Studies of Antimicrobial Properties of Different Leaf Extract of Tulsi (*Ocimum tenuiflorum*) against Human Pathogens

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Abstract

Objective: *To study the antimicrobial activity of the different leaf extracts of Tulsi (Ocimum tenuiflorum), also known as Ocimum sanctum, against three human pathogens Escherichia coli, Staphylococcus aureus and Candida albicans* ***Methods:*** *Different extracts (Ethanol, Methanol, Ethyl acetate and chloroform) of dried leaf of O. tensanctum were tested against three human pathogens strains such as Escherichia coli, Staphylococcus aureus and Candida albicans through the well diffusion and the poison plate method. The Minimum inhibitory concentration (MIC) values of the crude extract of the tested plant leaves were determined.* ***Result:*** *Both methods (well diffusion and poison plate) showed the strongest activity in methanol extract. Among four methanol extracts, they show more inhibition against ~~in~~ S. aureus than E. coli and C. albicans.* ***Conclusion****: The result showed methanol leaf extract inhibits S. aureus growth.*

Keywords: *Ocimum tenuiflorum,* well diffusion, poison plate, antimicrobial activity, leaf extract

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1. Introduction

A natural product is a substance produced by a living organism which is found in nature and which usually has a biological or pharmacological activity that can be used in drug discovery and drug design. Natural products are important in the treatment of life-threatening conditions. Natural products may be obtained from the extraction of tissues of plants, marine organism or from micro-organism fermentation. Plant-derived substances have recently become of great interest pharmaceutical researchers owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs for traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharamaceutical intermediates and chemical entities for synthetic drugs [1].

Ociumum tenuiflorum also known as *Ocimum sanctum*, Holy basil or Tulsi is an aromatic plant. It is native throughout the Eastern world and tropics and is wide spread as cultivated plant. The variety of *Ocimum tenuiflorum* used in Thai cuisine is referred to as Thai Holy Basil [2].

Ocimum is a genus of about 35 species of aromatic annual and perennial herbs and shrubs. Some species include~~s~~ *Ocimum basilicum* or Thai basil; *O. campechianum* or Amazonian basil; *O. gratissimum* or African Basil; *O. tenuiflorum* or *O. sanctum* or Tulsi or Holy Basil; *O. citriodorum* or Lemon Basil, *O. sanctum* grow up to 60 cm high with red or purple sub quadrangular branches. Leaves are simple, serrate and hairy. Flowers are purple in color. Fruits are smooth and not mucilaginous when wetted.

It is propagated by means of seeds. Seeds are planted directly in the ground. Young plants are transplanted when they attain 8-10 cm height [3]. Krishna Tulsi has purple leaves while Shri Tulsi has green leaves. Tulsi is used to reduce skin disorders, pain, swelling, headache and disease of the head and neck. Tulsi leaves are very useful for lung intestinal and cardiovascular diseases. They are also effective in reducing stress, blood sugar and blood cholesterol. Prakash and Gupta [4] have described a short review on therapeutic uses of *Ocimum Sanctum* with a note on eugenol and its pharmacological actions.

A critical review on phytochemical, antibacterial and immunomodulatory properties of *O. sanctum* is reported by Kumar et al [5]. Joshi et al [6] has investigated phytochemical and an antimicrobial property of aqueous ethanolic extracts of Tulsi, Cloves, Datiwan, Neem medicinal plants. Prasad et al [7] have explained antibacterial, phytochemical and antioxidant potential of some Ocimum species. The qualitative phytochemical screening and GC-MS analysis of *Ocimum sanctum* leaves extracts is discussed by Devendran and Balasubramanian [8]. A comparative study of antimicrobial activity and phytochemical screening of aqueous and alcoholic leaf extract of Tulsi on *E. coli* is presented by Sadul Rama et al [9]. Choudhury et al [10] studied pharmacognostical and phytochemical screening of various Tulsi plants available in South Eastern Odisha State of India. Comparative analysis of Tulsi stem and leaves for phytochemicals and inorganic constituents has been described by Shafquatullah et al [11]. In vitro antioxidant potential of *Ocimum sanctum* and *Ocimum basilicum* is reported by Ramesh and Satakopan [12].

