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Phytochemical estimation and Antimicrobial activity of Aqueous and Methanolic extract of *Ocimum Sanctum L.*

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ABSTRACT

Plants have served human kind as sources of medicinal agents since its earliest beginnings. In fact natural product once served as the source of all drugs. OcimumSanctumL. is an aromatic plant in the family Lamiaceae. The main chemical constituents of Tulsi are: Oleanolic acid, Ursolic acid, Rosmarinic acid, Eugenol, Carvacrol, Linalool, and β-caryophyllene, have been used extensively for many years in food products, perfumery, and dental and oral products. Recent studies suggest that Tulsi may be a COX-2 inhibitor, like many modern painkillers, due to its high concentration of eugenol. The present study was to evaluate the qualitative estimation of phytochemicals and antimicrobial activity ofaqueousand methanol extracts of root and leaves of Ocimum sanctumagainst pathogenic bacteria i.e. Escherichia coli, Proteus mirabilis, Staphylococcus aures.Study has been shownthe presence of steroids, alkaloids and tannins. Significant antimicrobial activity of plant extract has been observed.

Key words:*Ocimum sanctum,* antimicrobial activity, *Escherichia coli, Proteus mirabilis, Staphylococcus aures.*

INTRODUCTION

Infectious diseases are the leading cause of deaths world-wide. So, antibiotic resistance has become a global concern but the clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens [1].The increasing prevalence of multidrug resistant strains of bacteria and the recent appearance of strains with reduced susceptibility to antibiotics raises the specter of untreatable bacterial infections and adds urgency to the search for new infection-fighting strategies [2]. Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents [3]. According to World Health Organization [4], medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency [5]. The medicinal plants are rich in secondary metabolites [which are potential sources of drugs] and essential oils of therapeutic importance .These products are known by their active substances, for example, the phenolic compounds which are part of the essential oils [6], as well as in tannin [7]. Despite the existence of potent antibiotic and antifungal agents, resistant or multi-resistant strains are continuously appearing, imposing the need for a permanent search and development of new drugs, which is safe, more dependable than costly drugs and which have no adverse side effects[2]. The important advantages claimed for therapeutic uses of

medicinal plants in various ailments are their safety besides being economical, effective and their easy availability [8; 9]. There are more than 35,000 plant species being used in various human cultures around the world for medicinal purpose. India is one of the richest countries in the world in regard to genetic resources of medicinal plants. Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections [Jantan, 1998 10].

Tulsi has also been recognized by the rishis for thousands of years as a prime herb in Ayurvedic treatment. In Ayurveda Tulsi[*Ocimum sanctum L.*]has been well documented for its therapeuticpotentials and described as DashemaniShwasaharni[antiasthmatic] and antikaphicdrugs [Kaphaghna][11].In last few decades several studies have been carried out by Indian scientists and researchers to suggest the role of essential oils &eugenol in therapeutic potentials of *Ocimumsanctum L.* [12; 13]. Eugenol is a phenolic compound and major constituent of essential oils extracted from different parts of Tulsiplant [14; 15]. The main chemical ingredients in this plant are eugenol, carvacrol, methyl eugenol and caryophyllene.. One of the qualities that make the Tulsi plant such a potent medicinal herb is its ability to reduce stress. Tulsi is abundant in essential oils and antioxidants, which are tremendously effective in reducing the effects of stress on the body. Therefore the aim of present study was to evaluate the antimicrobial activity of extracts from the different parts *Ocimum sanctum* plant against three pathogenic bacteria *Escherichia coli, Proteus mirabilis*.

MATERIALS AND METHODS

Collection and Identification of Plant Material

Fresh plant part of both *Ocimumsanctum*was collected randomly from the semi-arid region of Jaipur Rajasthan, India and taxonomic identification of these plants from Department of Botany University of Rajasthan [RUBL 20848]. Fresh plant materials were washed under running tap water, air dried and then homogenized to fine powder and stored in airtight bottles.

