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ANTIBACTERIAL ACTIVITY OF *OCIMUM* SPECIES AND THEIR PHYTOCHEMICAL AND ANTIOXIDANT POTENTIAL

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Abstract- Since ages, many plants have been used for preservative and medicinal purposes due to the presence of secondary metabolites referred as Phytochemicals. Phytochemicals are biologically active plant compounds having disease hampering capabilities and preservative action. The genus *Ocimum* contains more than 200 species of herbs and shrubs which have been shown to have medicinal properties and also are used as a culinary herb, preservative and flavoring agents. In this study methanol extracts from the leaves of *Ocimum species* (*O. sanctum purple, O. sanctum green, O. gratissimum, O. basilicum and Camphor basil*) were investigated for their phytochemical constituent and antioxidant activity. Also, six extracts (Ethanol, Methanol, Propanol, Chloroform, Petroleum Ether and Isoamyl alcohol) were assayed to test their ability to inhibit the clinically isolated Enterobacteria (*S. pneumoniae, Proteus sp., E. faecalis, S. typhi, S. aureus and B. subtilis*). The *Ocimum sp.* were screened for the presence of phenolic content, glycosides, anthraquinones, terpenoids, proteins, flavinoids, tannins, lignin and Saponins. The test results were positive for all Phytochemicals in methanolic extracts of *Ocimum sp.* The antioxidant activity was measured by reducing power assay, 1-1-diphenyl-2-picrylhydrazyl (DPPH) assay and Thiobarbituric acid (TBA) which showed positive. In the antimicrobial assay studies *O. gratissimum and O. basilicum* showed maximum antibacterial activity compared to other species with maximum activity in isoamyl alcohol extract. The Present investigation suggests that the phytochemical content and its antioxidant properties can be further studied for its application in health and in food industries. Furthermore, these species can be used as a source of novel drugs for the treatment of infectious diseases caused by pathogenic microorganisms.

Keywords- *Ocimum*, Phytochemicals, Antioxidant Activity, Antibacterial Activity

Abbreviations- Osp- *O. sanctum purple*, Osg- *O. sanctum green*, Og- *O. gratissimum*, Ob- *O. basilicum*, Cb- *Camphor basil*

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Introduction

Plants are the richest resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [1]. Traditional medical system has great value and also many medicinal plants have been identified from indigenous pharmacopoeias, because of which plants are still making imperative contribution to healthcare regardless of modern medicines which has many advances [2]. The medicinal values of plants lie in their Phytochemicals, which produce definite physiological actions on the human body. Phytochemicals are compounds present in plants that are used as food and medicine to protect against illness and to maintain human health [3]. Phytochemicals have antioxidant or hormone-like effect which helps in fighting against many diseases including cancer, heart disease, diabetes, high blood pressure and preventing the formation of carcinogens on their target tissues [4]. Medicinal plants have been found to be helpful in curing many diseases and have always promoted the search for different extracts from plants which could act as a potential source of new antimicrobial agent [5,6]. Spice plants have been used traditionally as coloring agents, flavoring agents, preservatives, food additives and as well as antiparasitic, antihelmintic, analgesic, expectorant, sedative, antiseptic and anti-diabetic substances in many parts of the world [7]. In addition, they possess biological activities such as that of antioxidants [8] and hypocholesterolemics [9]. Thousands of medicinal species found in different parts of the India are being used from the ancient time and are of great medicinal and economical value [10].

In recent years, it has been seen that because of indiscriminate use of commercial antimicrobial drugs, rate of acquiring multiple resistances in human pathogenic microorganisms has increased [11]. This fact has led the scientists to search for novel antimicrobial substances and screen medicinal plants regularly for new antibacterial agents. The genus *Ocimum* comes under Lamiaceae

family and is found in many part of the world like tropical and subtropical regions of Asia, Africa and Central and South America. It is a source of essential oils and aroma compounds, a culinary herb and an attractive, fragrant ornamental plant. Tulasi is considered as a sacred plant and its various medicinal properties have been mentioned in ancient medicinal text, *Ayurveda*. Different parts of this plant are used for treatment of various ailments. *Ocimum* is believed to decrease lipid peroxidation and increase the activity of superoxide dismutase [12]. The constituents of *Ocimum* species have antibacterial, antifungal, antioxidant and radio protective activity [13,14]. Studies show that many *Ocimum* species are useful for the treatment of disorders in central nervous system (CNS) and also as antidepressant [2,15].

