Paper for International Conference on 'Emerging Trends in Waste Management Technology' to be held at MIT, Pune on 3rd – 4th Dec.2009

HERBAL DISINFECTION OF WATER

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ABSTRACT

Provision of clean and safe water in rural areas is a great challenge for the developing countries of the world since most communities rely on poor traditional sources that often provide unsafe domestic water. It is estimated that world over, about three million people die annually from water-borne diseases (World Health Organization [WHO], 1999). Conventional water treatment relies on the addition of chemicals such as alum (aluminium sulfate) as coagulants and the addition of chlorine as a bactericide. The availability of these chemicals, which depends on foreign exchange, is unreliable and unpredictable. Because of economic and political constraints, the universal provision of piped water is not currently feasible. This circumstance leaves millions without access to safe drinking water (WHO, 1999). Interim solutions are clearly needed. A vast rural Indian population is dependant on the supply of untreated water, despite of spending billions of rupees for water purification. Thus the rural population is thriving on the contaminated water supply which is the root cause of their ailments. Safe water is vital for improving the health and quality of life and for alleviating poverty. A lot of agencies have taken up the initiative to supply safe drinking water, various policies have been put forth, however these policies do not reach rural India due to unorganized structure and management.

In the process of developing a plant based substitute for economical safe approach for water purification against conventional chemical constituents, in vitro antibacterial studies were carried out on the Alcoholic and Aqueous leaf extracts on *E.coli*. The choice of E.coli was done since it is an important indicator organism indicating faecal pollution of water. Phytochemical screening was also performed to check the active antimicrobial components present in the leaf extracts. *Ocimum sanctum* is effective against *E.coli* and shows increase in antibacterial activity with increase in concentration and specified contact time. The aqueous leaf extract showed antibacterial activity with a hundred times more concentration and increased exposure time as compared to the alcoholic leaf extract. The Alcoholic leaf extract showed better reduction of microbial load in contaminated water during the Coliform reduction test (MPN test) as compared to the aqueous leaf extract. A qualitative phytochemicals analysis was performed on both the extracts for the detection of Alkaloids, Glycosides, Terpenoids, Steroids, Flavonoids, Tannins and Reducing sugar. The alcoholic leaf extract revealed the presence of Alkaloids, Steroids and Tannins while the aqueous leaf extract contained only Alkaloids and Steroids which are the active antimicrobial components in plants.

Keywords: *Ocimum sanctum*, Alcoholic extract, Aqueous extract, Water purification, Effective against *E.coli*, Phytochemical analysis.

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1. INTRODUCTION:

About 1.6 million people die every year from diarrhoeal diseases (including cholera) attributable to lack of access to safe drinking water and basic sanitation and 90% of these are children under 5, mostly in developing countries. Approximately 150 million people are infected with schistosomiasis causing tens of thousands of deaths yearly; approximately 500 million people are at risk of trachoma from which 146 million are threatened by blindness and 6 million are visually impaired. Intestinal helminthes (Ascariasis, trichuriasis and hookworm infection) are plaguing the developing Countries due to inadequate drinking water with 133 million suffering from high intensity intestinal Helminthes infections, there are around 1.5 million cases of clinical hepatitis every year (World health Organization 2009). Even after spending billions of rupees through different State and Central government schemes, several parts of Rural India are still deprived of organized system of collecting, treating and supply of safe drinking water and thus attributes to 90% of rural diseases. Moreover improper maintenance of scheme, inadequate supply of chemicals, unskilled manpower adds to the drinking water related problems. Different agencies like Ministry of Water resources, Ministry of Urban development and Poverty alleviation, Ministry of rural development, Ministry of Environment and Forest and Ministry of health and family welfare have got different roles to play in order to provide drinking water of adequate quantity and potable quality to meet the health needs of the community. The provision of clean drinking water has been given priority in the constitution of India; with article 47 conferring the duty of providing clean drinking water and improving public health standards. Several policies have been implemented to supply safe drinking water to rural India since 1949 till 2007. Despite of so many policies, a major part of rural India is deprived of safe drinking water.

Water that has been disinfected (by UV treatment, boiling, chlorination, micro-filtration, ozone, etc.) may still be polluted with other contaminants that are not affected by the disinfection treatment. There are several disadvantages associated with conventional water treatment methods; either a heavy cost is incurred or unskilled labor to handle the chemicals. In both the oral and written traditions, knowledge of alternative methods of water treatment is still available (Vandana Shiva, 1988). Principal among them are the Charaka Samhita and Sushruta samhita (300 A.D.) which are the foundations of the Ayurveda, the Indian system of natural healing. According to Shiva the Sushruta samhita lists seven modes of purifying water. The wood of Amla (Planthus emblica) is used to clear small rain ponds in the Indian peninsula. Tulsi (Oscimum sanctum) is a water purifier with antibacterial and insecticidal properties. Drumstick tree (Moringa oliefera) which is Sudan is called the clarifier tree produces seeds, which are used for water purification. Seeds of honge (Pongamia glabra) and nuts of Nirmali tree (Strychnos potatorium) are used as water clarifiers. This is virtually costless way to render contaminated water fit for human consumption.

