

Antibacterial Activity of Aqueous, Ethanol and Acetone Extracts of *Ocimum sanctum* Linn

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Abstract: Aqueous, ethanol and acetone leaf and root extracts of *Ocimum sanctum* Linn were investigated for their antibacterial activities at various concentrations against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* using standard methods. Antimicrobial studies indicated that both the acetone leaf and root extracts of *O. sanctum* were found comparatively more effective against these bacteria than any other extract tested while aqueous extract being the least effective against the tested microbes. The highest mean zone of inhibition of acetone leaf extract against *P. aeruginosa* was 20.74 ± 0.68 followed by 19.36 ± 0.29 at concentration 150mg/ml and 100 mg/ml respectively. The aqueous and ethanol root extracts of *O. sanctum* for had no inhibitory effect against the test microorganisms. Generally the acetone crude extracts showed activity against the three bacteria species with highest average zone of inhibition compared to other extracts. The antimicrobial activity of the leaf extract was more pronounced against test microbes than root extract. The study revealed that the plant possessed antimicrobial properties and could be a potential source of antibacterial agent in the treatment of bacterial infections.

Keywords: Antibacterial Activities, Extract, Inhibition, Medicinal Plant, *Ocimum sanctum*

1. Introduction

Ocimum sanctum Linn known as Holy Basil is an aromatic plant native to the tropics of Asia and Africa being medicinally important plant in the family Lamiaceae. The family Lamiaceae is one of the most exploited medicinal plant family worldwide not only as a source of medicinal plants but also with its valuable essential oils being used as spices and flavours for various food products [1, 2]. Morphologically *O. sanctum* is an erect about 75 cm tall, much branched with hairy stems and simple opposite green leaves that are strongly scented. Leaves have petioles, and are ovate, up to 5 cm long, usually slightly toothed [3]. *Ocimum sanctum* is known as a consolidated source of extracts with many applications in folk medicine ranging from strong antibacterial and antioxidant properties to particularly in Asia and Africa [4, 5]. The presence of essential oils in *O. sanctum* has been reported to exhibit repellence activity against insects [2, 6].

The World Health Organization (WHO) estimates that up to 80% of the world's population in developing countries depends on locally available plant resources for their primary healthcare because they are easily accessible and less

expensive compared to western pharmaceuticals which are often expensive or inaccessible [7, 8, 9]. Several modern drugs have been isolated or derived from natural sources based on their use in traditional medicine as herbal remedies or crude drugs, approved various regulatory agencies [10, 11]. With consideration of *O. sanctum* as reservoir plant for many therapeutic applications, various studies have reported its usages in complementary and alternative medicine particularly as anticancer [12-15]. As an aromatic medicinal herb, *O. sanctum* is used customarily in the treatment of headaches, coughs, diarrhea, constipation, antifertility, worms, kidney malfunctions and dysentery [16, 17].

Furthermore, studies have revealed *O. sanctum* to possess anti-inflammatory, analgesic, antipyretic, antidiabetic, hepatoprotective, hypolipidemic and antistress activities [13]. In the latest assessment for its clinical efficacy of herbal medicines, studies have shown its potential for the treatment of oral submucous fibrosis [18]. Traditionally as medicinal plant *O. sanctum* is taken in a variety of forms being hot or dried leaf tea, powdered leaf as well as seed, root, stem formulations, both systemically and topically [19]. Although several studies have been conducted for ethnomedicinal potential of *O. sanctum*, only scarce information is available

for its different plant parts being extracted and screened for antimicrobial activity. The aim of this study was to evaluate the antimicrobial effects of aqueous, ethanol and acetone leaf and root extracts of *O. sanctum* against pathogenic bacteria to determine their potentials as antibacterial agent.

2. Materials and Methods

2.1. Plant Sample Collection

The plant samples of *O. sanctum* were collected from Ukonga ward, Dar es Salaam at the latitude 6° 38' 39"S, longitude 39° 10' 29" °E. The identification of the plant was further confirmed at the Herbarium of the Botany Department, University of Dar es Salaam. The leaves and roots of *O. sanctum* were washed with distilled water to remove any impurities and then cut into pieces and oven dried at 70°C for 12 hrs to remove all moisture. The dried samples were then ground into fine powder with warring commercial laboratory blender and further milled (mesh size 850 µm).

