforms and *E.coli* with respect to different herbs are shown in Tables:1-3. The MPN count of one herb (*Ocimum tenuiflorum*) is shown in Table: 4

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 Baeur 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 was determined. A quantity of 15 ml (3 teaspoon) 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 the Table: 5 and the acceptance level of different households are shown in the Table: 6. A graph was also plotted showing the percentage of removal of Total coliforms, Faecal coliforms and *Escherichia coli* in the different households and was 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 method, the percentage of removal of *E.coli* using 15 ml *Ocimum sanctum* extract in 100 ml well water sample was 100 %. The percentage reduction 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 where as 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 reduction 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* extract can be considered as a suitable material for use for 16 days and after that its life ceases.

According to the Kirby Baeur 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 sp.*. 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 sp.*. 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* were eugenol (57.94%), β-caryophyllene (15.32%), β-elemene (7.57%) and germacrene D (9.10%). (Mondal S. *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 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. (Shahedur Rahman *etal*., 2011)

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 Manakkadavu (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 Manakkadavu, 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.

Plant essential oils and extracts have been used for many thousands of years, in food preservation, pharmaceuticals, alternative medicine and natural therapies. It is necessary to investigate those plants scientifically which have been used in traditional medicine to improve the quality of healthcare. Plant extracts are potential sources of novel antimicrobial compounds especially against bacterial pathogens. (Bishnu Joshi et al., 2009).

An important characteristic of plant extracts and their components is their hydrophobicity, which enable them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable. Extensive leakage from bacterial cells or the exit of critical molecules and ions will lead to death. (Rastogi and Mehrotra, 2002).

The beneficial medicinal effects of plant materials typically result from the secondary products present in the plant although, it is usually not attributed to a single compound but a combination of the metabolites. The medicinal actions of plants are unique to a particular plant species or group, consistent with the concept that the combination of secondary products in a particular plant is taxonomically distinct (Parekh et al., 2005).

Awareness of local community should be enhanced incorporating the traditional knowledge with scientific findings. (Bishnu Joshi et al., 2011). The extracts of natural herbs Tulsi (Ocimum sanctum), Neem (Azadirachta indica), Wheatgrass (Triticum aestivum), Amla (Phyllanthus emblica) and Katakphala (Strychnos potatorum) were tested at different contact time and concentration against *E-coli*. (Sunil et al., 2011).

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, reachable for all and the components present in herbs have no side effects to human compared to chemical treatment. More over the water treated with these extracts serve both as germ free and medicinal water. In rural areas 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 Percentage reduction values of Total Coliforms with respect to different herbs

Sl .No.	Herbal extracts	Concentration (ml)	Percentage reduction values of TC (%)
1	*Ocimum sanctum*	2.5	55.1
		5	63.3
		7.5	70
		10	85.3
		12.5	87.3
		15	89.2
2	*Ocimum kilimandscharicum*	2.5	44.2
		5	59.1
		7.5	64.7
		10	71.1
		12.5	75
		15	85.3
3	*Ocimum tenuiflorum*	2.5	40.9
		5	53.1
		7.5	60.2
		10	70
		12.5	72.3
		15	78.7
4	*Azadirachta indica*	2.5	34.1
		5	47.2
		7.5	50
		10	59.7
		12.5	60
		15	62.1
5	*Simarouba glauca*	2.5	26.1
		5	38.7
		7.5	43.1
		10	48
		12.5	49.1
		15	50.2
6	*Caesalpinia sappan*	2.5	36
		5	48.3
		7.5	52.1
		10	60.2
		12.5	64.1
		15	68.7
7	*Cuminum cyminum*	2.5	13.8
		5	14.3
		7.5	15
		10	15.8
		12.5	16.9
		15	18.1
8	*Vetiveria zizanioides*	2.5	39.8
		5	49
		7.5	58.1
		10	67.7
		12.5	68
		15	78.3
9	*Saraca indica*	2.5	30
		5	43
		7.5	45.3
		10	55.9
		12.5	56
		15	56.5

10	*Murraya Koenigii*	2.5	32.2
		5	45.1
		7.5	46.5
		10	57.4
		12.5	57.9
		15	58.2

