



## Journal of Medicinal Plants Studies

# Queen of herbs tulsi (*ocimum sanctum*) removes impurities from water and plays disinfectant role

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Tulsi is a Sanskrit word which means “matchless one”. Several medicinal properties have been attributed to the Tulsi plant not only in Ayurveda and Siddha but also in Greek, Roman and Unani systems of medicine. In Ayurveda, Tulsi used as antiasthmatic and antikaphic drugs. It is also used in treatment of fever, bronchitis, arthritis, convulsions etc. Scientific explorations of traditional belief of medicinal properties of Tulsi have got momentum mostly after the middle of the 20th century. *Ocimum sanctum* (Tulsi or holy basil) has a very special place in the Hindu culture. The present study was focused on evaluation of antimicrobial activity of *Ocimum sanctum* leaf extract in normal tap water and local river water. The antimicrobial effect was studied with different concentration (100 to 600 mg l<sup>-1</sup>) of Tulsi leaf extract in tap and river water. In this, 600 mg l<sup>-1</sup> concentration of plant extract treated water showed effective antimicrobial activity at 15 to 16 hrs than the other concentration of extract. The 500 mg l<sup>-1</sup> of extract treated water showed 95 to 98% antibacterial activity in 14 to 16 hrs. The minimum bacterial concentration (MBC) was observed in 500 and 600 mg l<sup>-1</sup> extract concentration. The concentration of the bacterial cells inhibited gradually for an hour was studied by spread plate method.

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**Keyword:** *Ocimum sanctum*, Antimicrobial effect, Minimum Bactericidal Concentration, Microbial growth.

### 1. Introduction

Plants are of the important sources of medicine & a large numbers of drugs in use are derived from plants. The therapeutic uses of plant are safe, economical & effective as their ease of availability <sup>[1]</sup>. Among the plants known for medicinal value, the plants of genus *Ocimum* belonging to family Lamiaceae are very important for their therapeutic potentials. *Ocimum sanctum* has two varieties i.e. black (*Krishna Tulsi*) and green (*Rama Tulsi*), their chemical constituents are similar <sup>[2]</sup>. Tulsi is a Sanskrit word which means “matchless one”. Several medicinal properties have been attributed to the Tulsi plant not only in Ayurveda and Siddha but also in Greek, Roman and Unani systems of medicine <sup>[3]</sup>. The medicinal use of plants is very old. Literatures indicate that therapeutic use of plants is as old as 4000-5000 B.C and Chinese used first the natural herbal

preparations as medicines <sup>[4]</sup>. Earliest references are available in Rigveda which is said to be written between 3500-1600 B.C <sup>[5]</sup>. Tulsi (*Ocimum sanctum*), Queen of Herbs, the Legendary, “Incomparable One” is one of the holiest and most cherished of the many healing and health-giving herbs distributed mainly in the oriental region <sup>[6]</sup>. Tulsi a widely grown, sacred plant belongs to the lamiaceae family. It is called by names like Rama Tulsi Krishna Tulsi in Sanskrit and Holy Basil in English. The natural habitat of Tulsi varies from sea level to an altitude of 200 m. It is found growing naturally in moist soil nearly all over the globe <sup>[7]</sup>. In Nepal, Aryan people grow Tulsi as a religious plant in their homes, temples and their farms. They use Tulsi leaves in routine worship. Three main forms are generally recognized Rama tulsi with stems and leaves of green, Krishna tulsi with stems and sometimes also leaves of purple and Vana Tulsi

which is unmodified from its wild form. Variations in soil type and rainfall may also equate to a difference in the size and form of the plants as well as their medicinal strength and efficacy. *Ocimum* genus contains between 50 to 150 species of herbs and shrubs from the tropical regions of Asia [8]. Plants have square stems, fragrant opposite leaves and whorled flower on spiked inflorescence [9]. The essential oil of basil extracted via steam distribution from the leaves and flavouring tops are used to flavour foods, dental and oral products, in fragrances and in traditional rituals and medicines [10, 11]. Extracted essential oils have also been shown to contain biologically active constituents that are insecticidal [12], nematocidal [13] and fungistatic [14]. These properties can be frequently attributed to predominate essential oil constituents such as methyl chavicol, eugenol linalool, camphor and methyl cinnamate. Two minor components of the essential oil of sweet basils (*Ocimum basilicum*): Juvocimene I and II have been reported as potent juvenile hormone analogs [15]. With this background the main aim of present study was focused on evaluation of antimicrobial activity of *Ocimum sanctum* leaf extract in normal tap water and local river water.