Oxygen derived free radicals and reactive oxygen species (ROS) plays role in many diseases and in food deterioration and spoilage [4], by inhibiting the initiation or propagation of oxidative chain reactions, Antioxidants can delay or inhibit the oxidation of lipids or other molecules [16]. Synthetic antioxidants used in foods are very unstable and thought to be carcinogenic, because of which interest in finding out new natural and non toxic antioxidants is increasing [17]. Though much antibacterial and phytochemical studies has been conducted on *Ocimum species*, keeping in view the pharmacology, the present work has been designed to screen antimicrobial agents by using various solvent extracts and their action on various pathogens and also to evaluate the antioxidant potential of the extracts to explore the basis for its traditional use.

Materials and Methods

Plant Material

The plant material of *Ocimum species* were obtained from the local area in and around Ernakulam District, Kerala and University of agriculture, Bangalore India. The plants were indentified based on their description given in the literature. The plant leaves were air dried under shade and powdered. 1gm powder sample was dissolved in 100ml distilled water and used for qualitative phytochemical analysis.

Preliminary Phytochemical Analysis

Phytochemical screening of plant extracts was done by the standard procedure [18]. All the prepared plant extracts were subjected to preliminary phytochemical screening for the presence of phenolic content, glycosides, anthraquinones, terpenoids, proteins, flavinoids, tannins, lignin and Saponins.

Quantitative Analysis of Phytochemicals

Alkaloid Determination

0.15gm of the sample was taken in test tube and 5ml of 20% acetic acid in ethanol was added and covered to stand for 4 hrs. This was centrifuged and the supernatant was concentrated using a water-bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected by filtration and weighed [18,19].

Tannin Determination

0.1gm of the sample was weighed to which 10ml of distilled water was added and shaken for 1 hr. in a mechanical shaker. This was centrifuged and 1ml of the filtrate was pipette out into a tube and mixed with 200µl of 0.1 M FeCl3 in 0.1 N HCl and 0.008 M potassium ferrocyanide. The absorbance was measured in a colorimeter at 670nm wavelength, within 10 min. A blank sample was prepared with the reagent and distilled water and the absorbance taken at same wavelength [20].

Determination of Total Phenols

Estimation of phenols was done as described [21], 50µl of the aqueous extract of the sample was mixed with 1.5mL of Folin-Ciocalteau's reagent (diluted 1:2) and 1.2mL of 20% Na_2CO_3. The contents were mixed and incubated in dark at room temperature for 30mins. Phenol reacts with the phosphomolybdic acid present in the FC-reagent in alkaline medium to form a blue colored complex which can be measured at 765nm using a spectrophotometer.

Flavonoid Determination

20 µg of the plant samples were extracted repeatedly with 2ml of 80% aqueous methanol at room temperature. The whole solution was centrifuged. The supernatant was later transferred into an eppendorf tube and evaporated to dryness in a hot air oven and weighed [22].

Antioxidant Assay

Extract Preparation for Antioxidant Activity

For extraction, 5gm powder of *Ocimum* was mixed with 50ml of methanol. Extraction continued until the extraction solvents became colorless. The obtained extracts were filtered over Whatmann No.1 paper and the filtrate was collected, then methanol was removed by evaporation.

DPPH (1, 1-diphenyl-2-picrylhydrazyl) free radical scavenging assay

Scavenging activity on DPPH was assessed according to the method reported by [23] with a slight modification. Briefly, 500µl of extracts (0.2 to 1mg/ml) were mixed with 3ml of methanolic solution of 0.1mM DPPH. The mixture was shaken well and incubated at room temperature for 30 min and absorbance was measured at 517nm in a spectrophotometer. Experiment was performed in triplicate and average was taken for determination of percentage inhibition.

Reducing Power Assay

The reducing power of extracts was determined by following method 0.5ml of extracts (0.2 to 1mg/ml) was mixed with 0.5ml of 0.2 M phosphate buffer (pH 6.6) and 0.5ml potassium ferrocyanide (1%). After incubating the mixture at 50ºC for 20 min., 0.5ml of 10% trichloroacetic acid was added and then mixture was centrifuged at 3000 rpm for 10 min. 1ml of supernatant was mixed with 1ml of distilled water and 0.2ml FeCl3 (0.1%) and the absorbance was measured at 700.

