

# ANTIMICROBIAL ACTIVITY OF WATER DROP DEVELOPED FROM MIXTURE OF TULSI (*OCIMUM SANCTUM*) DISTILLATE AND COW URINE DISTILLATE

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**ABSTRACT:** *Ocimum sanctum* (Tulsi) is one of the holiest plant which exhibits tremendous healing potential. Tulsi covers many domains of medicines like Ayurveda, Siddha, Greek, Roman and Unani medicine system. Extract of Tulsi is used in ayurvedic treatments for common cold, heart diseases, and stomach disorders, poisoning cases, convulsions, epilepsy, malaria, fever, bronchitis and certain inflammatory problems. Therefore, extract of Tulsi is also known as "Extract of Life" and considered to grant longevity. Tulsi plant contains the various pharmacological compounds which are responsible for its antimicrobial activity. Many research and Review articles have been already published on this topic and they proposed to antimicrobial function of Tulsi. In the present study, efforts have been made to investigate antimicrobial activity of Water drop made by mixture of different concentration of Tulsi distillate and cow urine distillate. Leaf distillate of Tulsi was prepared in steam distillation column. In conclusion water drop found to be containing chemical compounds useful in food products and to deactivate the microbes present in drinking water as a disinfectants and development of drugs against food born and infectious microorganism

**Key Words :** *Ocimum sanctum*, antimicrobial activity, cow urine.

## 1. Introduction

Holy basil has a rich and fanciful history known since the Vedic age for its immense curative and multi-purpose utility. It has been the 'Herb royale' to the French, a sign of love by Italians, and a sacred herb in India. In the first century A.D. Roman naturalist Pliny reported that basil relieves flatulence, which had been subsequently proven true. In the Far East, the herb had been used as a cough medicine, and in Africa, it has been used to expel worms. American colonists considered holy basil is the essential ingredients in a snuff used to ease headaches.

The plant grows all over India up to 2000 meters height. It is grown in houses, temples and gardens. An erect annual grows 0.5-1.5 meters in height and has red or purple quadrangular branches.

Botanically, Tulsi is known as *Ocimum sanctum* and it belongs to family aminacea. The leaves contain an essential oil, which contains eugenol, eugenal, carvacrol, methylchavicol, limatrol and caryophylline. The seeds contain oil composed of fatty acids and sitosterol. The roots contain sitosterol. All parts of Holi basil have medicinal values.

### 1.1 Ayurveda

Tulsi has been used for thousands of years in Ayurveda, a Hindu form of medical science, for its diverse healing properties. It is mentioned in the CharakaSamhita, an ancient Ayurvedic text. Tulsi is considered to be an adaptogen, balancing different processes in the body, and helpful for adapting to stress. Marked by its strong aroma and astringent taste, it is regarded in Ayurveda as a kind of "elixir of life" and believed to promote longevity. If sprinkled over cooked food in stored water, tulsi leaves prevent bacterial growth during the eclipses.

### 1.2 Properties

Tulsi is pungent and bitter in taste, pungent in the post digestive effect and has hot potency. It alleviates kapha and vatadoshas, but slightly aggravates the pitta dosha. It possesses light and dry attributes. On the contrary the seeds are oily and slimy in attributes and have a cold potency. Tulsi is a stimulant, aromatic herb and effectively reduces the fever.

### 1.3 Chemical composition:

Oleanolic acid, ursolic acid, rosmarinic acid, eugenol, carvacrol, linalool,  $\beta$ -caryophyllene (about 8%),  $\beta$ -elemene (11.0%), and germacrene D (about 2%)

Table 1: Chemical composition of Tulsi

Chemical composition	Molar mass g/mol
Eugenol	164.2
Lutein	568.871
Apigenin	270.24
Caryophyllene	204.36
Ursolic acid	456.711
Oleonolic acid	456.7
Linalool	154.25
Carvacrol	150.217
Cirsilineol	314.293

#### 1.4 Pharmacological Properties of Extract Tulsi:

- Effective in dental plaque and gingival inflammation
- Antibacterial properties
- Antidiabetic properties
- Hepatoprotective properties
- Antimicrobial
- Immunomodulatory effect

