



Antibacterial Activity of *Ocimum Sanctum* Linn. and It's Role in Water Purification

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ABSTRACT

Many medicinal properties have been attributed to *Ocimum sanctum* L. The leaves, flowers, stems, roots, seeds, etc. of the plant are known to have therapeutic potential and are used by traditional medicine practitioners, as expectorant, analgesic, anticancer, antiasthmatic, antiemetic, diaphoretic, antidiabetic, antifertility. The major importance of the study lies in the cost-effective treatment of 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, environment-friendly and accessible for all and the constituent present in herbs have no side effects to human compared to chemical treatment. These findings will support the traditional knowledge of local users and it will be a scientific, validation for the use of these plants for antibacterial activity.

Keywords: *Ocimum sanctum*, antibacterial, therapeutic potentials.

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INTRODUCTION

Holy basil, (*Ocimum sanctum*), also called tulsi or tulasi, flowering plant of the mint family (Lamiaceae) grown for its aromatic leaves. Holy basil is native to the Indian subcontinent and grows throughout Southeast Asia. The plant is widely used in Ayurvedic and folk medicine, often as an herbal tea for a variety of ailments, and is considered sacred in Hinduism. It is also used as a culinary herb with a pungent flavour that intensifies with cooking. The plant is a small annual or short-lived perennial shrub, up to 1 metre (3.3 feet) in height. The stems are hairy and bear simple toothed or entire leaves oppositely along the stem. The fragrant leaves are green or purple, depending on the variety. The small purple or white tubular flowers have green or purple sepals and are borne in terminal spikes. The fruits are nutlets and produce numerous seeds [1]. Tulsi is often enjoyed as a simple herbal tea and is frequently blended with other herbs and spices for various medicinal and culinary purposes. In the ancient Ayurvedic text, the Charaka Samhita, Tulsi has been documented to be of immense use in the treatment of headaches, rhinitis, stomach disorders, inflammation, heart diseases, various forms of poisoning and malaria [2-3]. Each part of the plant has proven to offer protection against various diseases; the aqueous and alcoholic extract from the leaves have various pharmacological activities such as anti-inflammatory, antipyretic, analgesic, antiasthmatic, antiemetic, antidiabetic, hepatoprotective, hypotensive, hypolipidemic, and antistress agents. Further, distillation of the leaves yields oil of the plant which is known to possess antibacterial, antioxidant, and anti-inflammatory properties and is used extensively in the pharmaceutical industry mainly for skin cream preparations [15, 16]. Plants are of the most important sources of medicines and a spacious numbers of drugs in use are derived from plants. The remedial uses of plants are safe, economical and effective as their ease of availability [4, 5]. There are two varieties of *Ocimum santcum* i.e. Kali (Krishna Tulsi) and Hari (Rama Tulsi) [8]. *Ocimum santcum* having antimicrobial activity, anticancer, antidiabetic [9] and antibiotic protection [11] against many of the microorganism. Tulsi, the queen of herbs, is the most sacred and most cherished of the many healing and health-giving herbs of the orient, the mythical "incomparable" of India. [12]. Tulsi is often enjoyed as a simple herbal tea and is often mixed with other herbs and spices for various medicinal and culinary purposes. A variety of compounds such as ursolic acid, apigenin and luteolin have been isolated from the tulsi leaves. Phytochemical compounds in the leaf include eugenol (volatile oil), ursolic acid (triterpenoid) and rosmarinic acid (phenyl propanoid), with other active compounds including caryophyllene and oleanolic acid. Nutrients include vitamin A and C, minerals calcium, iron and zinc as well as chlorophyll, Tannins, alkaloids,

glycosides and saponins are abundant in Tulsi. Tulsi plant is also known to purify the environment and also acts as a repellent for mosquitoes, flies and other harmful insects. Major contribution of Tulsi leaves as anti-fertility effect [13], anti-diabetic effect, anti-allergic, immunomodulatory effect [14] and as stress reliever potent [6, 7].

MATERIAL AND METHODS

Water Sample: The water samples were collected from different sources: Tap water from zoology laboratory, Poddar International College Jaipur, Pond water and Storage tank water in a sterile plastic can.