Thiobarbituric Acid (TBA) Method

The method of [24] was referred; 1ml of 20% trichloroacetic acid and 1ml of 0.67% 2-thiobarbituric acid was added to 0.5ml of sample solution prepared in ethanol. The mixture was placed in a boiling water bath. After cooling, absorbance of supernatant was measured at 552nm.

Antibacterial Activity

Collection of Microorganisms for Antibacterial Activity

The pathogenic microorganism were isolated from clinical samples collected from diagnostic testing labs and indentified on the basis of morphological, biochemical and physiological characteristics according to Bergey's manual of determinative bacteriology. The isolated microorganisms were found to be *K. pneumoniaee, Proteus sp., E. faecalis, S. typhi, S. aureus* and *B. subtilis.*

Solvent Extraction

Plant leaves were dried under shade and dried leaves were crushed using mortar and pestle. Then 5gm of crushed plant material was kept on rotary shaker along with 50ml of different solvents for 2 days. The extract was concentrated by solvent evaporation and used for antimicrobial activity.

Determination of Antimicrobial Activity

The antimicrobial activity was determined by the agar well-diffusion method. Overnight grown bacterial culture was transferred to sterile Petri plate with Mueller Hinton agar medium (Hi Media Laboratories Limited, Mumbai, India) and was spread with sterile spreader to create a lawn. Wells of 6mm were punched into the previously seeded MH agar plates using sterile cork borer. About 80µl of the different *Ocimum* extract was placed in the wells and allowed to diffuse for 2 hrs. at 4ºC and the plates were incubated at 37°C for 24 hrs. The activity was determined by measuring the diameter of the inhibition zones for each well and expressed in millimeter.

Determination of Minimal Inhibitory Concentration (MIC)

The extracts that exhibited considerable activity were used for MIC determination. The extracts of the test samples were tested in four dose levels of 20µl, 40µl, 60µl and 80µl. The overnight grown bacterial culture was transferred on MH agar plate and wells were punched out using a sterile 6mm cork borer. Different concentration (20-80µl) of the extract was placed in separate wells, allowed to diffuse for 2 hrs. at 4°C and then the plates were incubated at 37°C for 24 hrs. The zone of inhibition was observed and the lowest concentration of the test sample showing zone of inhibition was recorded as the MIC.

Results

Phytochemical screening of the methanol extracts of *Ocimum* showed the presence of phenolic content, glycosides, anthraquinones, terpenoids, flavinoids, tannins, lignin and Saponins as chemical constituents.

Table 1- Qualitative Assay for Phytochemicals

Test	SAMPLES				
	Osp	Osg	Og	Ob	Cb
FEHLING'S TEST	-	-	-	+	+
CARDIAC GLYCOSIDES	+	+	+	+	+
ANTHRAQUINONES	+	+	+	+	+
TERPENOIDS (SALKOWSKI)	+	+	+	+	+
PROTEINS	-	-	-	-	-
FLAVINOIDS	+	+	+	+	+
TANNINS	+	+	+	+	+
LIGNIN	+	+	+	+	+
SAPONINS	+	+	+	+	+

The results for phytochemical screening are shown in [Table-1] and the quantitative estimation of Phytochemicals is shown in [Table-2].

Table 2- Quantitative Assay for Phytochemicals

Phytochemical	Samples				
	Osp	Osg	Og	Ob	Cb
Tannins (OD_{670})	0.81	0.24	0.26	0.54	0.36
Phenols (mg GAE/g dw)	111.6	174	171.8	125.2	134.6
Flavanoids (%)	6	4.66	3.83	5.5	2.83
Alkaloids (%)	5.56	7.56	4.67	8.67	9.33

The antioxidant activity of *Ocimum* species was determined by three different assays such as reducing power assay, 1-1-diphenyl-2-picrylhydrazyl (DPPH) assay and Thiobarbituric acid (TBA) assay. *Camphor basil* showed maximum activity in reducing power assay at different concentrations as shown in [Fig-1].