#### 1.5 Uses:

- Used for bronchial asthma; expectorant and bronchodilator effects.
- Used against respiratory ailments including bronchitis and tuberculosis.
- Used for rhinitis (inflammation of nasal mucus membrane).
- Can serve as a cure and prophylactic as well for the severe acute respiratory syndrome (SARS) – The root of the Tulsi plant should be crushed and boiled with turmeric powder for a few minutes, after which it should be filtered. Consuming two spoonful of this portion twice daily will cure SARS and prevent contracting of the disease.
- Tulsi tea with honey is a good expectorant especially in cases where fever is involved.
- The juice of the leaves is given in catarrh and bronchitis in children.
- Chewing the leaves relieves cold and flu. A decoction of the leaves, cloves and common salt also gives immediate relief in case of influenza.
- The essential oil of basil extracted via steam distillation from the leaves and flavoring tops are used to flavor foods, dental and oral products, in fragrances and in traditional rituals and medicines. Extracted essential oils have also been shown to contain biologically active constituents that are insecticidal and fungistatic. These properties can be frequently attributed to predominant essential oil constituents such as methyl chavicol, eugenol linalool, and camphor and methyl cinnamate. Two minor components of the essential oil of sweet basil (*Ocimum basilicum*): Juvocimene I and II have been reported as potent juvenile hormone analogs. 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.

#### 1.6 Cow Urine:

Cow Urine has a great pharmacological importance its medicinal utility has been greatly mentioned in depth in Ayurveda. Cow urine is found to be effective against reversal of certain cardiac and kidney diseases, indigestion, stomach ache, edema, skin disease etc. The cow urine distillate has been patented as an activity enhancer and availability facilitator for bio molecules including anti-infective and anti-cancer agents. Cow urine has certain volatile and nonvolatile components which might have high antimicrobial activity. Cow urine exhibits both antioxidant and antimicrobial activities which was confirmed by Edwin. Cow's urine is widely used in the Ayurvedic pharmaceuticals for enhancing the properties of many drugs.

## 2. MATERIALS AND METHODS

### 2.1 Sample

Fresh leaves of *Ocimum sanctum* with its stalks were obtained from local areas. The leaves were carefully cleaned manually to remove dirt and damaged ones, and were removed from the stalks. Cow Urine Extract, extracted by using steam distillation method.

**2.2 Preparation of Tulsi Leaf Crude Extract**

Fresh Tulsi leaves were collected from local areas. The leaves were carefully cleaned manually to remove dirt and damaged ones, and were removed from the stalks. Then 800 g of leaves were extracted with sterile distilled water using steam distillation column. Initial temperature is 30 °C and final temperature is about 80 °C. Distillation time was about 135min and rate of distillation were 0.503 Cm<sup>3</sup>/s. The extract contains pale yellow color oily stresses which are removed using separating funnel. 2.3 g of essential oil which is rich in eugenol was obtained in this distillation and extract contains 6.77% of water. After removal of oil content the filtrate was passed through 0.2 micron cellulose acetate membrane to get the highly refined extract. The basil distillate passed through the cellulose acetate membrane yields clear distillate and this was used for making of water drop.

**2.3 Product Development (Water Drop):**

Drop was made by using mixture of basil distillate and cow urine distillate because both have same property like antimicrobial, antifungal, antibacterial, anticancer. The drop formed purely organic and used for drinking water and hence named as Water Drop.

Herbal based product is the recent fad in the market and this continues to be one of the strategies employed by manufacturers to attract consumers' attention in more mature markets. Because chemical based product shows their result immediately but their voluminous side effects. Hence this point to be noticed we made organic drop. This Organic drop has lot of health benefit for human being and it has no side effect. After making Organic drop, some laboratory testing like pH, TDS, Antimicrobial testing, colour, odour, chemical analysis, TSS etc. were examined.

For making the organic drop various sample were made by mixing both distillate in different volume. The table given below shows the drop sample with different volumes

Table 2: The drop samples with different volumes

Sr. No.	Basil Distillate (ml)	Cow Urine Distillate (ml)	Total (ml)
1	40	10	50
2	30	20	50
3	25	25	50
4	20	30	50
5	10	40	50

**2.4 Antimicrobial testing:**

The bacterial strains used in this study were E.coli. 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.