## 2. Materials and Methods

### 2.1. Water Sample

The water samples were collected from Bagmati river of Kathmandu Valley and local tap waters from Biotechnology Research Laboratory, Amrit Science Campus and Thamel and stored in a sterile plastic can.

### 2.2. Preparation of Tulsi Leaf Crude Extract

Rama Tulsi was cultivated in the local garden. Tulsi leaves were plucked from the plant, washed with tap water and subsequently with sterile distilled water (3 times). The preweighed leaf materials were crushed by mortar & pestle and extracted with sterile distilled water using soxhlet apparatus. Finally the extract was weighed gravimetrically.

### 2.3. Optimization of Treatment Period

Tulsi leaf crude extract (100mg in 2ml of sterile distilled water) was dissolved in 1 litre of tap and river water maintained at a pH of 6.8-7.0 and treated for different treatment periods (1-16 hrs). Water samples (5ml) were withdrawn for every hour and the sample was subjected to microbiological analysis by plate method and counted as CFU/ml.

### 2.4. Estimation of Microorganisms by Plate Count Method.

The original inoculum is diluted in a series of dilutions. Each succeeding dilution will have only one-tenth the numbers of microbial cells as the preceding tube. Then the samples of the dilution samples are used to inoculate (spread plate) the Petri plate on which the colonies grow and can be counted. The water sample containing bacteria (*E. coli*) are streaked on to the Nutrient Agar medium to check the growth of bacteria. The water sample was used as a control. If there were no colonies on the medium, the results indicate that the Disinfectant is highly effective against the bacteria. All the treatments were carried out in triplicates and the average values were accounted.

### 2.5. Optimization of Treatment dosage

After optimizing the treatment period, treatment dosage is optimized. Different treatment dosages (100, 200, 300, 400, 500 and 600 mg *Ocimum sanctum* leaves extract in 2 ml sterilized distilled water) were prepared as mentioned earlier. To 1 litter of tap and river water, the treatment dosages were treated individually and incubated for 16 hrs at room temperature and plating was done for microbial analysis.

### 2.6 Statistical Analysis

All data were analyzed by using one-way ANOVA (Sigma Stat v2.01, Jandel Corporation) followed by Duncan's Multiple Range tests<sup>16</sup> to test for the effects of dietary treatment. When a significant treatment effect was observed, a least significant difference test was used to compare means. Treatment effects were considered with the significant level at  $P < 0.05$ .

### 3. Results

From 100g of leaf 1.0g of extract were recovered. Evaluation of the antimicrobial potential of plant extracts were performed by optimizing the treatment period and disinfectant dosage for different kind of drinking water. For the fixed amount of disinfectant dose  $100 \text{ mg l}^{-1}$  treated water was subjected to plate counting. The results revealed that the microbial populations were gradually reduced from 22,000 cells in control to 16,000 cells at 16 hours treatment and after which the reduction stabilizes. Hence 16 hours treatment period was taken as the optimization period. After six different dose treatments of plant extract, two different dose range  $500 \text{ mg l}^{-1}$  and  $600 \text{ mg l}^{-1}$  concentration treated water samples shows best

antimicrobial activity in both water samples. In tap water  $600 \text{ mg}$  of extract treated water gave 45% activity at 1 hour and 100% activity while reaching 15 hours treatment and  $500 \text{ mg l}^{-1}$  treated water showed 95% of antimicrobial activity and rest of the dosage gave lesser activity. In river water  $500$  and  $600 \text{ mg l}^{-1}$  dosage treated sample showed 100% activity at 14 hours and the other dosage ( $200, 300, 400 \text{ mg l}^{-1}$ ) also gave 73 to 94% activity at 15 hour treatment shown in fig (1-6). From this experiment the higher concentration  $600 \text{ mg l}^{-1}$  shows 100% antibacterial activity at 16 hrs treatment which indicates that the minimal bacterial concentration was observed in  $600 \text{ mg l}^{-1}$  of leaf extract and then in  $500 \text{ mg}$ .

