

Disinfection of Drinking Water in Rural Area Using Natural Herbs

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Abstract:- Rural population in India obtains their water supply from unprotected sources such as open dug wells, small streams, ponds and rivers which are often polluted. Due to economical and political constraints, the provision of piped water is not currently feasible. This circumstances leaves millions without access to safe drinking water. There are various methods available for disinfecting drinking water but not economically feasible for poor people. Interim solutions are clearly needed. *Ocimum sanctum* (Tulsi) and *Azadirachta indica* (Neem) are herbal plants which have antimicrobial activity against many of the microorganisms commonly found in water sources causing diseases to people. The present study was focused on evaluating the efficiency of *Ocimum sanctum* and *Azadirachta indica* to disinfect water from Lake, River and Well. *In vitro* antibacterial studies were carried out using aqueous leaf extract and fresh leaf juice against *Salmonella typhi*, an indicator microorganism. *Ocimum sanctum* and *Azadirachta indica* showed increase in antimicrobial activity with increase in concentration at specified contact time (18hrs).The antimicrobial effect of Aqueous extract showed better MPN reduction [i.e. from 313 to 7 (Neem) &10 (Tulsi) for Well water ; from 175000 to 16000 (Neem) & 40000 (Tulsi) for Lake water ; from 125000 to 70000 (Neem) & 26000 (Tulsi) for River water] than the fresh leaf juice [i.e. from 313 to 345 (Neem) & 1800 (Tulsi) for well water ; from 175000 to 152000 (Neem) and 175000 (Tulsi) for Lake water ; from 125000 to 180000 (Neem) & 210000 (Tulsi) for River water]. The alcoholic extract showed the best result [i.e. from 313 to 0 (Neem) & 2 (Tulsi) for Well water ; from 175000 to 4000 (Neem) & 2000 (Tulsi) for Lake water ; from 125000 to 2000 (Neem) & 2000 (Tulsi)].

Keywords:- Azadirachta indica, Ocimum sanctum, Etahnolic extract, Aqueous extract, Antimicrobial activity.

I. INTRODUCTION

Out of 121 crore Indians ,83.3 crore live in rural areas [16]. Supplying safe drinking water to such a large population is economically a daunting task. As a result most of the rural population in India are depended on traditional water sources such as wells, lakes , rivers etc. and often these sources are contaminated with various microbial pathogens. Meeting the drinking water needs of such a large population can be a daunting task. Around 37.7 million Indians are affected by waterborne diseases annually, 1.5 million children are estimated to die of diarrhea alone and 73 million working days are lost due to waterborne disease each year. The standard methods of disinfection include initial filtration of various sorts, and Ultraviolet treatment is sometimes employed in special situations; but reliance is mainly placed on chemical treatment species such as Chlorine and Chlorine dioxide. [1]. There are certain disadvantages associated with conventional methods of disinfecting water; either they are costly or unskilled labor to handle the chemicals leads to the formation of unwanted by-products obtained from chemical disinfectants which have got the potential to cause hemolytic anemia, cancer risk, nervous system disruption. In both the oral and written traditions, knowledge of alternative methods of water treatment is still available. According to traditional belief Tulsi leaves (*Ocmium sanctum*) & Neem leaves (*Azadirachta indica*) have the capacity to purify water.[15]. This is virtually cost less way to render contaminated water fit for human consumption.

II. MATERIALS AND METHODS

II.a Plant materials

Mature leaves of *Ocimum sanctum* were collected from local Market, Pune and leaves of *Azadirachta indica* were collected from Botanical Garden near Environment Science Dept. Bharati Vidyapeeth, Pune,

Maharashtra, India. Leaves were segregated and washed with tap water followed by distilled water and dried before use.

II.b Preparation of Juice / Extract:

Fresh juice : Clean leaves (50 gms) were made into paste with distilled water using mortar and pestle. The paste was then diluted and filtered through muslin cloth. The extract then obtained was made upto 100ml using distilled water.

Aqueous leaf extract:- Dissolve 200gms of leaf powder in 500 ml of distilled water and keeping for 8 hours at room temperature. It was then filtered through muslin cloth and centrifuged at 5000 rpm at 4⁰C for 8 minutes. The extract was adjusted to the volume and stored in the refrigerator.[4].

Alcoholic extract :- 50 gms dried leaf powder were taken in separate container. To this 250ml of ethanol was added and kept for 24 hours with periodic shaking. The extract was then filtered through Watmann No.1 filter paper and filtrate was made upto volume and stored.[2].

II.c Collection of water sample:

River water was collected from from Bhima river at Pune, well water from Baoda vill. Satara dist. ,Pune and lake water from Jambhulwadi lake in Pune. These sources were generally used for potable water by rural people. All samples were collected during the month of July,2012. The samples were stored in pre cleaned, autoclaved plastic cans and immediately used for experiment.

II.d Inhibition of bacterial growth:

Bacterial growth inhibition was checked using standard strain of *Salmonella typhi*. The bacterial suspension (1% 1 OD A600nm) in was distributed in in different beakers. From the mother solution, different volumes of aqueous leaf extract (0.4,0.8,2,4,8,12,16,20 ml) and fresh leaf juice (0.5,1,2.5,5,5,10,15,20 ml) were added in each beaker and the volume of 25ml was made up with distilled water. One beaker without any extract or juice kept as control. The beakers were incubated for 18 hours and serially diluted before plating in Nutrient agar plates to find out the total viable count. All the experiments were repeated minimum two times and average was reading were taken for analysis.