2.2. Extraction of Plant Material

Crude extracts were obtained by using aqueous (distilled water), acetone and ethanol. The aqueous extract was also prepared by mixing 20g of each powdered leaf and root samples with 100 mL boiling distilled water for 5 minutes. For ethanol and acetone extracts the powdered leaf and root samples (20g) each were extracted individually using 100mL of extraction solvent of 95% ethanol and 95% acetone for 24 hrs with intermittent shaking at room temperature. The extracts were filtered by using Whatman filter paper number 1 and air dried at 28 °C. The extracts were then concentrated by evaporating the solvents using a water bath at 40°C. After evaporation for 45 min to 1hr depending on the type of the solvents, the weight of crude extract was determined by subtracting the flask's weight from total weight of the flask after evaporation. The dried extracts were then labeled and kept in airtight bottles in the refrigerator at 4 °C prior to determination of antibacterial activity.

2.3. Antibacterial Activity

In testing the antibacterial activity *O. sanctum* leaf and root extracts the following bacterial strains were used;

Escherichia coli-ATCC 25923, *Staphylococcus aureus*-ATCC 25923, *Pseudomonas aeruginosa*-ATCC 27853. These microorganisms were obtained from the Microbiology Department of Muhimbili National Hospital and they were maintained at 4°C in the refrigerator. Three different concentrations (50, 100 and 150 mg/ml) of each prepared aqueous, ethanol and acetone extracts were used for assessing the presence of zone diameter of inhibition.

Antibacterial activity was determined by using the well diffusion method. Each Petri dish containing Muller-Hinton agar medium was inoculated with one bacterial strain by scattering the suspension of the organism with a bended tip sterile glass rod. In each plate wells were made at equal distances using sterile cork borer. Gentamycin was used as a positive control and was prepared at a concentration of 25 µg/ml. Plates were kept in the refrigerator for 40 minutes to allow the diffusion of sample to the surrounding agar medium. The petri dishes were incubated at 37°C for 24 hrs. Plates were examined for zone of inhibition of that different extracts and the diameter of the zone of inhibition measured using a digital venial caliper and the average diameter for each sample was calculated.

2.4. Statistical Analysis

All experiments were carried out in four replicates and means and standard mean errors were calculated for the zones of inhibition measured for the replicate sets of experiments in each case. Results analyzed using Duncan's multiple range test (DMRT) to test the difference among the treatments.

3. Results

Results for the aqueous leaf and root extract of *O. sanctum* at concentrations 50, 100 and 150 mg/ml is indicated (Table 1). The antimicrobial activity of aqueous leaf extract against *E. coli*, *S. aureus* and *P. aeruginosa* was revealed only at concentration 150 mg/ml with mean zone of inhibition 4.28 ± 0.68 against *E. coli*, 7.42 ± 0.36 against *S. aureus* and 11.42 ± 0.76 mm against *P. aeruginosa* (Table 1). Generally in the aqueous extract, the leaf showed significantly high inhibition against *P. aeruginosa* compared to what was revealed against *E. coli* and *S. aureus* while the aqueous root extract had no inhibition against all three tested microbes (Table 1).

Table 1. Mean zone of inhibition (in mm) of aqueous leaf and root extracts of *O. sanctum*.

Extract Type	Crude extracts Conc. (mg/ml)	Test organisms		
		<i>E. coli</i> ATCC 25922	<i>S. aureus</i> ATCC 25923	<i>P. aeruginosa</i> ATCC 27853
Leaf	50	0.0 ± 0.00^a	0.0 ± 0.00^a	0.0 ± 0.00^a
	100	0.0 ± 0.00^a	0.0 ± 0.00^a	0.0 ± 0.00^a
	150	4.28 ± 0.68^b	7.42 ± 0.36^b	11.42 ± 0.76^b
	Control	24.62 ± 0.36^{ab}	24.14 ± 0.12^{ab}	23.92 ± 0.84^{ab}
Root	50	0.0 ± 0.00^a	0.0 ± 0.00^a	0.0 ± 0.00^a
	100	0.0 ± 0.00^a	0.0 ± 0.00^a	0.0 ± 0.00^a
	150	0.0 ± 0.00^a	0.0 ± 0.00^a	0.0 ± 0.00^a
	Control	23.86 ± 0.58^{ab}	25.62 ± 0.86^{ab}	21.86 ± 0.52^{ab}

Means with the same letter superscript on the same column are not significantly different at $p < 0.05$.