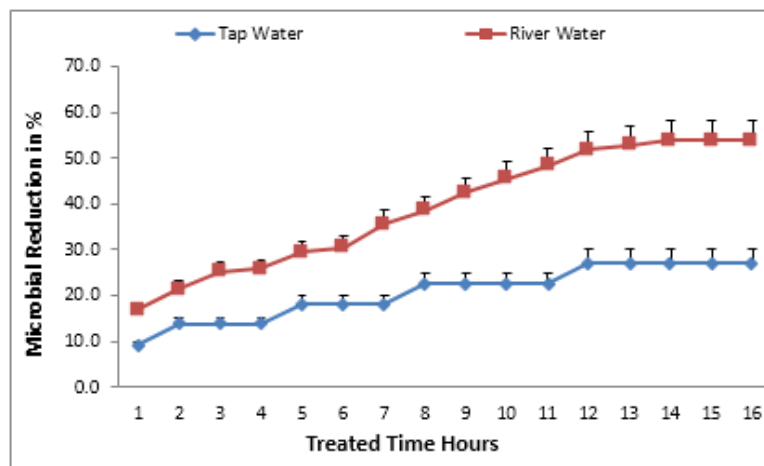


Fig 1: Treatment of water samples with 100 mg of *Ocimum sanctum* extract.

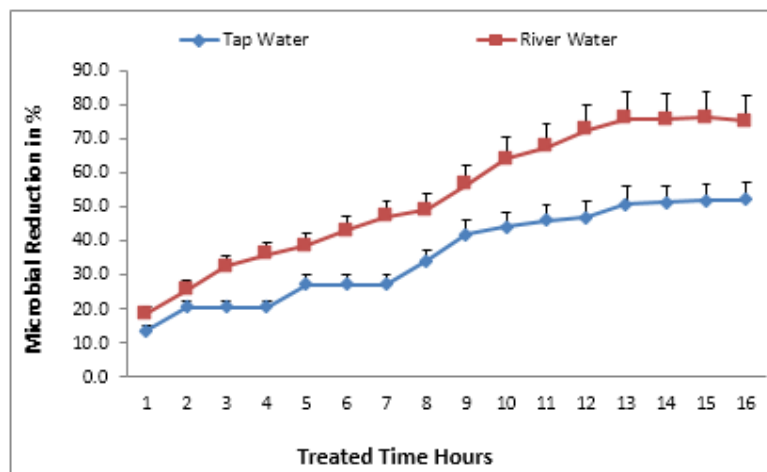


Fig 2: Treatment of water samples with 200 mg of *Ocimum sanctum* extract.

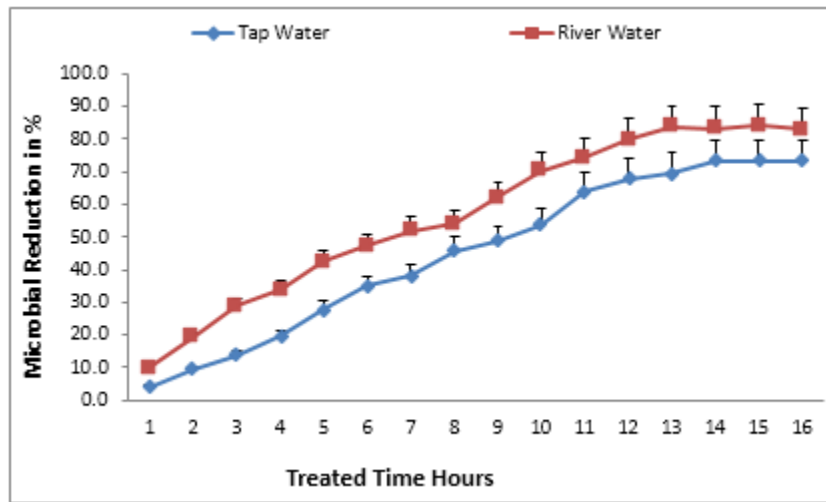


Fig 3: Treatment of water samples with 300 mg of *Ocimum sanctum* extract.

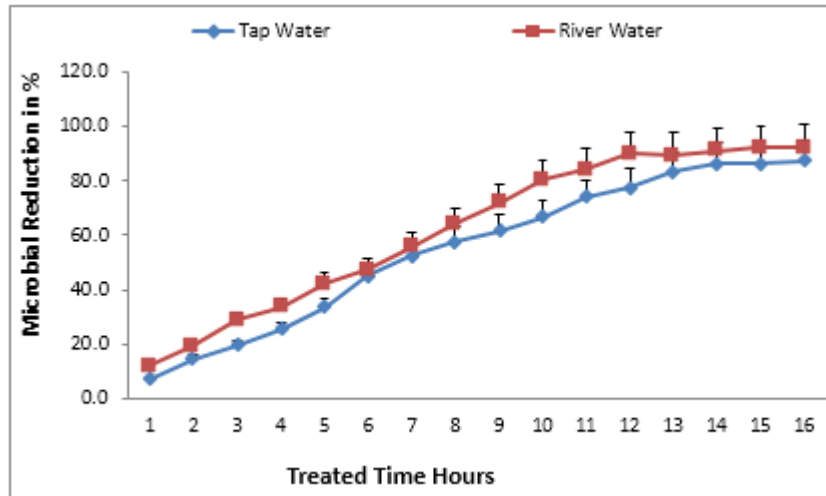


Fig 4: Treatment of water samples with 400 mg of *Ocimum sanctum* extract.