Extraction of Plant Material

Aqueous extraction

20 gm., of air-dried powder of leaves and root was added to distilled water and boiled on slow heat for 24 h. It was then filtered through filter paper. The supernatant was collected. This procedure was repeated twice. The supernatant concentrated to make the final volume one-fourth of the original volume with the help of water bath [16].

Methanol extraction

20 gm., of air-dried powder was taken in 100 ml of methanol in a conical flask, plugged with cotton wool and then kept on a rotary shaker at 150 rpm for 24 h. After 24 hours the supernatant was collected and the solvent was evaporated to make the final volume one fourth of the original volume the help of water bath and stored at 4^0C in airtight bottles [17].

Phytochemical studies

The methods described by Harborne were used to test for the presence of the active ingredients in the test sample[18].

Test for steroids

A 10 ml of plant extract [methanol-leaf and root, aqueous-leaf and root extract] was evaporated to a dry mass and the mass is dissolved in 0.5 ml of chloroform. Acetic anhydride [0.5 ml] and 2 ml of concentrated sulphuric acid were added to above [19].

Test for alkaloids

The plant extract [0.5 g] was stirred with 5 ml of 1% HCl on a steam bath. The solution obtained was filtered and 1 ml of the filtrate was treated with two drops of Mayer's reagent. The two solutions were mixed and made up to 100 ml with distilled water [19; 20].

Test for tannins

About 1 g of plant extract powder was weighed into a beaker and 10 ml of distilled water added. The mixture was boiled for five minutes. Two drops of 5% $FeCl_3$ were then added[21].

Test for flavonoids
A few drops of 1% NH_3 solution is added to the plant extract [0.5 g] in a tube for observation of Yellow coloration[19].

Test for reducing sugar
To 0.5 ml of extract solution, 1 ml of water and 5 - 8 drops of Fehling's solution were added at hot and observed for brick red precipitate [22].

Antibiotics
Two commercial antibiotics [Erythromycin and Ciprofloxacin] were used to evaluate and control the pattern of antibiotic sensitivity of the different target strains. Erythromycin and Ciprofloxacin Stock solutions were freshly prepared at 10 mg/ml in sterile distilled water.

Bacterial Strains
Microorganisms were provided by the institute S.P. Biotech. Three microorganisms were investigated namely *Proteus mirabilis, Staphylococcus aureus*, *Escherichia coli*. All the Microorganisms were maintained at 4^0C on nutrient agar slants.

Media Preparation
5g peptone dissolve in 850 ml of distilled water, 3g of beef extract was added in the solution from step 1, Then 15g of agar was dissolved in the solution from step 2, Adjust *p*H to 7.0. Final volume made up to 1000 ml with distilled water and sterilized the medium in autoclave at 121^0C for 20 minutes. Beef Extract [3.0g] + Peptone [5.0g] + Agar [15g] + NaCl[5g].

Bacterial culture and susceptibility test
The Antimicrobial activity of each plant extract was determined [examined] on *E.Coil*, *Proteusmirabilius* & *Staphylococcus aureus*using a modified Kirby-Bauer disc diffuse ion method. The appropriate molten agar media [Mueller Hinton or Luria Bertani] was inoculated with of the inoculum [1 x 10^8cfu/ ml] and poured into the Petri plate [Hi-media]. For agar disc diffuse ion method, paper discs of 5mm diameter were prepared by keeping dipped for overnight in three different concentrations i.e. 50 mg/l, 100 mg/l, 200 mg/l of the test compound. Sterile paper discs of 5mm diameter [containing 500 ppm drug] along with one standard antibiotic containing disc were placed in each plate [23], after spreading of microbial strain. The plates were incubated overnight at 37^0C. Microbial growth was determined by measuring the diameter of zone of inhibition with the help of inhibition zone scale [Hi-media]. The experiment was done three times and the mean values are presented by figures

RESULTS

The qualitative phytochemical analysis was performed for the detection of alkaloids, steroids, flavonoids, tannins and reducing sugars. In vitro antimicrobial activity was examined for aqueous and methanol extracts [Root and Leaf] of *Ocimum sanctum* plant. The antimicrobial assay was performed by agar disc diffusion method for solvent extract.