**3. RESULTS AND DISCUSSION**

**3.1 Results :**

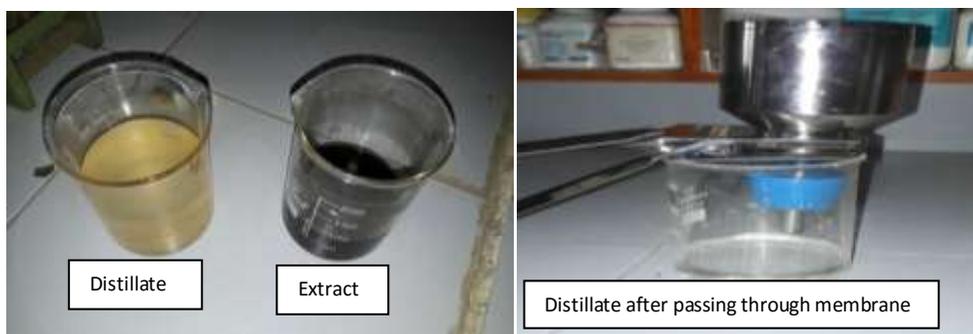


Figure 1: samples of tulsi distillate and tulsi extract



Figure 2: Water drop samples

• **Tulsi distillate analysis:**

Table 3: Tulsi distillate analysis

Sr. no.	Parameter	Distillate
1	Color	Pale yellow color
2	Odor	Basil
3	TDS	88 ppm
4	TSS	0
5	pH	7.0

• **Cow urine distillate analysis:**

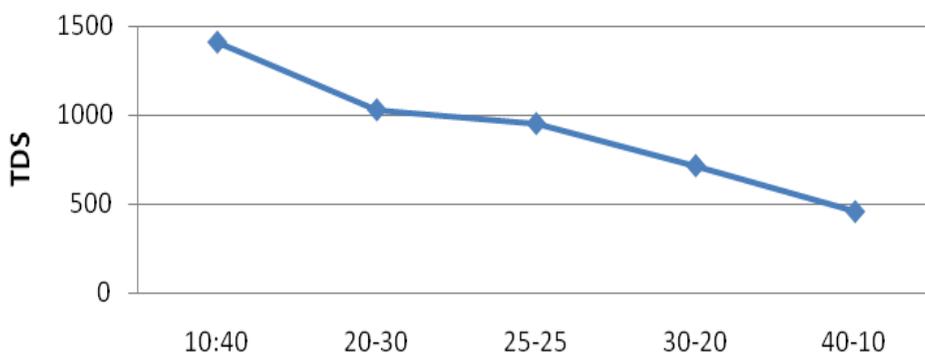
Table 4: Cow urine distillate analysis

Sr. no.	Parameter	Distillate
1	Color	Yellow color
2	Odor	Ammonia
4	TDS	169 ppm
5	TSS	0
6	pH	8.19

• **TDS in water drop:**

Table 5: TDS of water drop with varying volumes of tulsi and cow urine distillate

Concentration of basil distillate in ml	Concentration cow urine distillate in ml	TDS
10	40	1410
20	30	1030
25	25	953
30	20	716
40	10	458



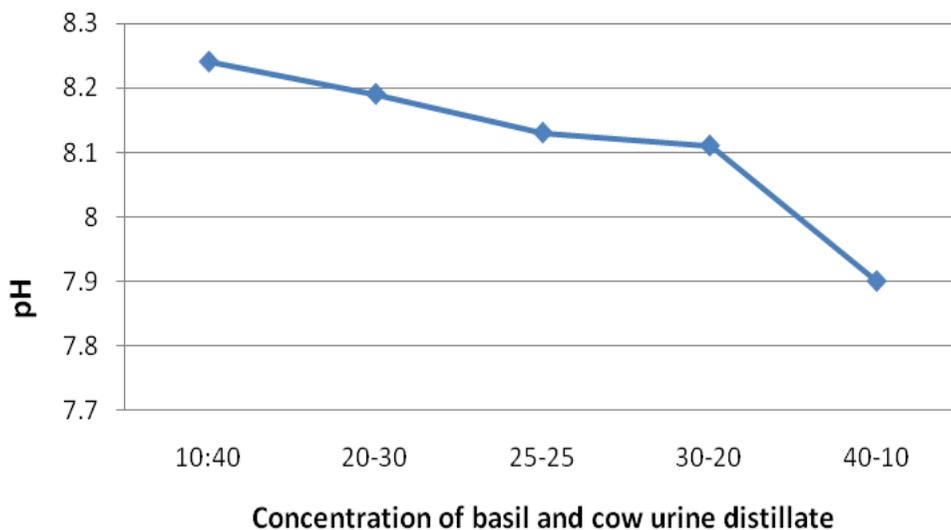
concentration of basil and cow urine distillate

Graph 1: TDS verses concentration of basil and cow urine distillate

- **pH in Water Drop :**

Table 6: pH of water drop with varying volumes of tulsi and cow urine distillate

Concentration of basil distillate in ml	Concentration cow urine distillate in ml	pH
10	40	8.24
20	30	8.19
25	25	8.13
30	20	8.11
40	10	7.9