2. MATERIALS AND METHODS:

2.1. PLANT MATERIALS:

Mature leaves have been collected from healthy plants grown at Loni, Maharashtra, India. The leaves were shade dried at room temperature and were ground to get a very fine powder followed by sieving it under 75 microns sieve and stored at room temperature.

2.2. PREPARATION OF HERBAL EXTRACT:

Alcoholic leaf extract was prepared by extracting 50 gm leaf powder in 100 ml of pure ethanol by three cold percolations. Extract was dried in incubator at 37 degree Celsius for 24 to 36 hours. This treatment gave rise to dry powder or paste of extract. Extract was stored in refrigerator. Then this plant extract was dissolved in 10% ethanol to obtain 1.138 mg/ml solution. This mother solution was used and serial dilutions were made with sterile distilled water to get concentrations as follows:-
1.138 mg/ml, 0.113 mg/ml, 0.0113 mg/ml, 0.0011 mg/ml

Aqueous leaf extract was prepared by extracting 50 gm of leaf powder with distilled water for 6 h at slow heat. Then it was filtered through a four layers muslin cloth and kept in the water bath at slow heat till a dry paste is obtained. This extract was stored in the refrigerator. A concentration of 113.8 mg/ml of aqueous leaf extract was prepared by dissolving extract in sterile distilled water. This mother solution was used and serial dilutions were made with sterile distilled water at follows:-
113.8 mg/ml, 11.38 mg/ml, 1.138 mg/ml, 0.113 mg/ml

2.3. ANTIBACTERIAL ACTIVITY OF ALCOHOLIC AND AQUEOUS LEAF EXTRACT:

The standard strain of E.coli was taken and suspended in saline to make 1 O.D cell suspension. 9 ml of cell suspension was distributed in each of the 5 test tubes and 1 tube containing 10 ml cell suspension which was kept as control. 1 ml of different concentrations of leaf extract was added in 4 test tubes and 1ml of 10% ethanol was added in the 5^{th} tube kept as control. An exposure time of 6 hrs was given to all the tubes and then was diluted 6 times and spread plate on nutrient agar plates in duplicates. The plates were observed for inhibition after 18 hours. The same test was performed by spread plate of exposed leaf extract at the 0^{th} hour and 6^{th} hour respectively followed by spread plate every hour for an exposure period of 6 hours. The antibacterial activity for aqueous leaf extract was studied by exposing different concentrations of leaf extract with equally distributed cell suspension for an exposure of 6 hrs and spread plate at 6^{th} hour followed by spread plate at 0^{th} and 6^{th} hour, finally spread plate every hour for an exposure period of 6 hours . The observations were done for inhibition after 18 hrs incubation of the plates. Colony forming units were counted and recorded.

2.4. MPN TEST TO CHECK THE COLIFORM REDUCTION IN WASTE WATER SAMPLE BY AQUEOUS AND ALCOHOLIC EXTRACT:

Aqueous and alcoholic extracts of concentrations 113.8 mg/ml and 1.138 mg/ml were used for the test. A known volume of waste water was taken and divided into 3 parts each of 250 ml. One part was kept untreated while the remaining two parts were treated with 20 ml aqueous extract of 113.8 mg/ml concentration and alcoholic extract of 1.138 mg concentration respectively. An exposure time of 60 minutes was given. Bacteriological test was performed on all the three samples. Since isolation of bacteria was a time consuming and cumbersome process, simple tests were carried to determine the presence of intestinal bacteria. It was observed that at 37 degree Celsius, coliforms in lactose medium ferment within 48 hours with formation of gas indicating a positive test. Combination of positive tubes is observed and Macrady's table is referred for recording the number of coliforms per

100 ml of untreated sample and subsequent reduction in coliforms by treatment with aqueous and alcoholic extracts.