Phytochemical studies
Qualitative phytochemical investigation discovered presence of steroidal compounds [Appearance of blue or green color or a mixture of the two shades]; alkaloids and tannins[The turbidity or yellow precipitation shows the presence of alkaloids and greenish precipitate indicated the presence of tannins] and absence of flavonoids[Not observed yellow coloration] in all mentioned extracts of plant. Fehling test [formation of yellow or brownish-red precipitate] showed positive result for aqueous extract [Root and Leaf] only, [Table S1].

Antibacterial activity
The antimicrobial activity of the extracts was quantitatively assessed by the presence of zone diameter of inhibition at three different concentrations of 50mg/l, 100 mg/l and 200 mg/l methanol and aqueous extract. Erythromycin and Ciprofloxacin antibiotic were used as control for bacterial strains *Escherichia coli [E. coli]*, *Proteus mirabilis [P. mirabilis] and Staphylococcus aureus[S. aureus]*. Both antibiotics showed no effect on the growth of E.*coli* and *P.mirabilis* whereas 17 and 19 mm zone diameter of inhibition [ZDI] was seen in *S.aureus* for Erythromycin and Ciprofloxacin respectively, [Figure 1].

Table 1:Presence of phytochemicals in different extracts of *Ocimum sanctum*

Phytochemicals	Methanol leaf extract	Methanol root extract	Aqueous leaf extract	Aqueous root extract
Steroids	+	+	+	+
Alkaloids	+	+	+	+
Tannins	+	+	+	+
Flavonoids	-	-	-	-
Reducing sugars	-	-	+	+

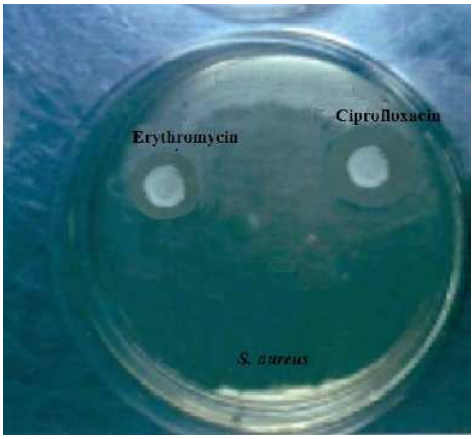

Figure 1: Antimicrobial activity of antibiotic against the *Staphylococcus aureus*

