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SCREENING OF ANTIMICROBIAL ACTIVITY OF OILS FROM DIFFERENT SPECIES OF OSCIMUM AGAINST PATHOGENIC BACTERIAL STRAINS

PRAJAPATHI DN, SAROJA K* AND NAROLKAR SN

Ashok & Rita Patel Institute of Integrated Study & Research In Biotechnology and Allied Sciences, New Vallabh Vidyanagar, Gujarat

***Corresponding Author: E Mail: sarojavalarivan@ymail.com**

ABSTRACT

Drug resistance in microbes is a very serious problem we are facing today. Hence plant origin herbal medicines are considered as safe alternative of synthetic drugs. Plant materials having non-toxic substances are considered to be a good alternative for drugs. Many plant oils and the chemicals present in them have good fighting potential against many drug resistant pathogens. Many plant oils and extract shows high degree of antibacterial, antifungal, antiviral and antioxidant properties. The present study was to evaluate antimicrobial activity of Oscimum oil against pathogenic bacterial cultures like *Pseudomonas aeruginosa* (MTCC- 7436), *Proteus vulgaris* (MTCC- 426), *Serratia marcescens* (MTCC- 8780), *Bacillus megaterium* (MTCC-3353), *Staphylococcus aureus* (MTCC-96), *Escherichia coli* (MTCC-302) and *Streptococcus pyrogenes* (MTCC- 1923). The genus *Oscimum*, a herbaceous plant belongs to the family Labiatae (Lamiaceae) is represented by many species used for centuries for the treatment of various ailments like fever, common cold, cough, sore throat, kidney stone, heart disorders, stress, mouth infections, skin diseases etc.

Keywords: *Oscimum sp*., Pathogenic Bacteria, Aerial Parts, Herbal Medicines

INTRODUCTION

Disease caused by bacteria is widespread. The treatment of these infections is mainly based on the use of antibiotics. In recent years, a number of antibiotics have lost their effectiveness due to resistant strains mostly, through the expression of resistant genes **[1, 2].** In addition to this problem, antibiotics are sometimes associated with adverse effects

including hypersensitivity, immune suppression and allergic reactions **[3, 4].** Therefore, there is a need to develop antibacterial drugs for the treatments of infectious diseases from various sources such as medicinal plants. There are many methods of medicines like Ayurveda, Homeopathy and Unani which utilizes plant materials for drug production. Plant materials having non-toxic substances are considered to be a good alternative for drugs. Many plant oils **[5, 6]** and the chemicals present in them **[7]** have good fighting potential against many drug resistant pathogens **[3, 8].** Many plant oils and extract shows high degree of antibacterial, antifungal, antiviral and antioxidant properties **[9, 10].** In the present study the crude extracts and oils extracted from tulsi are used for screening antimicrobial activity against many bacteria like *Pseudomonas aeruginosa* (MTCC- 7436), *Proteus vulgaris* (MTCC-426), *Serratia marcescens* (MTCC- 8780), *Bacillus megaterium* (MTCC- 3353), *Staphylococcus aureus* (MTCC-96), *Escherichia coli* (MTCC-302) and *Streptococcus pyrogens* (MTCC- 1923). The present study mainly aims to check the antimicrobial activity of different plant oils (*Ocimum sanctum var1, Ocimum basilicum, Ocimum sanctum var-2, Ocimum americanum, Ocimum gratissimum var-1, Ocimum gratissimum var-2).*The oils extracted from various species of *Ocimum* may be used against microbes if found antimicrobial. *Ocimum* species is very cosmopolitan in occurrence and it is very easy to collect. As Tulsi is well-known to common man and its medicinal properties are known it can easily be used as an antimicrobial agent. The genus *Oscimum* , a herbaceous plant belongs to the family

Labiatae (Lamiaceae) is represented by many species used for treatment of various ailments for centuries.

MATERIALS AND METHODS

Present study was conducted during December, 2010 to March, 2011 at Ashok & Rita Patel Institute of Integrated Study & Research in Biotechnology and Allied Sciences (ARIBAS), New Vidyanagar, Anand, Gujarat. Six different *Oscimum* species **(Table 1)** were collected from the pollution free botanical garden of Directorate of Medicinal and Aromatic Plant Research (DMAPR), Boriavi, Anand, Gujarat.