2.5. PHYTOCHEMICAL SCREENING:

The Phytochemical screening for major constituents was undertaken using standard qualitative method as described by Siddiqui and Ali, (1997), Evans, (2002) and Iyengar, (1995). The Phytochemical screening was performed for alcoholic and aqueous extract respectively. The Alkaloids were detected by evaporating the extract to dryness and then heating the residue with 2% hydrochloric acid, cooled, filtered followed by addition of Mayer's or Dragendroff's reagent. White or yellow precipitate by addition of Mayer's reagent and orange or brown precipitate by addition of Dragendroff's reagent indicates presence of Alkaloids (Siddiqui and Ali, 1997). The presence of glycosides was detected by addition of few drops of ferric chloride and concentrated Sulphuric acid in solution of extract in glacial acetic acid, reddish brown color at the junction of two layers and bluish green color in the upper layer indicates presence of glycosides (Siddiqui and Ali, 1997). The presence of Terpenoids and Steroids were detected by treating four milligrams of extract by 0.5 ml of acetic anhydride and 0.5 ml of chloroform, addition of concentrated Sulphuric acid, red violet color indicates presence of Terpenoids and green bluish color indicates presence of Steroid (Siddiqui and Ali, 1997). The presence of Flavonoids was detected by treating four milliliters of extract with 1.5 ml of 50% methanol solution, warmed and addition of metal magnesium and 5-6 drops of concentrated Sulphuric acid, red color indicates presence of Flavonoids and orange color indicates presence of flavones (Siddiqui and Ali, 1997). The presence of Tannins was detected by adding 1 ml of water and 1-2 drops of ferric chloride solution to extract, blue color indicates presence of Gallic tannins and green black color indicates presence of catecholic tannins (Iyengar, 1995). The presence of Reducing Sugar was detected by adding 1 ml of water and 5-8 drops of Fehling's solution was added to 0.5 ml of extract, brick red precipitate indicates presence of Reducing Sugar.

3. RESULTS:

Antibacterial activity of alcoholic leaf extract

Table1. Results of Antimicrobial activity of different concentrations of alcoholic leaf extract by the spread plate technique

Concentration of exposed leaf extract.	**No. of cell/ml in the original cell suspension. C.F.U'S x 10^6**
1.113 mg/ml	0
0.113 mg/ml	90 x 10^6
0.011 mg/ml	105 x 10^6
0.001 mg/ml	120 x 10^6
E.coli	132 x 10^6
10% ethanol	12 x 10^6

Table 2 Results of Antimicrobial activity of alcoholic leaf extract at the 0^{th} and the 6^{th} hour

Concentration of the alcoholic leaf extract	**C.F.U'S x 10^7 at the 0^{th} hour**	**C.F.U'S x 10^7 at the 6^{th} hour**
5.69 mg/ml	30 x 10^7	3 x 10^7
1.038 mg/ml	27 x 10^7	6 x 10^7
50% Ethanol	1 x 10^7	1 x 10^7
10% Ethanol	48 x 10^7	40 x 10^7
E.coli	36 x 10^7	150 x 10^7

Table 3. Results of Antimicrobial activity of the aqueous leaf extract each hour from the 0^{th} hour till the 6^{th} hour

Hour	**Number of cells/ml C.F.U'S x 10^7**	**Number of cells/ml C.F.U'S x 10^7**	**Number of cells/ml C.F.U'S x 10^7**
0^{th}	300 x 10^7	302 x 10^7	301 x 10^7
1^{st}	310 x 10^7	200 x 10^7	303 x 10^7
2^{nd}	312 x 10^7	191 x 10^7	306 x 10^7
3^{rd}	315 x 10^7	110 x 10^7	315 x 10^7
4^{th}	323 x 10^7	0	317 x 10^7
5^{th}	330 x 10^7	0	327 x 10^7
6^{th}	336 x 10^7	0	330 x 10^7

Antibacterial activity of aqueous leaf extract

Table 4. Results of Antimicrobial activity of the different concentrations of aqueous leaf extract

Concentration of exposed leaf extract.	**No. of cell/ml in the original cell suspension C.F.U'S x 10^6**
113.8 mg/ml	0
11.38 mg/ml	72 x 10^6
1.138 mg/ml	102 x 10^6
0.113 mg/ml	114 x 10^6
E.coli	116 x 10^6
Distilled water	120 x 10^6

Table. 5 Results of Antimicrobial activity of aqueous leaf extract with respect to different time intervals that is at the 0^{th} hour and the 6^{th} hour

Concentration of the exposed leaf extract	**Number of colonies at the 0^{th} hour C.F.U'S**	**Number of colonies at the 6^{th} hour C.F.U'S**	**Number of cells/ml in the original cell suspension, C.F.U'S X 10 X 10^6**
113.8 mg/ml	10	0	0
11.38 mg/ml	10	3	$3 X 10^7$
1.138 mg/ml	19	4	$4 X 10^7$
Distilled water	37	150	$15 X 10^7$
E.coli	35	153	$153 X 10^7$

Table.6. Results of Antimicrobial activity of the aqueous leaf extract each hour from the 0^{th} hour till the 6^{th} hour