Graph 2: pH verses concentration of basil and cow urine distillate

- **Antimicrobial Activity of Tulsi (Ocimum Sanctum) Against E-coli:**

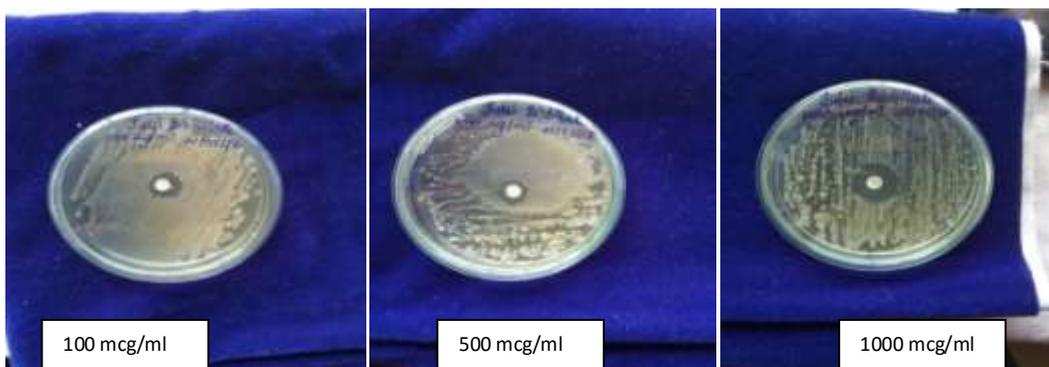


Figure 3: Antimicrobial activity of tulsi against E-coli

Table 7: concentrationwise inhibition zone seen of tulsi distillate

Concentration (mcg/ml)	Inhibition zone (in mm)
100	10
500	11
1000	15

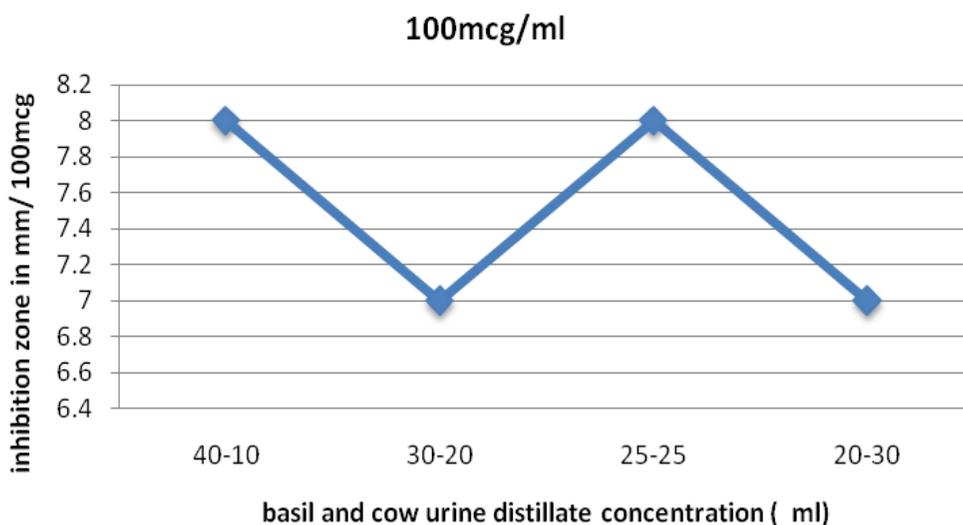
• **Antimicrobial Activity of Water Drop (Tulsi and Cow Urine Distillate):**



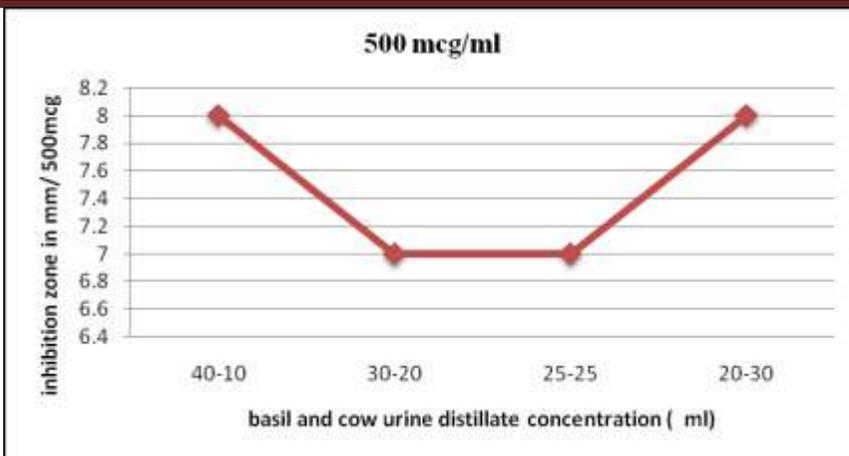
Figure 4: Antimicrobial activity of water drop against E-coli