Note: TC-Total Coliforms

Table: 2 Percentage reduction values of Faecal Coliforms with respect to different herbs

Sl. No.	Herbal extracts	Concentration(ml)	Percentage reduction values of FC (%)
1	*Ocimum sanctum*	2.5	55.7
		5	64.1
		7.5	70.8
		10	86.1
		12.5	90.4
		15	92.2
2	*Ocimum kilimandscharicum*	2.5	45.2
		5	60
		7.5	65.2
		10	72.3
		12.5	82.8
		15	87.9
3	*Ocimum tenuiflorum*	2.5	44.2
		5	54.6
		7.5	63.1
		10	70.4
		12.5	78.7
		15	79.1
4	*Azadirachta indica*	2.5	37.2
		5	48.5
		7.5	50.2
		10	62.1
		12.5	63
		15	69.1
5	*Simarouba glauca*	2.5	29.1
		5	40
		7.5	46
		10	55.2
		12.5	64.1
		15	65.1
6	*Caesalpinia sappan*	2.5	38.3
		5	49.5
		7.5	56.1
		10	68
		12.5	70.1
		15	74.1
7	*Cuminum cyminum*	2.5	14.1
		5	14.4
		7.5	15.1
		10	16.2
		12.5	18.3
		15	20.4
8	*Vetiveria zizanioides*	2.5	42.9
		5	50.3
		7.5	60.3
		10	68.1
		12.5	72.2
		15	80

9	*Saraca indica*	2.5	32.7
		5	44.2
		7.5	47.2
		10	52.2
		12.5	65.7
		15	69.1
10	*Murraya Koenigii*	2.5	33
		5	46.3
		7.5	49.1
		10	60.2
		12.5	60.7
		15	68.1

Note: FC-Faecal Coliform

Table: 3 Percentage reductionvalues of *E.coli* with respect to different herbs

Sl. No.	Herbal extracts	Concentration(ml)	Percentage reduction values of *E.coli* (%)
1	*Ocimum sanctum*	2.5	56
		5	65.3
		7.5	71.1
		10	87.9
		12.5	96.2
		15	100
2	*Ocimum kilimandscharicum*	2.5	49.4
		5	60.1
		7.5	70.5
		10	86
		12.5	91.9
		15	93.3
3	*Ocimum tenuiflorum*	2.5	45
		5	55.2
		7.5	64.1
		10	73.1
		12.5	81.7
		15	85
4	*Azadirachta indica*	2.5	38.7
		5	49.4
		7.5	54.2
		10	63.3
		12.5	74.2
		15	75.1
5	*Simarouba glauca*	2.5	30.8
		5	42.7
		7.5	50
		10	58.3
		12.5	68.2
		15	68.9
6	*Caesalpinia sappan*	2.5	40.1
		5	51.2
		7.5	57.1
		10	68.5
		12.5	76.4
		15	76.8
7	*Cuminum cyminum*	2.5	15.2
		5	16.8
		7.5	20.2
		10	22.3
		12.5	24.7
		15	26.2

8	*Vetiveria zizanioides*	2.5	43.2
		5	53.1
		7.5	60.5
		10	70
		12.5	77.7
		15	80.2
9	*Saraca indica*	2.5	33.3
		5	45.5
		7.5	51
		10	60.1
		12.5	71.8
		15	72.3
10	*Murraya Koenigii*	2.5	34.1
		5	47.3
		7.5	52.1
		10	62.6
		12.5	73.7
		15	74.1

Table 4: The MPN count of *Ocimum tenuiflorum* (Total Coliform)

Sl. No.	Concentration of *Ocimum tenuiflorum* herbal extract (ml)	Total Coliform count		
		Initial count (Dilution done)	Final count (Dilution done)	Percentage reduction value
1	2.5	4600±0	2720±0	40.9
2	5	640±28	300±14	53.1
3	7.5	460±0	183±1	60.2
4	10	300±0	90±0	70
5	12.5	390±7	108±1	72.3
6	15	300±0	64±1	78.7

Table 5: Details of sampling stations

Sl. 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	Kandamkulangara	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 6: Acceptance level of different households

Sl. 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	-

6. FIGURE CAPTIONS

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.koengii* 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