The mean zone of inhibition of ethanol leaf and root extracts of *O. sanctum* at different concentrations is indicated in Table 2. For the three concentrations used at 50, 100 and 150 mg/ml only concentrations at 100 and 150 mg/ml for ethanol leaf extract revealed antimicrobial activity. The effect of the ethanol leaf extracts on *E. coli* had mean values for the zones of inhibition 16.30 ± 0.22 and 18.52 ± 0.82 at concentrations 100 and 150 mg/ml respectively. The mean

values for the zone of inhibition on *S. aureus* were 13.44 ± 0.12 and 16.62 ± 0.72 at concentration 100 and 150 mg/ml respectively. The mean zones of inhibition on *P. aeruginosa* were 17.38 ± 0.1 and 19.72 ± 0.34 also at concentration 100 and 150 mg/ml respectively (Table 2). The ethanol root extract at all concentration tested had no inhibition against *E. coli*, *S. aureus* and *P. aeruginosa* (Table 2).

Table 2. Mean zone of inhibition (in mm) of ethanol leaf and root extracts of *O. sanctum*.

Extract Type	Crude extracts Conc. (mg/ml)	Test organisms		
		<i>E. coli</i> ATCC 25922	<i>S. aureus</i> ATCC 25923	<i>P. aeruginosa</i> ATCC 27853
Leaf	50	0.0 ± 0.00^a	0.0 ± 0.00^a	0.0 ± 0.00^a
	100	16.30 ± 0.22^b	13.44 ± 0.12^b	17.38 ± 0.14^b
	150	18.52 ± 0.82^b	16.62 ± 0.72^b	19.72 ± 0.34^b
	Control	24.86 ± 0.76^{ab}	25.26 ± 0.86^{ab}	25.44 ± 0.47^{ab}
Root	50	0.0 ± 0.00^a	0.0 ± 0.00^a	0.0 ± 0.00^a
	100	0.0 ± 0.00^a	0.0 ± 0.00^a	0.0 ± 0.00^a
	150	0.0 ± 0.00^a	0.0 ± 0.00^a	0.0 ± 0.00^a
	Control	25.60 ± 0.87^{ab}	28.32 ± 0.64^{ab}	27.22 ± 0.26^{ab}

Means with the same letter superscript on the same column are not significantly different at $p < 0.05$.

The result in Table 3 indicates that both the acetone leaf extract and acetone root extract of *O. sanctum* showed inhibitory effect against *E. coli* and *P. aeruginosa*. The mean zone of inhibition of acetone leaf extract on *E. coli* was 14.30 ± 0.36 and 17.53 ± 0.29 at concentration 100 and 150 mg/ml respectively. The acetone root extract at concentration 150 mg/ml was the only concentration which revealed zone of inhibition against *E. coli* as other concentrations had no inhibition against the bacteria (Table 3). The mean zone of inhibition of acetone leaf extract against *P. aeruginosa* was revealed in all three tested concentrations at 50, 100, and 150 mg/ml and the effect of extracts was 3.2 ± 0.56 , 19.36 ± 0.29 and 20.74 ± 0.68 respectively for the concentrations while 20.74 ± 0.68 showed significantly high inhibition against *S.*

aureus compared to other concentrations (Table 3). The mean zones of inhibition of acetone root extract against *P. aeruginosa* were 15.57 ± 0.24 and 16.83 ± 0.58 at concentration 100 and 150 mg/ml only as the 50 mg/ml concentration had no inhibition (Table 3). While the acetone leaf and root extract showed inhibition against *E. coli* and *P. aeruginosa*, zone of inhibition for *S. aureus* was only through the acetone leaf extract ranging from 12.67 ± 0.12 to 17.12 ± 0.34 at 100 and 150 mg/ml respectively. The acetone root extract had no inhibition against *S. aureus* for all concentrations tested (Table 3). The gentamycin used as a positive control at a concentration of 25 µg/ml showed significantly high inhibition against all tested microbes (Table 1-3).

Table 3. Mean zone of inhibition (in mm) of Acetone leaf and root extracts of *O. sanctum*.