Hour	**E.coli**	**Number of cells/ml**	**Distilled Water**	**Number of cells/ml**	**113.8 mg/ml Concentration of leaf extract**	**Number of cells/ml**
	C.F.U'S	**C.F.U'S X 10 X 10^6**	**C.F.U'S**	**C.F.U'S X 10 X 10^6**	**C.F.U'S**	**C.F.U'S X 10 X 10^6**
0^{th}	67	$67 X 10^7$	61	$61 X 10^7$	64	$64 X 10^7$
1^{st}	73	$73 X 10^7$	75	$75 X 10^7$	38	$38 X 10^7$
2^{nd}	79	$79 X 10^7$	76	$76 X 10^7$	15	$15 X 10^7$
3^{rd}	81	$81 X 10^7$	83	$83 X 10^7$	10	$10 X 10^7$
4^{th}	90	$90 X 10^7$	93	$93 X 10^7$	3	$3 X 10^7$
5^{th}	101	$101 X 10^7$	102	$102 X 10^7$	0	0
6^{th}	110	$110 X 10^7$	120	$120 X 10^7$	0	0

Table :7. Results of MPN Test for alcoholic and aqueous leaf extract

Type of the sample	**Combination of the positives**	**Number of cells/100ml**
Untreated sample	5-5-5	1600
Sample treated with 113.8 mg/ml of aqueous leaf extract	5-5-2	500
Sample treated with 11.38 mg/ml of alcoholic leaf extract	5-5-0	240

Table 8: Results of phytochemical screening

Active chemical constituents in plants	Presence or absence in alcoholic leaf extract	Presence or absence in aqueous leaf extract
Alkaloids	Present	Present
Glycosides	Absent	Absent
Terpenoids	Absent	Absent
Steroids	Present	Present
Flavonoids	Absent	Absent
Tannins	Present	Absent
Reducing sugar	Absent	Absent

5. DISCUSSION

The wealth of India is stored in the enormous natural flora which has been gifted to her, endowed with a wide diversity of agro-climatic conditions. The study shows simple and effective method to disinfect water at consumer end. In rural areas bacterial contamination of Water could be due to poor sanitary conditions around water sources, improper drainage facilities near hand pumps and bore wells and located cattle sheds and latrine soak pits. The significant increase in bacterial contamination has been found between collection at water sources and eventual use. The substantial faecal pollution occurring between collection and use may be attributed to several unhygienic reasons. The tentative findings of researchers show that Neem, Tulsi and Wheat seem to exert germicidal effects on coliform bacteria. From the previous experiments it has been concluded that Tulsi gives the best result followed by Wheat and then Neem. Water treated with Tulsi gives a pleasant taste as well. The effective antimicrobial activity of plant extracts is due to the extensive leakage from bacterial cells or the exit of critical molecules and ions that lead to death. Perhaps may also be due to the synergistic effect of the active components present in the plant parts.

Sewage water may not be completely disinfected by this method due to its high turbidity which encourages microbial proliferation. Perhaps clarifying the sewage of turbid water samples by filtration through charcoal, clay or sand could be done prior to the treatment to achieve reliable disinfection. The technique can be effective for water obtained from nearby streams and other sources. Harmful pathogenic organisms present in such water are killed to a large extent, provided the initial degree of water contamination is not severe. The suspension time required is substantially high. It remains to be seen if sunlight experiments can be combined with herbal disinfection of Water to achieve complete destruction of enteric bacteria.

6. CONCLUSION

The antimicrobial activity of the alcoholic leaf extract of Oscimum sanctum was observed on different concentrations of leaf extract, however complete inhibition was observed at 1.138 mg/ml concentration. The antimicrobial activity of alcoholic leaf extract of Oscimum sanctum was observed with respect to different time intervals that are at the 0^{th} and the 6^{th} hour and complete inhibition was observed at the 4^{th} hour.

The antimicrobial activity of aqueous extract of Oscimum sanctum was observed on different concentrations of leaf extract, however complete inhibition was observed at 113.8 mg/ml

concentration. The antimicrobial activity of aqueous leaf extract of Oscimum sanctum was observed with respect to different time intervals that are at the 0^{th} and the 6^{th} hour and complete inhibition was observed at the 5^{th} hour. The MPN tests were carried out using aqueous and alcoholic leaf extracts and it was observed that the untreated sample contained 1600 coliforms per 100 ml and that treated with 113.8 mg/ml aqueous leaf extract and 1.138 mg/ml of alcoholic leaf extract contained 500 coliforms per 100 ml and 240 coliforms per 100 ml respectively (i.e. reduction of approximately 68.75% and 85% microbial load with Aqueous and Alcoholic leaf extract respectively). The Phytochemical screening was performed on aqueous leaf extract, the presence of Alkaloids, Steroids and Tannins were detected and in alcoholic leaf extract, the presence of Alkaloids and Steroids were detected.

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