Table 8: concentrationwise inhibition zone seen of water drop with varying volumes of tulsi and cow urine distillate

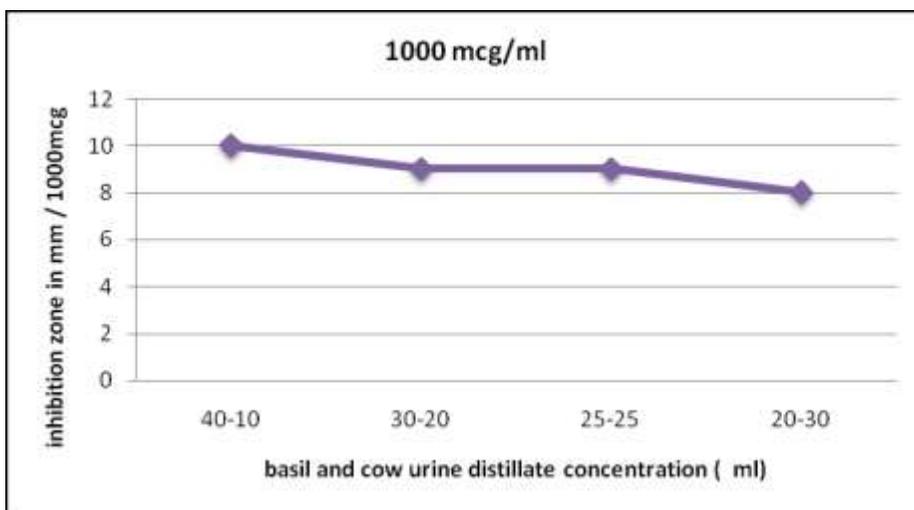
Microorganism	Basil and cow urine distillate concentration	Concentration (mcg/ml)	Inhibition concentration (in mm)
E-coli	40-10	100	8
		500	8
		1000	10
E-coli	30-20	100	7
		500	8
		1000	9
E-coli	25-25	100	8
		500	8
		1000	9
E-coli	20-30	100	7
		500	8
		1000	8



Graph 3: Water drop concentration Vs inhibition zone at 100 mcg



Graph 4: Water drop concentration Vs inhibition zone at 500 mcg



Graph 5: Water drop concentration Vs inhibition zone at 1000 mcg

Evaluated the antimicrobial activity of basil and cow urine distillate against E-coli by using antimicrobial testing for different concentration of water drop (Tulsi and Cow Urine Distillate) such as 40-10, 30-20, 25-25, 20-30. As compare to cow urine distillate the higher concentration of antibacterial property of basil distillate. We take a different concentration such as 100, 500, and 1000 mcg/ml and got a different inhibition zone. We had found better result in the water drop of 40ml Tulsi distillate and 10ml cow urine distillate mixture for 1000mcg/ml concentration.

**3.2 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. Tulsi is renowned for its important role in the traditional ayurvedic and Unani systems of holistic health and herbal medicine of the East. In this study the antimicrobial activity of the leaf extract was analyzed, the maximum activity was observed in Water Drop of 40ml Tulsi distillate and 10ml cow urine distillate mixture for 1000mcg/ml concentration sample. From this we concluded the harmful organisms were inactivated by this organic drop at 1000 mcg/ml concentration. We can use this water drop to reduce the microbial activity in drinking water and in foods products also as it showed the positive effect on TDS removal of water and considerable pH values and it is safe for drinking and has no side effects.

**3.3 CONCLUSION:**

Tulsi distillate is good for health. Tulsi leaves 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.

Herbal based product is the recent fad in the market and this topic to be noted for herbal based product (water drop) from tulsi and cow urine distillate because both showing common property i.e. microbial activity. In scientific research the TDS value above 500 is not better for health and this point to be remarked for making organic drop below 400 TDS value. In antibacterial testing, different concentration such as 100, 500, and 1000 mcg/ml and got a different inhibition zone. And better result were found in water drop of 40ml Tulsi distillate and 10ml cow urine distillate mixture for 1000mcg/ml concentration and got a higher inhibition zone.

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