Preparation of Tulsi Leaf Crude Extract:

Tulsi leaves are plucked from the plant and washed with tap water and then washed 4 times with sterile distilled water. The pre weighed leaf materials were crushed by mortar & pestle. Extracted leaf materials were sterile distilled water using soxhlet apparatus and finally the extract was weighed gravimetrically.

Optimization of Treatment Period: Tulsi leaf crude extracts (100 mg in 2 ml sterile distilled water) were dissolved in 1 liter of tap, storage and pond water at a pH of 6.8 - 7.0 and treated for various treatment periods (1 -16 h) Water samples (5ml) were taken every hour and microbiological analysis of the sample as carried out by plate method and counted as CFU/ml.

Estimation of Microorganisms: The original inoculum is diluted in a series of dilutions. Each subsequent dilution will contain only one tenth of the number of microbial cells as the preceding tube. Then samples from dilutions are used to inoculate a Petri plate, on which colonies grow and can be counted. The water sample containing bacteria are streaked on to the Nutrient Agar and check for the growth of bacteria. The untreated water sample was used as a control. If there were no colonies on the medium, the results showed that the disinfectant is highly effective against bacteria. All treatments were performed in triplicate and the average values were considered.

Optimization of Treatment dosage: After optimizing the treatment duration, treatment dosage is optimized. Different treatment doses (100, 200, 300, 400, 500 and 600 mg of *Ocimum sanctum* leaf extract in 2 ml of sterilized distilled water) were prepared as mentioned earlier. To 1 liter of tap, pond and stored water, the treatment dosages were treated individually and incubated (16 hrs at room temperature) and then plating was done for analysis.

Table1: Optimization of Treatment Period of antibacterial activity of *Ocimum sanctum* leaf extract

Treatment period (hrs)	Leaf extract (mg/l)	No of cells	No of cells	Microbial reduction (%)
		control water	Treated water sample	
1	100	22,000	20,000	09
2	100	22,000	19,000	14
3	100	22,000	19,000	14
4	100	22,000	19,000	14
5	100	22,000	18,000	18
6	100	22,000	18,000	18
7	100	22,000	18,000	18
8	100	22,000	17,000	22
9	100	22,000	17,000	22
10	100	22,000	17,000	22
11	100	22,000	17,000	22
12	100	22,000	16,000	27
13	100	22,000	16,000	27
14	100	22,000	16,000	27
15	100	22,000	16,000	27
16	100	22,000	16,000	27

RESULT

From 100 g of leaf, 1.0 g of extract was recovered. Evaluation of the antimicrobial potential of plant extract was performed by optimizing the treatment period (Table 1) with disinfectant dosage for different kind of drinking water. For the fixed amount of disinfectant dose 100 mg/l, treated water was subjected to plate counting. The results showed that the microbial population gradually decreased from 22,000 cells to 16,000 cells in 16 h of treatment and after which the reduction stabilize (Table2). Hence 16 hours treatment period was concluded as the optimization period and 16 hours treatment period was taken as the optimization period. After treatment of six different doses of plant extract, two different dose range

500 mg l-1 and 600 mg l-1 concentration treated water samples shows best antimicrobial activity in the water samples. In tap water 600mg of extract treated water gave 40% activity at 1 hour and 99 % activity at 15 hours treatment and 500 mg l-1 treated water shown 95% of antimicrobial activity rest of the dosage gave lesser activity. In pond water 500 and 600 mg l-1 dosage treated sample shown 94% and 97 % activity at 14 hours and the other dosage (100, 200, 300, 400 mg l-1) (table 2, 3,4 and 5) also gave to 30-99% activity at 15 hour treatment shown in graphs. From this experiment the higher concentration 600 mg l-1 shows 99% antibacterial activity at 16 hrs treatment and it indicates the minimal bacterial concentration was observed in 600 mg l-1 of leaf extract and then in 500 mg (table 6 and 7).