Extract Type	Crude extracts Conc. (mg/ml)	Test organisms		
		<i>E. coli</i> ATCC 25922	<i>S. aureus</i> ATCC 25923	<i>P. aeruginosa</i> ATCC 27853
Leaf	50	0.0 ± 0.00^a	0.0 ± 0.00^a	3.2 ± 0.56^a
	100	14.30 ± 0.36^b	12.67 ± 0.12^b	19.36 ± 0.29^b
	150	17.53 ± 0.29^b	17.12 ± 0.34^b	20.74 ± 0.68^{ab}
	Control	23.17 ± 0.58^{ab}	25.58 ± 0.86^{ab}	25.38 ± 0.44^{ab}
Root	50	0.0 ± 0.00^a	0.0 ± 0.00^a	0.0 ± 0.00^a
	100	0.0 ± 0.00^a	0.0 ± 0.00^a	15.57 ± 0.24^b
	150	14.74 ± 0.67^b	0.0 ± 0.00^a	16.83 ± 0.58^b
	Control	24.76 ± 0.47^{ab}	25.43 ± 0.68^{ab}	25.54 ± 0.76^{ab}

Means with the same letter superscript on the same column are not significantly different at $p < 0.05$.

The average zone inhibition for the different extracts indicating antibacterial potential of *O. sanctum* is shown in Figure 1. The acetone extract was the most effective against all the tested bacterial strains with highest average zone of inhibition of 15.04 mm for *P. aeruginosa* followed by *E. coli* with average zone of inhibition 9.16. The aqueous extract had the least average zone of inhibition in against all tested

microbes. However, in comparison with the control, the inhibition of the microbes was significantly higher in the control than in plant extract namely aqueous, ethanol and acetone (Figure 1).

The average zone inhibition based on concentrations is shown in Figure 2. For the three different extract concentrations used (50, 100 and 150 mg/ml), the

concentration 150 mg/ml had the highest average zone of inhibition followed by 100 mg/ml. At concentration 100 mg/ml and 150 mg/ml the antimicrobial activity against *P. aeruginosa* high compared to other tested microbes.

Furthermore, the concentration 50 mg/ml showed antimicrobial activity only for *P. aeruginosa* as the concentration showed no inhibition against *E. coli* and *S. aureus* (Figure 2).

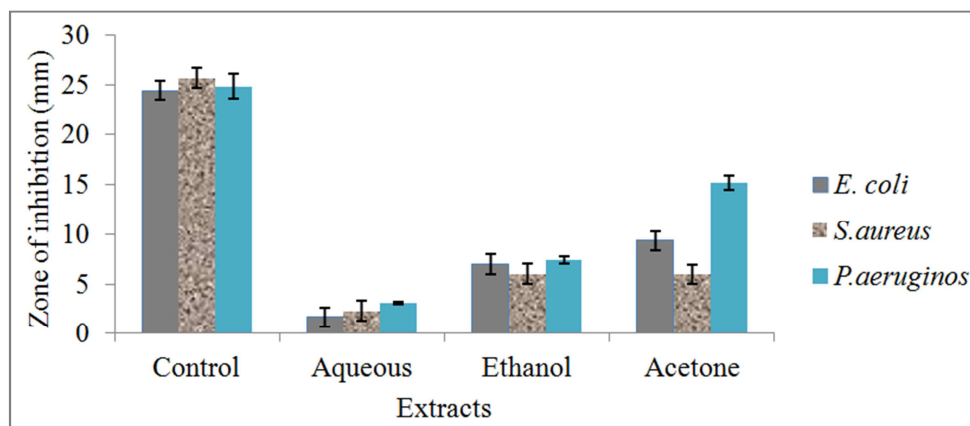


Fig. 1. Antimicrobial activities of *O. sanctum* for the average inhibition zone for the different extracts.

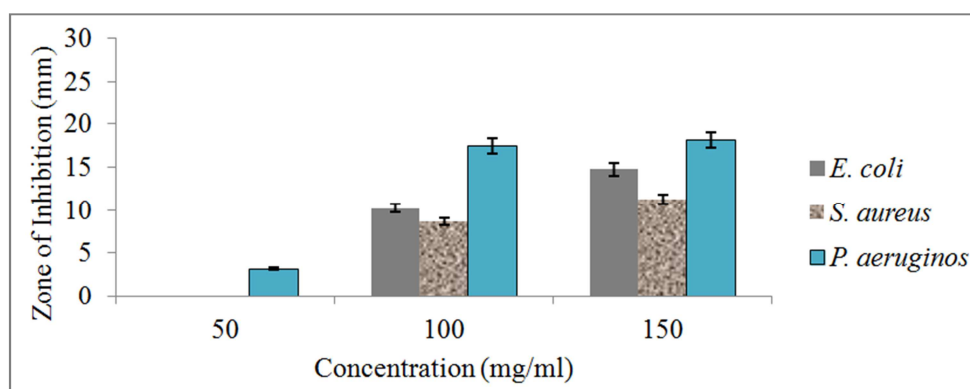


Fig. 2. Antimicrobial activities of *O. sanctum* for the average inhibition zone for the different concentrations.