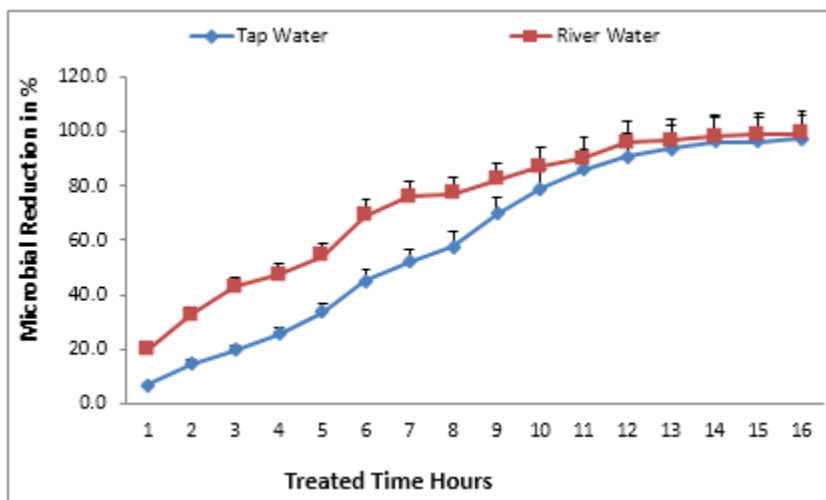


Fig 5: Treatment of water samples with 500 mg of *Ocimum sanctum* extract.

