



Studies on Antimicrobial Activity of Ocimum Species of Karnataka against Clinical Isolates

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ABSTRACT

Antimicrobial activity of the various species of *Ocimum* plants like *Ocimum basilicum*, *Ocimum gratissimum*, *Ocimum sanctum*, *Ocimum kilimandscharicum* against various pathogens like *E. coli*, *Bacillus subtilis*, *Streptococcus* species, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Serratia marcescens*, *Klebsiella pneumonia* were studied with the help of agar well diffusion method. The antimicrobial activity of the samples with the following solvent extracts Methanol, Ethanol, Chloroform, Acetone, Diethyl ether of *Ocimum gratissimum*, *Ocimum sanctum* purple were measured by using Muller-Hinton agar plates which showed the highest zone of inhibition against *Salmonella typhimurium*, *Pseudomonas aeruginosa* and *Serratia marcescens*. In *Ocimum kilimandscharicum*, all the pathogens were resistant against all the extract except for *Bacillus subtilis* which showed least susceptible to the Methanol extract.

Keywords: Muller-Hinton agar plate, Agar well diffusion method, Zone of inhibition, Pathogens, Medicinal plants.

INTRODUCTION

The Genus *Ocimum* belonging to the family Lamiaceae, subfamily Nepetoideae has a sharp, oval-shaped leaves which is found in Iran, Afghanistan, and India (Mann et al., 2000, Volak & Jiri, 1997, Zargari, 1990, Mirheidar, 1990). It is commonly known as Basil, an aromatic herb which has been traditionally used in treating Headache, cough, Diarrhoea, Constipation, warts, Kidney malfunctions (Sikmon et al., 1990). The presence of secondary metabolites like Alkaloids, Steroids, Tannins, Phenol

compounds, Flavonoids, Resins, Fatty acids, a gum has shown medicinal importance in giving the first line of defense in the body (Joshi et al., 2009). It is also used in the treatment of ulcers (Volak & Jiri, 1997). The inhibitory activity against HIV-1 reverse transcriptase and platelets aggregation were shown by them which has been induced by Collagen and ADP (Yamasaki et al., 1998, Okazaki et al., 1998). The essential oils of these plant have the effect of Insecticidal (Deshpande & Tipnis, 1997), Nematicidal (Chaterjee et al., 1982),

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Fungistatic (Reuveni et al., 1984) and antimicrobial properties (Yamasaki et al., 1998, Wannissorn et al., 2005).

In the present study antimicrobial activity of various species of *Ocimum* has been tested against various pathogens like *E.coli*, *Staphylococcus aureus*, *Streptococcus* species, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Bacillus subtilis*, *Salmonella typhimurium*, *Serratia marcescens* by using various extracts of Methanol, Ethanol, Chloroform, Acetone.

Escherichia coli, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Salmonella typhimurium* resides in the gastrointestinal tract of humans and other vertebrates. Some of the strains are harmful leading to serious infections while some of the other are beneficial helping in food digestion. *Staphylococcus aureus*, *Streptococcus species*, *Bacillus subtilis*, *Serratia marcescens* are Gram-positive bacteria. It is an anaerobe which grows in the absence of oxygen. Some of the strains are pathogenic which produce toxins on the surface of their cell wall proteins thereby inactivating the antibodies soon after they bind and lead to many infectious diseases. The antimicrobial activity of *Ocimum* species against pathogens has been studied by agar well diffusion method by measuring the zone of inhibition against the various extracts treated with pathogens. In our study it has found that Gram-positive bacteria like *Streptococcus species*, *Bacillus subtilis*, *Serratia marcescens* are more susceptible to the leaf extracts of *Ocimum* due to the presence of thick-walled Peptidoglycan which undergoes degradation treated with methanolic extracts of *Ocimum* whereas Gram-negative bacteria like *E.coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* are more resistant to all the leaf extracts of *Ocimum* due to the presence of three layers around their plasma membrane which is made up of Lipopolysaccharide (LPS).

MATERIALS AND METHODS

The plant samples of different species were obtained from the University of Agriculture and maintained in the greenhouse of

Visveshwarapura College of Science, Bangalore India. The obtained leaf samples were sterilised, powdered and stored in air tight bottles.

A. Solvent extract preparation:

50 mL of methanol is used to extract 5g of each powdered sample for 48 hours. After 48 hours the supernatant obtained was used to make the crude extract by the process of evaporation.