Devi [13] has presented reviews on antioxidant properties of the Indian Holy Basic, *Osciumum sanctum* (Tulsi). Joshi et al [14] studied antibacterial property of different medicinal plants viz: Tulsi, Ram Tulasi, Dalchini, and Timur for potential antibacterial activity against 10 medically important bacterial strains e.g. *S. aureus, E. coli, B. subtilis, S. typhi, Shigella, K. pneumnial* etc. Mishra and Mishra [15] have observed antibacterial activity of aqueous and chloroform extract of leaves of Tulsi against the bacteria i.e. *E. coli, S. aureus, P. aeruginosa, S. typhimurium* .Present investigation reveals that *Oscimum sanctum* may be used as a preservative in food industries since it is equally effective against pathogenic gram positive and gram negative bacteria. Jeba and Rameshkumar [16], studied antimicrobial activity of leave extracts of *Ocimum gratissiumum* by testing them against *Salmonella, Typhimurium, S. aureus*, and *E. coli*, pathogenic bacteria that cause diarrhea. Rathod et al [17] have reported antimicrobial activity of aqueous and ethanolic extract of Neem (leaves and bark) and Tulsi (leaves) against two gram positive bacteria (*B. Subtilis* and *E. Coli*) and two gram negative bacteria (*K. pheumaniae* and *E. Coli*). Rabeta and Lai [18] have determined antioxidant capacity of freeze drying, vacuum drying, fermented and unfermented leaves of *O. sanctum.* The vacuum drying method seems to produce a product which has a higher quality of antioxidant property than freeze drying. Khan et al [18] observed anti-fungal activities of aqueous extracts and oils of five (5) Indian medicinal plants against two Candida species causing *Candiasis, C. albicans* and *C. tropicalis*. Tulsi essential oil was found to be most effective.

Sanguri et al [19] have investigated and compared antibacterial and anti-fungal activity of leaves extracts taken from plants viz *Q. indica, C. procera, A. aspera, O. sanctum* against ten microorganism comprising five bacteria and five fungi. *Ocimum sanctum* extract was found to be more effective on bacterial species.

Singh et al [20] has evaluated the qualitative estimation of phytochemicals and antimicrobial activity of aqueous and methanol extracts of root and leaves of *Ocimum sanctum* against pathogenic bacteria *E. coli, P. mirabilis, S. aureus*. The study has shown the presence of steroid, alkaloids and tannins. Significant antimicrobial activity of plant extract had been observed. Chemical composition, antifungal and anti aflatoxigenic activities of oils of Ocimum species were investigated by some workers [21, 22]. An essential oil *Ociumum sanctum* and its major component, eugenol act against the fungi cause bio- deterioration of foodstuff during storage. Essential oil of *Ociumum gratissimum* is used as a preservative, an antimicrobial, and an antioxidant.

Review on therapeutic potential of *O. sanctum* in prevention and treatment of cancer was investigated by Singh et al [23].

Pingale et al [24] reported that extract of *O. tenuiflorum* was potent in radio protective, antimicrobial, antioxidant, anti-helmertic, antiviral, cardioprotective, anticancer, anti-distress, renal damage recovery and wound healing activity of Ocimum sanctum in albino rats observed by Asha et al [25]. Tropical *O. santum* was found to promote better ranulation tissue, complete epithelisation and better tensile strength. Antibacterial, antifungal, antioxidant, anticancer, anti-ulcer pharmacological properties of *Ocimum species* viz: *O. sanctum / tenuiflorum, O. gratissiumum, O. basilicum, O. americanum, O. kilimandcharicum* were discussed by Verma and Kothiyal [26]. A review on use of *Ocimum kilimand* for the treatment of disease like cold, cough, abdominal pain, anti-cancer, anorexia, memory disorder, anti-ulcer, memory disorder, diarrhea was conducted by Narwal et al [27].

Joseph and Nair [28] presented a comprehensive study on anti-cancerous effect of *O. sanctum* in numerous cancers such as lung, skin, oral, cervical, gastric, breast and prostate. Joshi et al [29] presented a review of the plant Tulsi from Ayurvedic texts and macroscopic and microscopic sections were taken to identify the species.