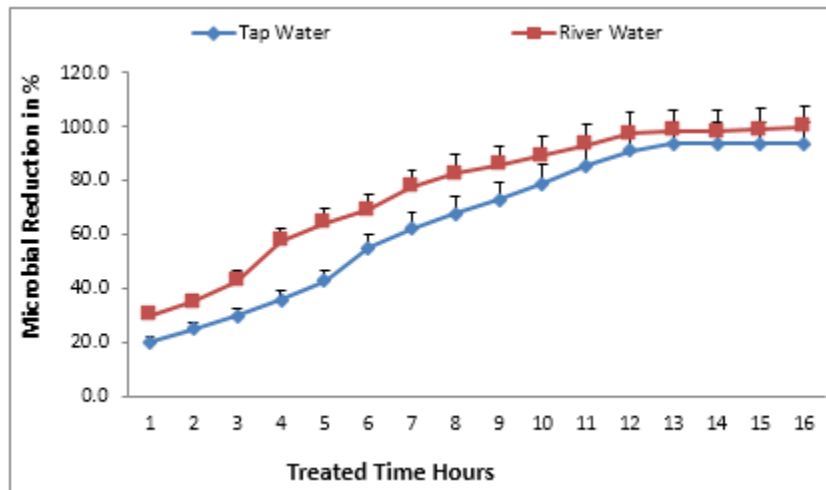


Fig 6: Treatment of water samples with 600 mg of *Ocimum sanctum* extract

#### 4. Discussion

Queen of herbs *Ocimum sanctum* plant (Tulsi/Holy Basil) has got very well documented beneficial effects of its different parts in many medical conditions<sup>17</sup>. Preliminary review shows that very few workers attempted to look into the various changes in reproductive system in detail after feeding *Ocimum sanctum* leaves extract<sup>[18]</sup>. Tulsi is renowned for its important role in the traditional ayurvedic and unani systems of holistic health and herbal medicine of the East<sup>[19]</sup>. Medicinal, religious and culinary use of Tulsi has also been documented for centuries in China and the rest of Asia, the Middle East, North Africa and Australia<sup>[20]</sup>. According to Ayurvedic system of medicines a large number of plants are employed in the traditional medicines for the treatment of several diseases like cancer<sup>[21, 22]</sup>, leprosy<sup>[23]</sup>, hepatic disease<sup>[24]</sup>, paralysis<sup>[25]</sup>, urinary stone track disease<sup>[26]</sup>, depression and other nervous disorder<sup>[27]</sup> and diabetes<sup>[28]</sup>. Ancient literature Rigveda and Atharveda mention 67 and 290 medicinal plants respectively<sup>[30]</sup>. The main properties of *Ocimum sanctum* lowers blood sugar levels, antispasmodic, analgesic, lowers blood pressure<sup>[31]</sup>. Tulsi leaves have anti-inflammatory<sup>[32]</sup>, anabolic hypoglycemic<sup>[33]</sup>, cardiac depressant<sup>[34]</sup>, antifertility<sup>[35]</sup>, antiulcer<sup>[36]</sup>, antidiabetic<sup>[37]</sup>, anticancer<sup>[38]</sup>, antioxidant<sup>[39]</sup>, antistress<sup>[40]</sup>, immunostimulant<sup>[41]</sup>, smooth muscle relaxant<sup>[42]</sup>,

adaptogenic and immuno modulator properties<sup>[43]</sup>. The Tulsi plant is even known to purify or de-pollute the atmosphere and also works as a repellent to mosquitoes, flies and other harmful insects<sup>[44]</sup>. The major effects of tulsi leaves are anti fertility effect<sup>[45]</sup>, anti diabetic effect<sup>[46]</sup>, anti allergic<sup>[47]</sup> and immuno modulator effects<sup>[48]</sup>, stress resilience<sup>[49]</sup>, anti-ageing effects<sup>[50]</sup>, anti oxidant activity<sup>[51]</sup>, immunity tune-up<sup>[52]</sup>, anti-inflammatory action<sup>[53]</sup>, antibiotic protection<sup>[54]</sup>, lung and bronchial support<sup>[55]</sup>, nutrition<sup>[56]</sup>, allopathic medicine complement<sup>[57]</sup>, antimicrobial properties<sup>[58]</sup>. Currently, *O. basilicum* and *O. sanctum* oils are being studied for their anti inflammatory and antiulcer activity<sup>[58]</sup>, Basil is also proved to be valuable source of anticarcinogenic agents<sup>[59]</sup>. Holy basil (*Ocimum sanctum*) may have the ability to prevent the early events of carcinogenesis<sup>[60]</sup>. Other studies provide evidence of potent anti HIV-I<sup>[59]</sup>, antioxidant<sup>[60]</sup> hypoglycemic<sup>[61]</sup> and hypolipidemic activities of basil leaves and leaf extracts<sup>[62]</sup>. Ancient literatures of world of medicine suggest that the primitive people of antiquity and those of earlier centuries have been using several kinds of medicinal plants for combating diseases<sup>[3]</sup>. The herbal medicines of ancient times practiced by the Assyrians (4000 B.C.), Sumerians (3500 B.C.) Indians (3500 B.C.), Chinese (3000 B.C.) and Egyptians (2500 B.C.) and which was temporarily subdued under

the impact of modern medicine have staged a comeback and a 'herbal renaissance' is blooming across the world.

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<sup>-1</sup> 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<sup>-1</sup> in 15 to 16 hrs

## 5. Conclusion

Current scientific research offers substantial evidence that tulsi protects against and reduces stress; enhances stamina and endurance; increases the body's efficient use of oxygen; boosts the immune system; reduces inflammation; protects against radiation damage; lessens aging factors; supports the heart, lungs and liver; has antibiotic, antiviral and antifungal properties; enhances the efficacy of many other therapeutic treatments; and provides a rich supply of antioxidants and other nutrients. Overall, tulsi is a premier adaptogen, helping the body and mind to adapt and cope with a wide range of physical, emotional, chemical and infectious stresses, and restore disturbed physiological and psychological functions to a normal healthy state. These general vitality enhancing and health promoting properties, in addition to tulsi's many more specific therapeutic actions, likely account for much of the exceptionally broad range of tulsi's traditional medical uses, as well as contributing to its mythological importance and religious sanctity.

Tulsi Leaf extract have great potential as antimicrobial agent for the treatment of water. The treatment is simple, cost-effective, eco-friendly, reachable for all and the components present in *Ocimum sanctum* leaves have no side effects to human compared to chemical treatment. More over the water treated with Tulsi extract serve not only as germ free but also as Medicinal water.

## 6. Acknowledgement

The author is grateful to the Campus Chief, Amrit Science Campus, Tribhuvan University, Kathmandu for providing us lab facility.

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