Extraction of Essential Oil

The fresh plant leaves were subjected to hydro distillation for four hours in a Clevenger's type apparatus **[7].** The distillate was extracted with diethyl ether. The ethereal layers were dried over anhydrous sodium sulphate and ether was removed by gently

heating in water bath. The oils obtained were stored at 8^0C for further antimicrobial studies. Dilution was made using fresh solvents while studying antimicrobial activities.

Bacterial Cultures

Antimicrobial activities were checked with the help of MICC cultures obtained from ARIBAS. Bacterial cultures such as *Pseudomonas aeruginosa* (MTCC- 7436), *Proteus vulgaris* (MTCC- 426), *Serratia marcescens* (MTCC- 8780), *Bacillus megaterium* (MTCC- 3353), *Staphylococcus aureus* (MTCC-96), *Escherichia coli* (MTCC-302) and *Streptococcus pyogenes* (MTCC-1923) were used in the present study.

Screening of Antimicrobial Activity

Antimicrobial activity of essential oil obtained from Tulsi on various bacterial growths was accessed using different increasing concentration. Every sample was assayed for antimicrobial activity in triplicate. All the experimental conditions were standardized to find out MIC *in vitro*.

Agar well diffusion method was used for screening antimicrobial activity. Various concentrations of oil were prepared by diluting the samples using solvent. Agar plate was prepared and well was made using borer of 0.5cm.size. Bacterial inoculums was spread evenly on the surface of each agar plate with sterile rubber pad spreader and Tulsi oil was poured into the well using sterile dropper up to 2/3 of the well. Sterile distilled water was used as a control. All the plates were incubated for 24 hours at 37^oC and zone of inhibition surrounding the well was measured.

Table 1: Plants and Plant Parts Used in Present Study

Plant Name	Common Name	Family	Part Used
Ocimum sanctun.var-1	**Tulsi**	**Lamiaceae**	**Aerial**
Ocimum basilicum	**Tulsi**	**Lamiaceae**	**Aerial**
Ocimum sanctum .var-2	**Tulsi**	**Lamiaceae**	**Aerial**
Ocimum americanum	**Tulsi**	**Lamiaceae**	**Aerial**
Ocimum gratissimum.var-1	**Tulsi**	**Lamiaceae**	**Aerial**
Ocimum gratissimum.var-2	**Tulsi**	**Lamiaceae**	**Aerial**

RESULTS AND DISCUSSION

Colony forming Units (CFU) of Cultures Used

In the present investigation seven pathogenic cultures were used to check the antimicrobial activity. Five pathogens are Gram- negative and two are Gram- positive. Minimum inhibitory concentration (MIC) values of six different oils against seven bacteria are given below **(Table-2)**. The colony forming units was calculated by checking the optical density at the 600nm and different dilutions were placed on nutrient agar plate and CFU/ml was calculated after 24 hours. Most of the cultures were adjusted to final colony number of 10^5 C.

P. vulgaris has high antimicrobial activity in sample-1 *(Ocimum americanum*) as compared to the sample-2 (*Ocimum basilicum*). Sample-3 (*Ocimum sanctum var-2)* and sample-4 (*Ocimum americanum*) has modrate antimicrobial activity. From this observation it is evident that *P. vulgaris* is more sensitive to sample-1 (*Ocimum sanctum var-1)* **(Table 3 and Figure 1-6)**.

S. pyogens has high antimicrobial activity in sample-4 (*Ocimum americanum*) as compared to the sample-1 *(Ocimum sanctum var-1),* sample-2 (Ocimum *basilicum*) and sample-3 (*Ocimum sanctum var-2). S. pyogens* is sensitive to sample-4 (*Ocimum americanum)* **(Figure.4)**.

Ocimum americanum (Sample-4) shows high antimicrobial activity in *B. megaterium* as compared to *Ocimum basilicum* (Sample-2) and *Ocimum sanctum var*-2 (Sample-3). *Ocimum sanctum var*-1 (Sample-1) has moderate antimicrobial activity. *B.megaterium* is sensitive to *Ocimum americanum* (Sample-4) **(Figure 1)**.

Ocimum sanctum var-2 (Sample-3) has high antimicrobial activity against *S.aureus* as compared to *Ocimum sanctum var*-1 (Sample 1) *Ocimum basilicum* (Sample 2) and *Ocimum americanum* (Sample 4) **(Table-3)**.

Sample-1 (*Ocimum sanctum var-1)* has high antimicrobial activity *in E. coli* as compared to sample-2 (*Ocimum basilicum)* and sample-3 (*Ocimum sanctum var-2).* Sample-4 (*Ocimum americanum)* has moderate antimicrobial activity. *E. coli* is more sensitive to sample-1(*Ocimum sanctum var-1). P. aeruginosa* has no antimicrobial activity in this plant extract **(Figure 6 and Table 3)**.