4. Discussion

The results of this study show that the acetone and ethanol extracts of *Ocimum sanctum* possess antimicrobial activities against the test bacteria. All the three extracts tested showed varying degree of antibacterial activity against the tested bacteria species. Effective extraction of biological compounds from plant material mostly is dependent on the type of solvent, in this study the plant extracts by acetone provided more reliable antibacterial activity followed by to ethanol extracts and the aqueous extract was ineffective to all tested microorganisms. This study is in agreement with other studies particularly for effectiveness of ethanol extracts and ineffective of aqueous extracts which revealed relatively lower antimicrobial activity compared to other extracts [20, 21, 22]. The effectiveness of acetone and ethanol extracts of *O. sanctum* against *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* respectively is an indication that the extracts from the selected parts of this plant have potential for use as an antimicrobial agent. This finding supports the ethnomedicinal use of *O. sanctum* in the management various microbes' diseases such as stomach aches and gastroenteritis [12, 19, 20].

Ocimum sanctum contain a wide range of essential oils rich in phenolic compounds and a comprehensive array of phytochemicals including flavonoids and anthocyanins, antioxidants and free radicals scavengers which could be the reason for many medicinal potentials of the plant [14, 19, 23, 24]. Several studies have revealed that the presence of bioactive compounds in plant materials of *O. sanctum* were responsible for antibacterial activity, particularly the presence of high content of linoleic acid which is reported to contribute towards its antibacterial activities against *staphylococcus bacillus* and *Pseudomonas aeruginosa* [25, 26].

In this study leaf extract had good result against all tested selected bacteria and this could be due to the presence of rich phytochemicals in leaves compared to roots. Although all parts of the plant might have the active compounds but in leaves the presence of phytochemicals such as eugenol, rosmarinic acid, apigenin, and carnosic acid in *O. sanctum* has previously been reported to be significant high which account for many medicinal activities of the plant [13, 26]. Findings of this study is in agreement with other studies reported antimicrobial activity of crude extracts from leaves of *O. sanctum* revealed to be the most active extract to inhibit a good number of bacteria tested [27, 28]. Other

antimicrobial effects of leaf extracts of this plant are also reported in its use on cotton fabrics [22].

In relation to medicinal potential *O. sanctum* and the frequently utilized plant parts, several studies have reported that regularly utilized parts were leaves [17, 29]. Consideration to use only specific parts particularly the leaves seems to be a sustainable means for harvesting and conservation of the species bearing in mind its medicinal values in traditional system. For conservation of medicinal plants several studies have recommended the leaf harvest and strongly discouraged unsustainable means such as root and bark harvesting [9, 30]. Harvesting medicinal plants through root excavation and bark striping can be very devastating and pose a serious threat to the plant survival. The high utilization of roots has also been reported as putting many plant species at a risk of extinction because of the damages inflicted on them in the course of uprooting the plants [31].

Several drug resistances have developed due to haphazard use of commercial antimicrobial drugs frequently used in the treatment of communicable diseases [32, 33]. Such an occurrence of drug resistance through the uses of commercial antibiotics has prompted numerous efforts in search for antimicrobial agents from plants with less vulnerable to many side effects but efficient for therapeutic potential to heal many infectious diseases [34, 35]. The antimicrobial activity of *O. sanctum* revealed in this study further supports its uses as alternative medication of ailments mostly caused by bacteria such as stomach ache and diarrhea, thus could safely be used as topical antibacterial agents where commercial antimicrobial drugs may not be utilized or may not be easily accessible.

5. Conclusion

The study has revealed that *O. sanctum* has good antibacterial activity, hence supporting the use of this plant as traditional medicine. Based on plant parts tested, the leaf extracts of *O. sanctum* showed higher potential for antibacterial effects than the root extract, thus recommending the sustainable use of leaf for medicinal harvesting compared to the use of roots. The plant can further be explored for the proper phytochemical compounds present in the leaves since this part showed good results for the antibacterial activity. Furthermore, the present study provides an important basis for the use of the acetone extracts which has not only revealed more potential for antimicrobial activity compared to other extracts but also the crude extract could be valuable for the development of new antibacterial drugs.

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