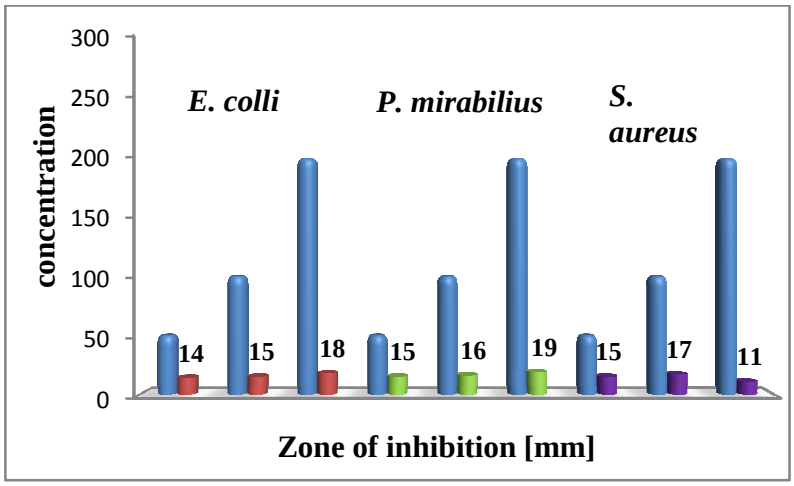

Figure 2: Antibacterial activity of methanol extract of *Ocimum sanctum* Root

Antibacterial activity of methanol and aqueous extract of O. sanctum Root

Both methanol and aqueous extracts showed zone of inhibition against all of bacterial strains used. The methanol extract demonstrating the highest activity [18, 19 and 17 mm ZDI] at 200 mg/l concentration followed by [15, 16, 15 mm ZDI] at the 100 mg/l and [14, 15, 13 mm ZDI] at the 50 mg/l for E.*coli*, *P.mirabilis* and *S.aureus* respectively, [Figure S2&S3]. While aqueous extracts also showed highest activity [15, 16 and 15 mm ZDI] at 200 mg/l concentration, [13, 14 and12 mm zone diameter of inhibition] at the 100 mg/l and [12, 11 and11 mm ZDI] at the 50 mg/l for E.*coli*, *P.mirabilis* and *S.aureus*respectively, [Figure S4].

Antibacterial activity of methanol and aqueous extract of O. sanctum LeafEscherichia coli were found to be resistant against methanol and aqueous extract of *Ocimum sanctum Leaf* show no effect. *Proteus mirabilis* also show the resistance against all of three concentration of leaf aqueous extract while themethanolleaf extract showed the highly antimicrobial effect [15, 17 and 20 mm ZDI] at different concentration of 50, 100 and 200 mg/l respectively. Both methanol *and* aqueous extract *of Ocimum sanctum* Leaf demonstrated the largest zone of inhibition [20mm and 16

mm ZDI respectively] at 200 mg/l against *Staphylococcus aureus*and followed by [16mm and 14 mm ZDI] at 100 mg/l and 50 mg/l concentration respectively of methanol extract, [Figure S5] while [11mm and 14mm ZDI] at respective 100 mg/l and 50 mg/l concentration of aqueous extract were observed, [Figure S6&S7].

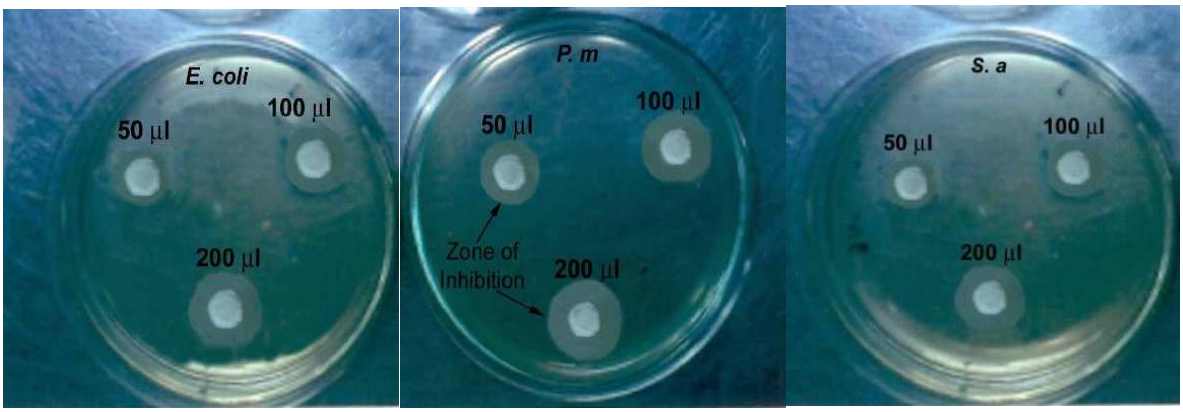

Figure 3: Antibacterial activity of methanol extract of *Ocimum sanctum* Root on *E. coli, P. mirabilius* and *S. aureus*