Mondal et al [30] presented a review of scientific studies of Oscimum species for its antimicrobial, anti-diabetic anti-inflammatory, mosquito repellant properties.

2. Materials and Methods

2.1. Collection of Plant Materials

The plant material leaves of *Ocimum sanctum* were collected from Cummings Lodge and the National Agriculture Research Institute (NARI), Georgetown, Guyana.

2.2. Preparation of Plant Materials:

The sample of *O. sanctum* leaves was weighed on Citizen CTG 3000 electronic balance. The leaves were dried in an oven (Gallenhamp Incubator Model IH-150) at 50-60ºC. The dried leaves were cooled at room temperature and weighed again on the same citizen electronic balance. The weight of green leaves, dried leaves and the value of percentage moisture content in various samples of *O. sanctum* are given in Tables 2.1. The weights of ground leaves of *O. sanctum* were found to be 320 g.

2.3. Collection of Text Organism

Human pathogens culture of *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*) and *Candida albicans* (*C. albicans*) used in this study were obtained from the Microbiology Laboratory of Georgetown Public Hospital Corporation, Georgetown (GHPC). All cultures were maintained in nutrient broth (Himedia, M002) at 37ºC and maintained on nutrient agar (Himedia MM012) slants at 4ºC.

2.4. Extraction and Preparation of Test Solutions

The grounded leaves of *O. sanctum* were extracted in each chloroform, ethanol, ethyl acetate and methanol solvents. At a time 20 g of dried pulverized leaves were soaked with 200 mL of solvent for 48 h. Solvent was decanted and the residue again soaked with the same solvent for 24 h. The total extract was combined and filtered. The evaporation of solvent was done on rotavapour (Buchi). The respective solvent was added to viscous semi solid liquid extract to make up the desired volume of extract solution.

2.5. Reducing Antioxidant Power

The reducing antioxidant power of the plants methanolic, ethanolic, ethyl acetate and chloroform extract was determined by the method of Oyaizu [63]. Different concentration of plant extracts (100- 1000 µL) in 1 mL of distilled water were mixed with phosphate buffer (2.5 mL, 0.2 M pH 6.6) and potassium free cyanide (K_3 $Fe(CN)_6$) (2.5 mL,1%). The mixture was incubated at 50ºC for 20 min. Then, 2.5 mL of trichloroacetic acid (10%) was added to mixture, which was then centrifuge for 10 min at 3000 rpm. The upper layer of solution (2.5 mL) was mixed with distilled water (92.5 mL) and $FeCl_3$ (0.5 mL,) 1%. The absorbance was measured at 700 nm against a blank using UV-Vis spectrophotometer (Phillips X 500). Increased absorbance of the reaction mixture indicates increase in the reducing power.

2.6 Anti-Microbial Assay

Antimicrobial assay was done by disc diffusion and poisons plate method (NCCLS, 1993, Awoyinka et al.,) [64] using plants (Tulsi,) extracts (ethanol, methanol, ethyl acetate and chloroform) and commonly used antibiotics. The test quantities of specific extracts dissolved, depending upon the solubility of the extracts.

The dissolution of the organic extracts (Chloroform, methanol, ethanol and ethyl acetate) were aided by water, which did not affect the growth of microorganism, in accordance with our control experiments. The surfaces of media were inoculated with bacterial from a broth culture. After 18 h of incubation at a specific temperature 28°C-30°C for *E. coli*, *S. areus* and *C. albicans*, the plates were examined and the diameters of the inhibition zones were measured to the nearest millimeter.