Antimicrobial activity of plant oil which include the plant extract of sample-5 (*Ocimum gratissimum var-1)* and sample-6 (*Ocimum gratissimum var-2).* Sample-5 (*Ocimum gratissimum var-1)* has a more antimicrobial activity in comparison to

sample -6 *(Ocimum gratissimum var-2)* **(Figure 7)**.

Antimicrobial activity of plant oil which include the plant extract of sample-5 and sample-6. Here the sample-6 has more antimicrobial activity as compared to sample -5(*Ocimum gratissimum var-1)* **(Figure 8)**.

Antimicrobial activity of plant oil which include the plant extract of sample-5 (*Ocimum gratissimum var-1*) and sample-6 (*Ocimum gratissimum var-2*). Sample-5 has a modrate antimicrobial activity as compared to sample -6 *(Ocimum gratissimum var-2)* which has no antibacterial activity **(Table-3)**.

Antimicrobial activity of plant oil includes the plant extract of sample-5 (*Ocimum gratissimum var-1*) and sample-6 (*Ocimum gratissimum var-2*). Here the sample-5and sample-6 has no antimicrobial activity. Antimicrobial activity of plant oil which includes plant extract of sample-5 and sample-6. Sample-6 (*Ocimum gratissimum var-2*) has antimicrobial activity more in comparison to the sample-5 (*Ocimum gratissimum var-1*).

Different methods were adapted to check the anti-microbial activity of six different *Ocimum* samples. Agar well diffusion method was used and for the homogenous distribution of oil, the plates were prepared using tween 80.

Sample-1 (*Ocimum sanctum var-1*) has higher antimicrobial activity against the bacteria *S. pyrogenes*, shows these bacteria are sensitive to sample 1 and it can be used to treat the *S.pyrogen*s infection including skin infection.

Sample-2 (*Ocimum basilicum*) has higher antimicrobial activity against the bacteria *E.coli*, and this bacteria are sensitive to this plant oil sample-2 (*Ocimum basilicum*) can be used to treat against the *E.coli* infection including diarrhea and vomiting.

Sample-3 (*Ocimum sanctum var-2)* has higher antimicrobial activity against the bacteria *S. aureus* and this bacterium is sensitive to tulsi extract. Sample-3 (*Ocimum sanctum var-2)* can be used to treat against the S. aureus infection including diarrhea, vomiting and food poisoning.

Sample-4 (*Ocimum americanum*) has higher antimicrobial activity against the bacteria *S. pyrogens*, and these bacteria are sensitive to this plant extract and sample -4 can be used to treat against the *S. payogens* infection including skin infection.

Sample-5 (*Ocimum gratissimum var-1*) has higher antimicrobial activity against the bacteria *B. megaterium* and these bacteria are sensitive to this plant extract and sample -

5 can be used to treat against the *B. megaterium* infection.

Sample-6 (*Ocimum gratissimum var-2*) has higher antimicrobial activity against the bacteria *S. pyrogens*, and these bacteria are sensitive to the plant extract and sample 6 can be used to treat against the *S.pyrogens* infection including skin infection.

Table 2: Minimum Inhibitory Concentration (MIC) Values of Six Different Oils Against Seven Bacteria

Name of the Organism	MTCC Number	CFU/ml
Pseudomonas aeruginosa	**MTCC-7436**	**3.86×10^4**
Proteus vulgaris	**MTCC-426**	**4.98×10^5**
Serratia marcescens	**MTCC-8780**	**3.06×10^5**
Bacillus megaterium	**MTCC-3353**	**4.48×10^4**
Staphylococcus aureus	**MTCC-96**	**2.10×10^5**
Esherichia coli	**MTCC-302**	**4.42×10^5**
Streptococcus pyogenes	**MTCC-1923**	**1.78×10^5**

Table 3: Antimicrobial Activity of Different Plant (Tulsi) Extract

Test Organisum	Plant extracts					
	Zone of inhibition in mm. diameter					
	Sample-1	**Sample-2**	**Sample-3**	**Sample-4**	**Sample-5**	**Sample-6**
B.megaterium	**10**	**8**	**8**	**12**	**8**	**7**
S. aureus	**12**	**7**	**16**	**10**	**6**	**8**
S. marcescens	**13**	**8**	**10**	**9**	**7**	**0**
P. vulgaris	**12**	**6**	**10**	**8**	**0**	**0**
E.coli	**10**	**9**	**12**	**13**	**0**	**0**
S. pyogenes	**14**	**8**	**9**	**16**	**6**	**9**
P. aeruginosa	**0**	**0**	**0**	**0**	**0**	**0**