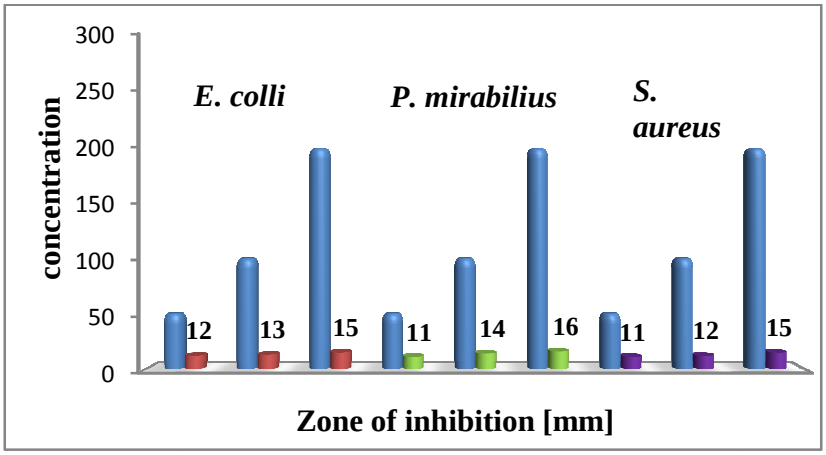

Figure 4: Antibacterial activity of aqueous extract of*Ocimum sanctum* Root

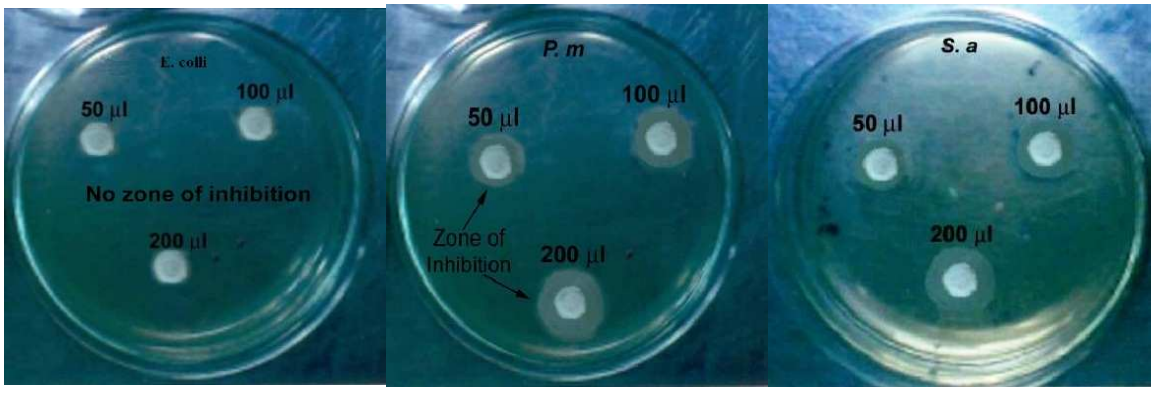

Figure 5: Antibacterial activity of methanol extract of *Ocimum sanctum* Leaf on *Pm and Sa*.