Table 2. Treatment of Tap water, Pond water and Stored water samples with 100 mg (*Ocimum sanctum* extract)

Time (hr)	Microbial Reduction in %		
	Tap Water	Pond water	Stored water
1	-	8	9
2	-	10	11
3	11	15	14
4	13	20	21
5	14	21	22
6	15	23	24
7	17	24	25
8	19	25	26
9	20	28	36
10	22	32	39
11	24	40	41
12	25	42	46
13	27	45	49
14	28	50	51
15	30	52	55

Table 3. Treatment of Tapwater, Pond water and Stored water samples with 200 mg (*Ocimum sanctum* extract)

Time (hr)	Microbial Reduction in %		
	Tap Water	Pond water	Stored water
1	5	7	9
2	10	11	12
3	15	18	19
4	20	21	22
5	25	26	27
6	28	29	30
7	30	32	34
8	35	39	41
9	38	40	42
10	40	45	46
11	42	48	49
12	46	52	54
13	47	59	60
14	48	63	65
15	51	68	70

Table 4. Treatment of Tap water, Pond water and Stored water samples with 300 mg (*Ocimum sanctum* extract)

Time (hr)	Microbial Reduction in %		
	Tap Water	Pond water	Stored water
1	2	7	9
2	12	13	15
3	15	16	16
4	20	22	25
5	27	29	30
6	38	39	42
7	42	45	50
8	46	48	52
9	50	54	56
10	52	56	58
11	55	58	60
12	65	66	69
13	68	70	72
14	69	73	75
15	72	81	85

Table 5. Treatment of Tap water, Pond water and Stored water samples with 400 mg (*Ocimum sanctum* extract)

Time (hr)	Microbial Reduction in %		
	Tap Water	Pond water	Stored water
1	30	15	12
2	32	20	21
3	39	38	39
4	40	41	45
5	42	45	50
6	45	46	52
7	50	51	53
8	55	57	60
9	60	64	63
10	72	78	79
11	78	80	81
12	80	84	85
13	82	88	89
14	87	90	92
15	90	94	95

Table 6. Treatment of Tap water, Pondwater and Stored water samples with 500 mg (*Ocimum sanctum* extract)

Time (hr)	Microbial Reduction in %		
	Tap Water	Pond water	Stored water
1	39	18	19
2	41	25	26
3	43	43	35
4	45	48	45
5	50	52	55
6	52	56	58
7	57	60	62
8	60	64	66
9	73	75	77
10	78	78	85
11	80	83	88
12	84	85	91
13	90	90	94
14	91	94	96
15	95	97	98

Table 7. Treatment of Tap water, Pond water and Stored water samples with 600 mg (*Ocimum sanctum* extract)

Time (hr)	Microbial Reduction in %		
	Tap Water	Pond water	Stored water
1	40	20	21
2	41	25	26
3	44	44	44
4	48	50	51
5	54	52	55
6	58	60	61
7	62	65	67
8	64	70	71
9	71	77	79
10	77	85	88
11	85	90	90
12	88	92	94
13	92	93	96
14	94	97	98
15	95	99	99

DISCUSSION

The findings of the study demonstrated that the *Ocimum sanctum* has the maximum antibacterial activity against *E.coli* and least activity for *P.aeruginosa* and *S. typhimurium*. This plant extract is effectively control *E.coli* than the other organisms In this study the antibacterial activity of the leaf extract was analysed with normal tap water and river water, the maximum activity was observed at 600mg l-1 concentrated water treatment in both sample at the pH range of 6.8 – 7.0 for 15 to 16 hrs. From this we concluded the human harmful organisms were inactivated by this plant extract at 600 mg l-1 in 15 to 16 hrs studied by Poonam et al. [17].

The findings of the study the evaluation of antimicrobial activity of *Ocimum sanctum* leaf extract in normal tap and river water. The antimicrobial effect was studied with different concentration (100 to 600 mg l-1) of Tulsi leaf extract in tap and river water. In this, 600 mg l-1 concentration of plant extract treated water shown effective antimicrobial activity at 15 to 16 hrs than the other concentration of extract. The 500 mg l-1 of extract treated water showed 95 to 98% antibacterial activity in 14 to 16 hrs studied by Pavithra et al.[9]

CONCLUSION

Tulsi leaf extract has great potential as an antimicrobial agent for water treatment. The treatment is simple, cost-effective, Environment friendly for all and the components present in *Ocimum sanctum* leaves have no side effects on human beings as compared to chemical treatment. Water treated with Tulsi extracts not only acts as germ free but also medicinal water.

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