II.e MPN Test to check coliform reduction in water samples by Aqueous leaf extract, Alcoholic leaf extract and Fresh leaf juice :

Fresh leaf juice, Aqueous and Alcoholic leaf extract of concentrations 50% , 14.72 mg/l (Neem) ; 26.24mg/l (Tulsi) , 7.68mg/l (Neem) ; 13.4mg/l (Tulsi) respectively were used for the test. A known volume of water sample was taken and divided into 4 parts each of 25 ml. One part was kept untreated while the remaining 3 parts were treated with 16ml of Aqueous extract, Alcoholic extract and 20 ml of Fresh juice taken from their mother solutions respectively. An exposure time of 18 hrs was given at 37⁰C. Presence of coliforms in Mackonkey broth medium, formation of gas and change in the colour of medium indicate positive test. Combination of positive tubes was observed and Macrady’s table was referred for recording the number of coliforms per 100ml of untreated sample and subsequent reduction in coliforms by treatment with Fresh juice, Aqueous and Alcoholic extract.

III. RESULTS

Inhibition of bacterial growth

Neem leaf juice was found to be more effective than the Tulsi leaf juice in inhibiting the bacterial growth. Neem juice could reduce the bacterial count from 1224 x 10⁶ to 20 x 10⁶ at maximum volume while in the case of Tulsi juice it could reduce the count to 30 x 10⁶ (Table 1). Whereas aqueous extract were very efficient in reducing the bacterial growth (Table 2). At concentration of 0.4mg Neem extract reduced to total bacterial count to 3x10⁶ and in the case of tulsi 1.42 mg powder reduced the bacterial count to 7x10⁶ within 18hr of incubation.

Table 1. Inhibition of bacterial growth by leaf juice of Neem and Tulsi	
Reaction mixture	Total viable count after 18 hr. (x 10⁶)
Control	1224
Tulsi juice 0.5 ml	800
Tulsi juice 1 ml	700
Tulsi juice 2.5 ml	670
Tulsi juice 5 ml	300

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Tulsi juice 10 ml	100
Tulsi juice 15 ml	50
Tulsi juice 20 ml	30
Neem juice 0.5ml	800
Neem juice 1 ml	600
Neem juice 2.5 ml	500
Neem juice 5 ml	100
Neem juice 10 ml	40
Neem juice 15 ml	28
Neem juice 20 ml	20

Table 2. Inhibition of growth by Neem and Tulsi aqueous extract.

Extract	Total viable count after 18 hr. ($\times 10^6$)
Neem extract 0.0092mg/0.4ml	750
Neem extract 0.0814/0.8ml	400
Neem extract 0.046mg/2ml	200
Neem extract 0.0.092mg/4ml	80
Neem extract 0.184mg/8ml	40
Neem extract 0.276mg/12ml	15
Neem extract 0.368/16ml	3
Neem extract 0.46/20ml	3
Tulsi extract 0.0284mg/0.4ml	700
Tulsi extract 0.0568mg/0.8ml	600
Tulsi extract 0.142mg/2ml	550
Tulsi extract 0.284mg/4ml	200
Tulsi extract 0.568mg/8ml	100
Tulsi extract 0.852mg/12ml	30
Tulsi extract 1.136mg/16ml	7
Tulsi extract 1.42mg/20ml	7

MPN Test

Neem and tulsi alcoholic extract were found to be more effective in reducing the MPN of all tested water sample. Alcoholic extract of Neem reduced the MPN of water from well, lake and river from 313, 175 and 125 to 0, 4 and 2, while Tulsi alcoholic extract reduced it to 2,2and 2 respectively. In the case of aqueous extract neem was found to be better than tulsi (Table 3). Amazingly MPN remained same or increased after treating with leaf juice of both plants.

Table 3. Results of MPN Test for leaf juice, aqueous and alcoholic extract.

	Initial	Leaf juice		Aqueous extract		Alcoholic extract	
		Neem	Tulsi	Neem (0.368mg/25ml)	Tulsi (1.136mg/25ml)	Neem (0.192mg/25ml)	Tulsi (0.336mg/25ml)
Well water	313	345	1800	7	10	0	2
Lake water ($\times 10^3$)	175	152	175	16	40	4	2
River water ($\times 10^3$)	125	180	210	7	26	2	2

IV. DISCUSSIONS

In rural areas high rate of water born diseases are due the contamination of water from different sources. The tentative findings of this study shows that Neem and Tulsi exert germicidal effects on coliform bacteria. From the present experiments it has been concluded that Neem gives the best result followed by Tulsi. The aqueous extracts of Neem and Tulsi gives better results than fresh leaf juices of Neem and Tulsi. However during the MPN test, the pre determined dose of leaf extracts and juices were applied which was not giving 0

mpn result, so the alcoholic extract of same dose was used to achieve 0 MPN result. But the water treated with Neem gave an unpleasant taste as compared to Tulsi. However, the taste and odour can be removed by simple filtration using charcoal and thus need extract can be used an effective method to disinfect water in rural area.

V. CONCLUSION

The major population in our country is living in rural areas, where these natural herbs are easily available. The conventional method of disinfecting water are economically non feasible & also people are reluctant to use chemicals as disinfectants. The effective antimicrobial activity of plants leaf extracts is due to the synergistic effect of the active components present in plant leaves. This technique can be effective for the water obtain from water sources having low degree of contamination or else water can be given prior filtration with charcoal or fine sand to reduce the contamination load. It remains to be seen if sunlight experiments can be combined with herbal disinfection of water to achieve complete destruction of enteric bacteria.

ACKNOWLEDGEMENTS:

The authors thank Bharati Vidyepeeth University for providing support to carry out the work.

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