3. Result

Table 1 – Percentage Moisture Content for *Ocimum Sanctum* Leaves

No. of Leaves Packets	Wt. of green leaves(g)	Wt. of leaves after 24 h	Wt. of leaves after 48 h	Wt of leaves after 72 h	Percentage moisture content
1	56.60	20.01	22.01	-	64.65
2	21.21	12.89	12.62	-	40.50
3	27.64	12.98	12.92	-	53.26
4	55.80	21.20	19.00	18.90	66.13
5	17.40	16.50	14.00	13.90	20.11
6	61.00	20.00	18.00	18.20	70.49
7	78.90	40.00	22.70	23.00	71.23
8	59.80	38.00	19.10	19.20	68.06
9	10.30	7.90	7.80	-	24.27

$$\text{Percentage moisture content} = \frac{\text{Weight of green leaves} - \text{weight of dry leaves}}{\text{Weight of green leaves}} \times 100$$

Table 2: Reducing Antioxidant Power of Ethanol Leaf Extract of Tulsi (*Ocimum Sanctum*)

S. No	Leaf Extract µL	Tulsi (nm)
1	Ethanol (control)	0.00
2	1	0.018
3	2	0.074
4	3	0.004
5	4	0.001
6	5	0.002
7	6	0.001
8	7	0.018
9	8	0.003
10	9	0.002
11	10	0.014

* replicates

Table 3: Reducing Antioxidant Power of Methanol Leaf Extract of Tulsi (*Ocimum Sanctum*)

S. No	Leaf Extract µL	Tulsi (nm)
1	Methanol (control)	0.00
2	1	0.051
3	2	0.054
4	3	0.057
5	4	0.074
6	5	0.099
7	6	0.147
8	7	0.179
9	8	0.182
10	9	0.186
11	10	0.195

* replicates

Table 4: Reducing Antioxidant Power of Ethyl Acetate Leaf Extract of Tulsi (*Ocimum Sanctum*)

S. No	Leaf Extract µL	Tulsi (nm)
1	Ethyl acetate (control)	0.00
2	1	0.010
3	2	0.034
4	3	0.020
5	4	0.031
6	5	0.033
7	6	0.036
8	7	0.036
9	8	0.039
10	9	0.040
11	10	0.041

* replicates

Table 5: Reducing Antioxidant Power of Chloroform Leaf Extract of Tulsi (*Ocimum Sanctum*)

S. No	Leaf Extract µL	Tulsi (nm)
1	Chloroform (control)	0.00
2	1	0.001
3	2	0.004
4	3	0.006
5	4	0.009
6	5	0.010
7	6	0.018
8	7	0.019
9	8	0.013
10	9	0.010
11	10	0.014

* replicates

Table 6: Antimicrobial Activity of Crude of Ethanol Leave Extract of Medicinal Plant Compared with Control by *Disc Diffusion* Method

Plants	Extract solvent (µ)L	Diameter of the inhibitory zone (nm)*		
		E. coli	*S. aures*	*C. albicans*
Tulsi *(Ocimum sanctum)*	Ethanol	0.00	0.00	0.00
	300	10.13	09.10	11.23
	600	14.46	11.13	13.45
	900	19.56	13.45	14.56

* replicates

Table 7: Antimicrobial Activity of Crude of Methanol Leave Extract of Medicinal Plant Compared with Control by *Disc Diffusion* Method

Plants	Extract solvent (µ)L	Diameter of the inhibitory zone (nm)*		
		E. coli	*S. aures*	*C. albicans*
Tulsi *(Ocimum sanctum)*	methanol	0.00	0.00	0.00
	300	19.12	14.23	18.34
	600	20.34	16.45	19.65
	900	22.67	17.98	20.89

* replicates

Table 8: Antimicrobial Activity of Crude of Ethyl Acetate Leave Extract of Medicinal Plant Compared with Control by *Disc Diffusion* Method

Plants	Extract solvent (µ)L	Diameter of the inhibitory zone (nm)*		
		E. coli	*S. aures*	*C. albicans*
Tulsi *(Ocimum sanctum)*	Ethyl acetate	0.00	0.00	0.00
	300	13.10	12.45	14.19
	600	15.70	13.00	14.98
	900	15.99	14.13	14.99

* replicates

Table 9: Antimicrobial Activity of Crude of Chloroform Leave Extract of Medicinal Plant Compared with Control by *Disc Diffusion* Method

Plants	Extract solvent (µ)L	Diameter of the inhibitory zone (nm)*		
		E. coli	*S. aures*	*C. albicans*
Tulsi *(Ocimum sanctum)*	chloroform	0.00	0.00	0.00
	300	19.78	18.67	17.13
	600	19.99	19.11	17.48
	900	20.10	20.11	18.00