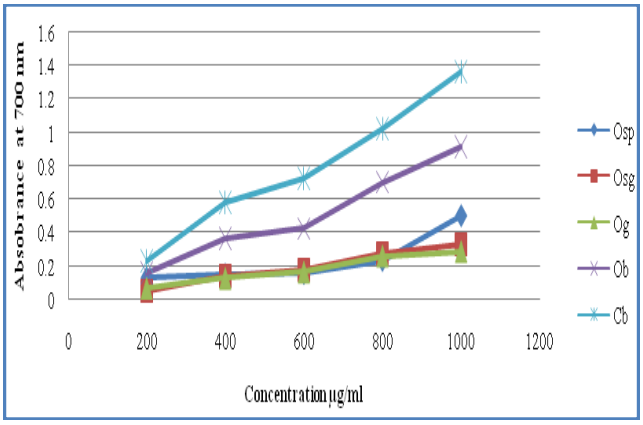

Fig. 1- Reducing Power Assay

In the DPPH method the absorbance was measured and the percent inhibition of the DPPH radical by *Ocimum* was calculated based on the measured absorbance. Antioxidant capacities in series of concentrations for *Ocimum* was used for calculating the percentage of antioxidant activity as showed in [Fig-2]. *Camphor basil* showed maximum antioxidant activity compared to other *Ocimum* species.

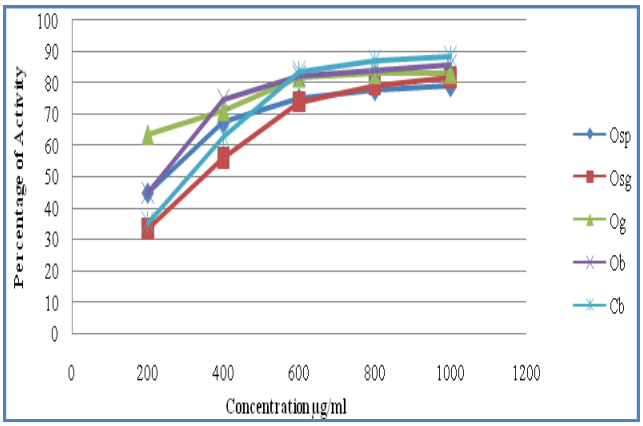

Fig. 2- Antioxidant activity by DPPH Assay

In the TBA assay *Camphor basil* was most active as an antioxidant followed by *O. sanctum purple* at various concentrations as shown in [Fig-3].

To determine the antibacterial activity, various solvent extracts like ethanol, Methanol, Propanol, chloroform, petroleum ether and Iso-

amyl alcohol was evaluated and compared using agar diffusion method. All the test extracts of *Ocimum* showed significant antibacterial activity against tested pathogens. The antibacterial activity of *O. sanctum purple, O. sanctum green, O. gratissimum, O. basilicum and Camphor basil are* presented in [Fig-4-8] respectively. The isoamyl extract of *Ocimum* was found to be the most active and produced highest zone of inhibition. Isoamyl extract of *O. sanctum purple* produced 24mm zone and *O. sanctum green produced* 32mm zone against *B. subtilis.* Isoamyl extract of *O. gratissimum* produced 26mm zone, *O. basilicum* produced 28mm zone *and Camphor basil* produced 22mm zone against *S. typhi.*