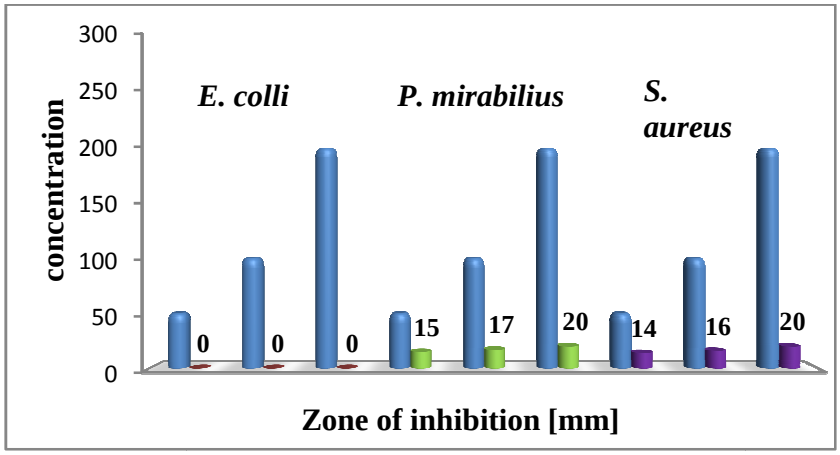

Figure 6: Antibacterial activity of methanol extract of *Ocimum sanctum* Leaf

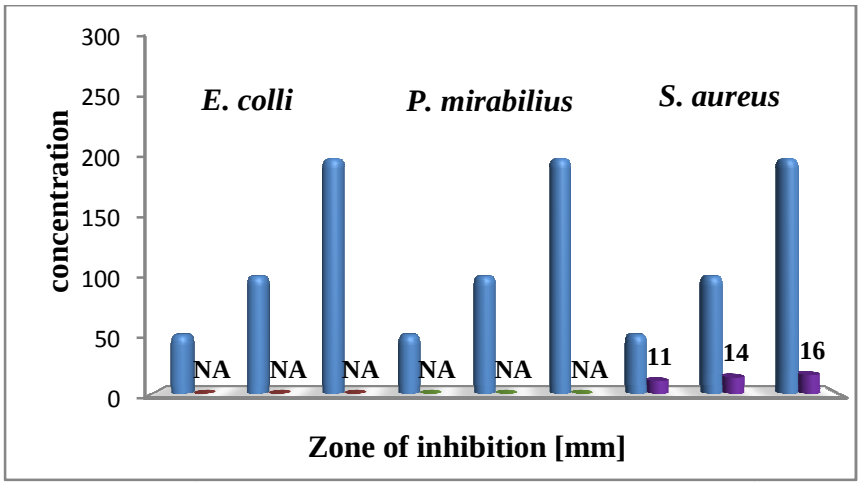

Figure 7: Antibacterial activity of aqueous extract of *Ocimum sanctum* Leaf
E.coli : *Escherichia coli,* ***P. m:****Proteus mirabilis,* ***S. a:*** *Staphylococcusaureus*
Concentration of extract, Zone diameter of inhibition [mm] E. coli, P. mirabilis, S. aureus.

DISCUSSION

Phytochemical constituents such as Steroids, alkaloids, flavonoids, tannins, phenol, and several other aromatic compounds are secondary metabolites of plants that serve a defense mechanism against prediction by many microorganisms, insects and other herbivores [24]. These secondary metabolites exert antimicrobial activity through different mechanisms.[25]worked on steroidal extracts from some medicinal plants which exhibited antibacterial activities on some bacterial isolates. [26]also confirmed the antiviral property of steroids. Another secondary metabolite Alkaloids which are one of the largest groups of phytochemicals in plants were observed in the all of extract of *Ocimum sanctum*. One of the most common biological properties of alkaloids is their toxicity against cells of foreign organisms like bacteria, [27]. Tannins have been found to form irreversible complexes with prolinerich protein [28] resulting in the inhibition of cell protein synthesis. Herbs that have tannins as their main components are astringent in nature and are used for treating intestinal disorders such as diarrhea and dysentery [29].

The differences in the spectrum of activities of the extracts show the concentrations at which the extracts have the best antimicrobial activities [30]. Largest zones of inhibition were observed at 200µ/l concentration of all extracts. Data indicated that the pattern of inhibition depends largely upon the extraction solvent and plant part. Organic extracts provided more potent antibacterial activity as compared to aqueous extracts. The polarity of Secondary

metabolites and antibacterial compounds make them more readily extracted by organic solvents because Secondary metabolites are more soluble in organic solvents then water, and using organic solvents does not negatively affect their bioactivity against bacterial species suggesting that organic solvents are clearly better solvents of antimicrobial agents.Among all the extracts, the methanol extract was found to be most active against all of the bacterial species tested when compared to aqueous extract. Furthermore, extracts prepared from root were shown to have better efficacy than leaf parts demonstrate that the secondary metabolites and antimicrobial agents are present in root in better amount than leaves.

The screening of plant extracts and plant products for antimicrobial activity has shown that higher plants represent a potential source of novel antibiotic protypes. These plants may prove to be a rich source of compounds with possible antimicrobial activities, but more pharmacological
Investigations are necessary [31; 32].

Concluding Remark:
The results confirm the validity of the use of *Ocimum sanctu*m plant as medicine in ancient medicinal traditions and suggest that some of the plant extracts possess compounds with antimicrobial properties that can be used as antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens. It is quit safer to use as an herbal medicine as compare to chemically synthesized drug.

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