* replicates

Table 10: Antimicrobial Activity of Crude of Ethanol Leave Extract of Medicinal Plant Compared with Control by *Poison Plate* Method

Plants	Extract solvent (µ)L	Diameter of the inhibitory zone (nm)*		
		E. coli	*S. aures*	*C. albicans*
Tulsi *(Ocimum sanctum)*	ethanol	0.00	0.00	0.00
	300	19.12	18.60	17.00
	600	19.00	19.13	17.00
	900	20.20	20.31	18.40

* replicates

Table 11: Antimicrobial Activity of Crude of Methanol Leave Extract of Medicinal Plant Compared with Control by *Poison Plate* Method

Plants	Extract solvent (µ)L	Diameter of the inhibitory zone (nm)*		
		E. coli	*S. aures*	*C. albicans*
* replicates	* replicates	* replicates	* replicates	* replicates
	300	19.18	18.27	17.33
	600	20.49	19.51	17.68
	900	21.70	20.81	18.90

* replicates

Table 12: Antimicrobial Activity of Crude of Ethyl Acetate Leave Extract of Medicinal Plant Compared with Control by *Poison Plate* Method

Plants	Extract solvent (µ)L	Diameter of the inhibitory zone (nm)*		
		E. coli	*S. aures*	*C. albicans*
Tulsi *(Ocimum sanctum)*	ethyl acetate	0.00	0.00	0.00
	300	19.78	18.67	17.13
	600	19.99	19.11	17.48
	900	20.10	20.11	18.00

* replicates

Table 13: Antimicrobial Activity of Crude of Chloroform Leave Extract of Medicinal Plant Compared with Control by *Poison Plate* Method

Plants	Extract solvent (μ)L	Diameter of the inhibitory zone (nm)*		
		E. coli	*S. aures*	*C. albicans*
Tulsi *(Ocimum sanctum)*	chloroform	0.00	0.00	0.00
	300	19.68	18.57	17.03
	600	19.89	19.01	17.38
	900	20.00	20.01	18.10

* replicates

4. Discussion

4.1 Reducing Power

Reducing power is to measure the reductive ability of antioxidant, and it is evaluated by the transformation of Fe (III) to Fe (II) in the presence of the sample extracts Gulcin et al. [66]. The reducing power of plant extracts are summarized in Tables 2, 3, 4 and 5. From the tables, reducing power increased with an increase in samples, increasing their reducing ability when the contraction of extracts was increased. The ability to reduce Fe (III) may be attributed to hydrogen donation from phenolic compounds Shimada et al, [67], which is also related to the presence of reducing agent Duh, [68]. In addition, the number and position of hydroxyl group of phenolic compounds also ruled their antioxidant activity Sawaddiwong et al [69].

It is observed from Tables 2 to 5 that:

Antioxidant power of *Ocimum sanctum* leaves extract in various solvents follow the order:

Methanol > ethyl acetate > chloroform > ethanol.

Ocimum sanctum leaves extract is found to have high reducing power in comparison to *Carica papaya* leaves extract in methanol and chloroform solvents.

4.2 Antimicrobial Activity

The results indicated that Tables 6, 7, 8 and 9 in disc diffusion and Tables 10, 11,12 and 13 in poison plate , all plants extracts showed antimicrobial activities toward the gram positive bacteria *S. aureus* as well as gram negative bacteria *E. coli* and *C. albicans*. The methanol extract of all the plants extracts showed more effective result against bacteria and fungi, with the two plants *O. sanctum* showing effective result in inhibition zones in both methods. Thus, it was evident that in the plant the organic extracts were more effective.

Table 6. Antimicrobial activity of crude of ethanol leave extract of *O. sanctum* compared with control in disc diffusion plate method. The result indicates that the ethanol leave extract showed more effective result than the other leave extract with significant inhibition against *E. coli* in well diffusion and poison plate. Thus, it was evident that the organic extracts were more effective than other extracts.

In conclusion, it is suggested that Tulsi plant leaves may be recommended as useful sources to prepare natural bioactive products from which we can develop new antimicrobial drugs which will be cost-effective because the plants are freely available.

5. Conflict of Interest Statement

We declare that we have no conflict of interest.

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