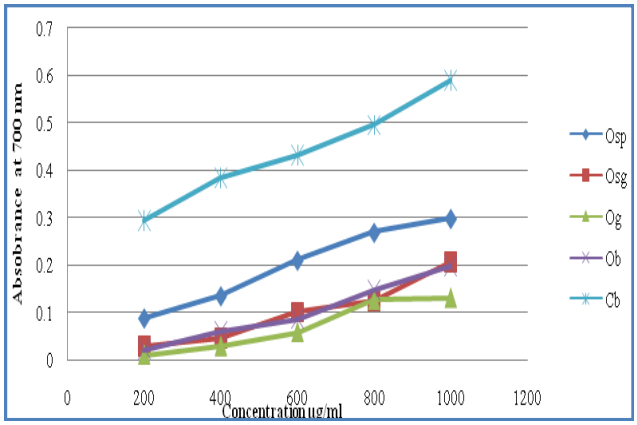

Fig. 3- Thiobarbituric Acid Assay

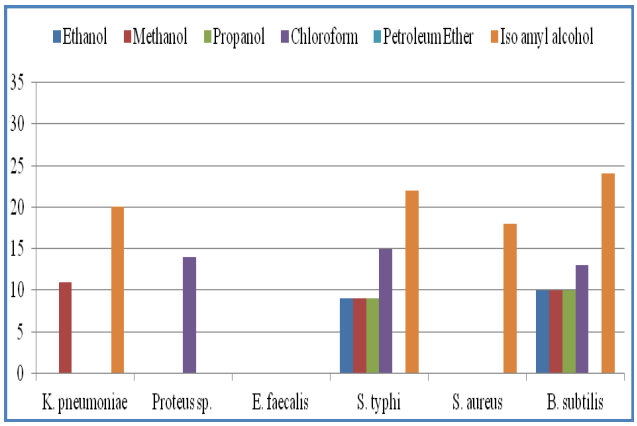

Fig. 4- Antibacterial Activity of *Ocimum sanctum purple*

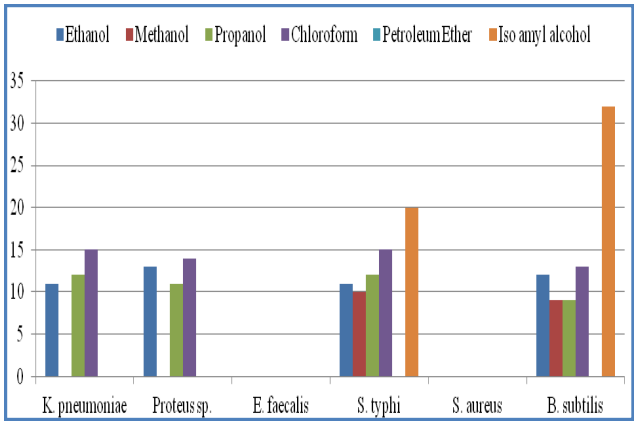

Fig. 5- Antibacterial Activity of *Ocimum sanctum green*

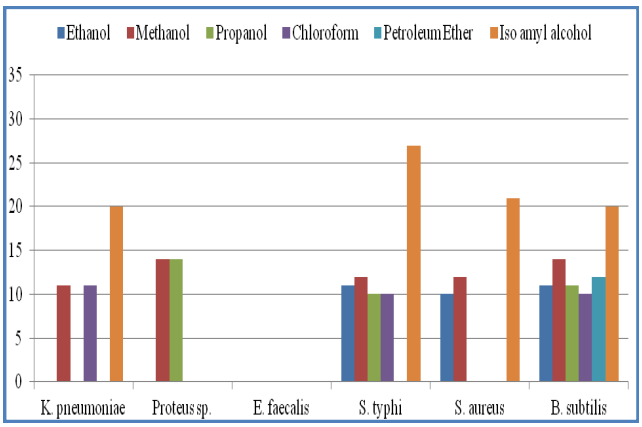

Fig. 6- Antibacterial Activity of *Ocimum gratissimum*

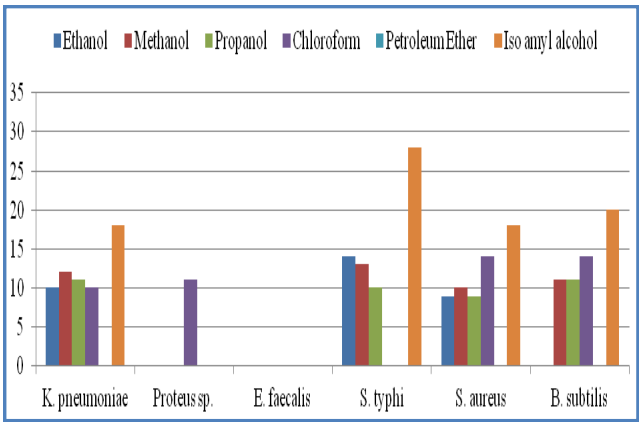

Fig. 7- Antibacterial Activity of *Ocimum basilicum*

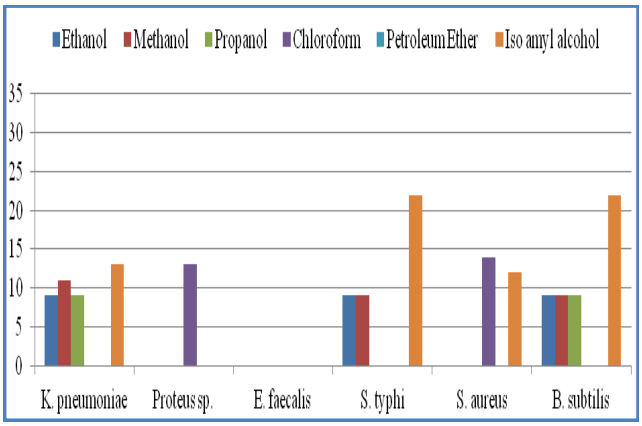

Fig. 8- Antibacterial Activity of *Camphor basil (Ocimum kilimandscharicum)*

In the MIC assay all the species exhibited diverse antimicrobial activity at varied concentrations as summarized in [Table-3].