Sample1-*Ocimum sanctum var-1,* Sample2-*Ocimum basilicum,* Sample3-*Ocimum sanctum var-2,* Sample 4- *Ocimum americanum,* Sample-5 *Ocimum gratissimum var-1,* Sample-6 *Ocimum gratissimum var-2*

Figure 1: ***B. megaterium***

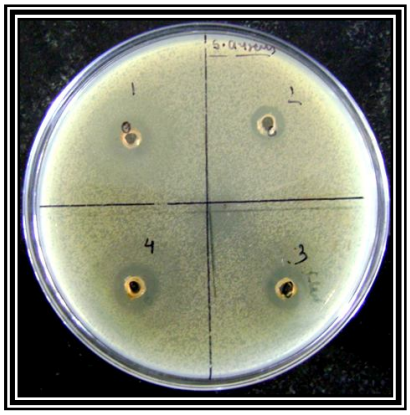

Figure 2: ***S. aureus***

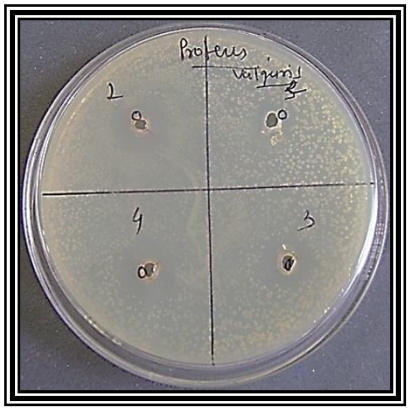

Figure 3: ***P. vulgaris***

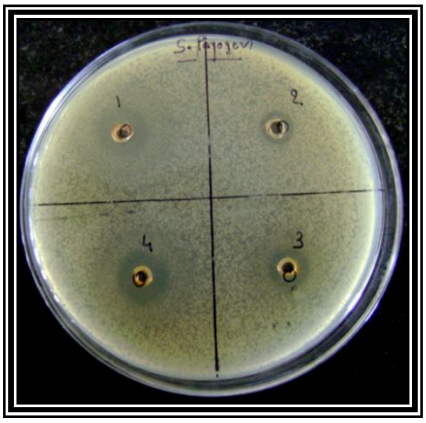

Figure 4: ***S. payogen***

Figure 5: ***E. coli***

Figure 6: ***P. aeruginosa***

Figures 1-6 Show the Plant Oil (Sample 1-4) Antimicrobial Activity Against the Selected Bacteria

Sample 1- ***Ocimum sanctum var-1,*** **Sample 2-** ***Ocimum basilicum,*** **Sample 3-** ***Ocimum sanctum var-2,***

Sample 4- ***Ocimum americanum***

Figure 7: *B. megaterium*

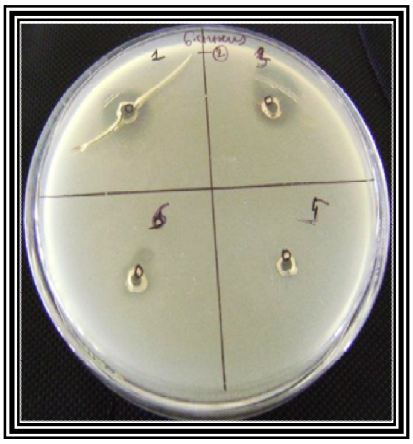

Figure 8: *S.aureus*

Figures 7-8 Show the Plant Oil (Sample 5-6) Antimicrobial Activity Against Few Selected Bacteria

CONCLUSION

Different methods were adapted to check the anti-microbial activity of six different *Ocimum* samples. Agar well diffusion method was used and for the homogenous distribution of oil, the plates were prepared using tween 80. All the samples used in the present study showed higher antimicrobial activity against the bacterial cultures like S. *pyrogens, E. coli, S. aureus and B. megaterium, so* these bacteria can be used to treat skin infections, diarrhea, food poisoning and vomiting. More importantly the plant samples can be included in the list of herbal medicines due to their higher antimicrobial activity and lesser side effects. Hence plants oils and their components can be recommended for therapeutic purposes and can be used as an alternative medicine.

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