The isoamyl alcohol extract was most active compared to other solvent extracts and *B. subtilis* and *S. typhi* were most sensitive among the tested microorganism.

Discussion

The Phytochemicals are either the product of plant metabolism or synthesized for defense purposes [4]. In the present investigation, the results of phytochemical analysis of *Ocimum* showed the pres-

ence of chemical compound such as phenolic compounds, glycosides, flavanoids, tannins and saponins which account for their usefulness as medicinal plants. The DPPH radical scavenging activities of *O. gratissimum* was higher compared to other species at lower concentration: whereas, antioxidant activity of *Camphor basil* was higher in other assays. These differences might be due to the different principles of these assays: hence, we can construe that antioxidant activity cannot be compared by different antioxidant assays. Our antioxidant assay result corresponds to the earlier studies [25,26].

Table 3- Minimal Inhibitory Concentration of Ocimum Species

Plant Sample and Extraction solvent	Pathogens					
	K. pneumoniae	*S. typhii*	*S. aureus*	*E. faecalis*	*Proteus sp.*	*B. subtilis*
Ocimum sanctum purple						
Ethanol Extract	_	80	_	_	_	60
Methanol Extract	60	80	_	_	_	80
Propanol Extract	_	80	_	_	_	60
Chloroform Extract		20	_	_	40	20
Petroleum Ether	_	0	_	_	_	_
Isoamyl alcohol Extract	20	20	20	_	_	20
Ocimum sanctum green						
Ethanol Extract	40	60	_	_	40	40
Methanol Extract	_	40	_	_	_	60
Propanol Extract	20	40	_	_	40	60
Chloroform Extract	40	20	_	_	20	40
Petroleum Ether	_	_	_	_	_	_
Isoamyl alcohol Extract	_	20	_	_	_	20
Ocimum gratissimum						
Ethanol Extract	_	40	60	_	_	60
Methanol Extract	80	40	80	_	60	40
Propanol Extract	_	60	_	_	60	60
Chloroform Extract	60	80	_	_	_	60
Petroleum Ether	_	_	_	_	_	40
Isoamyl alcohol Extract	20	20	20	_	_	20
Ocimum basilicum						
Ethanol Extract	60	20	80	_	_	_
Methanol Extract	60	40	80	_	_	60
Propanol Extract	40	60	80	_	_	60
Chloroform Extract	40	_	40	_	40	20
Petroleum Ether	_	_	_	_	_	_
Isoamyl alcohol Extract	20	20	20	_	_	20
Camphor basil						
Ethanol Extract	80	80	_	_	_	80

The antimicrobial activities of different extracts of *Ocimum* were evaluated qualitatively and quantitatively against the pathogenic microorganisms by the presence or absence of inhibition zones and zone diameter. The isoamyl extract showed stronger and broader spectrum of antimicrobial activity compared to other solvent extracts. These results predict that isoamyl alcohol is a better solvent for consistent extraction of antimicrobial substances from *Ocimum species*. In the antimicrobial assay, among the tested pathogens *B. subtilis* and *S. typhi* were most sensitive for *Ocimum* extract and no inhibition activity was observed against *E. faecalis*. Our results are different from results of Adiguzel A., et al [27], in our study zone of inhibition was seen for Ethanol and Methanol extract of *O. basilicum* on *Proteus sp.* and *S. aureus.* The minimal inhibitory concentration was determined at different concentrations and varied for different species. Among the tested microorganism most were sensitive for isoamyl alcohol extract of all *Ocimum* species. As *Ocimum* is widespread in India, it can be recommended as an easily available and renewal source of antimicrobial agent instead of synthetic chemicals. The present findings indicate that *Ocimum* possesses compounds with antimicrobial properties against pathogenic microorganisms.

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