The agar well diffusion method was used to assess the antimicrobial activity of plant samples. The Solvent extraction was done by the Soxhlet apparatus. Muller-Hinton agar plates were prepared to evaluate the antimicrobial activity of the samples with the following solvent extracts Methanol, Ethanol, Acetone, Chloroform and Diethyl ether against selected human pathogens viz., *E.coli*, *Bacillus sp.*, *Streptococcus sp.*, *Salmonella sp.*, *Pseudomonas sp.*, *Serratia marcescens*, *Klebsiella pneumonia* and *Staphylococcus aureus*. 100µl inoculum of each selected pathogen was uniformly spread on Muller-Hinton agar plates. After 5 minutes of incubation, sterile cork borer was used to punch the plates to obtain well of 6mm diameter well. 80 µl of the concentrated sample was added into the well. The incubation of plates was carried out at 37°C for overnight and after incubation plates were observed for the zone of inhibition. (HamidehJaberian, KhosroPiri, JavadNazari 2013; BiruhalemTaye et al., 2011)

Minimum Inhibitory Concentration:

The samples showing higher antimicrobial activity against the pathogens was assessed by Minimum inhibitory concentration. Different concentrations of the samples were checked for the antimicrobial activity to determine the concentration at which the samples showed the least activity. Five different concentration of the samples were used for the determination of the MIC. The chloroform extract of *H.mutabilis*, *P.chaba*, *O.gratissimum*, *O.sanctum purple*, and *O. basillicum* were used to determine the MIC as they showed a higher zone of inhibition against the pathogens (Emmanuel Jean Teinkela Mbosso et al., 2010).

RESULTS AND DISCUSSION

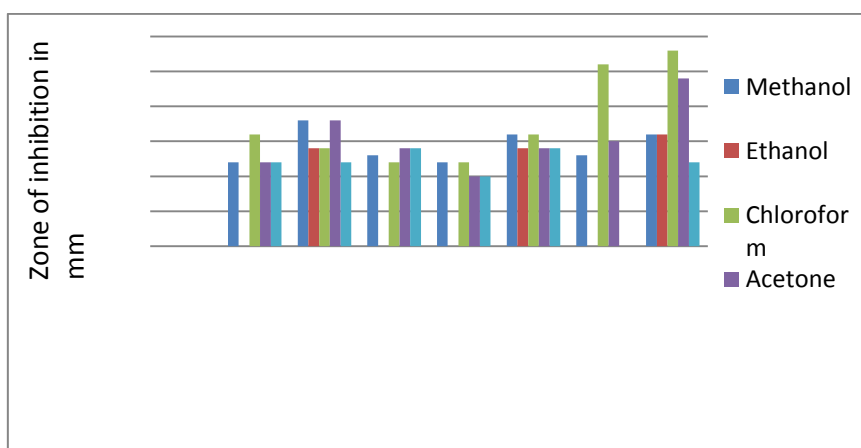
Plants having medicinal values play a significant role in treating diseases by acting against the pathogens. The antimicrobial properties of the *Ocimum* species are studied by agar well diffusion method. The plants find their application for the source of new drug compounds being contributed in large to human well being. The antimicrobial properties of plant extracts can be used for therapeutic purpose.

Ocimum species has been tested its antimicrobial activity by using various solvents of Methanol, Ethanol, Chloroform, Acetone, Diethyl ether against various pathogens like *Staphylococcus aureus*, *E.coli*, *Streptococcus species*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Bacillus subtilis*, *Salmonella typhimurium*, *Serratia marcescens*. *Ocimum basillicum* is not effective in inhibiting *Staphylococcus aureus* were as very effective in inhibiting *Salmonella*

typhimurium and *Serratia marcescens* (Table 1 and Graph1). Sowmen Saha et al. (2013) has studied the antimicrobial activity by using the methanol extract of *Ocimum basillicum*, *Ocimum kilimandscharicum*, *Ocimum gratissimum* against *Bacillus subtilis*, *E.coli* and *Vibrio cholera* ranging the zone of inhibition from 7-10mm respectively. They found that essential oils are exhibiting a stronger antibacterial activity than methanolic extracts against all the pathogenic bacteria including Gram-negative ones due to the high content of phenols (Burt, 2004, Gallucci et al., 2009, Bassolé & Juliani, 2012). The sensitivity to essential oils is more in Gram-positive bacteria compared to Gram-negative bacteria. The Gram-negative bacteria having the outer membrane are resistant to the antibacterial activity of essential oil. The antibacterial substances kills the Gram positive bacteria as they lack outer membrane (Kalemba & Kunicka 2003).

Table 1: Evaluation of Antimicrobial activity of *Ocimum basillicum* using various solvents against selected Human pathogens (The values represents the zone of inhibition in mm)

	<i>Staphylococcus aureus</i>	<i>E.coli</i>	<i>Streptococcus sps</i>	<i>Pseudomonas aeruginosa</i>	<i>Klebsiella pneumonia</i>	<i>Bacillus subtilis</i>	<i>Salmonella typhimurium</i>	<i>Serratia marcescens</i>
Methanol	0	12	18	13	12	16	13	16
Ethanol	0	0	14	0	0	14	0	16
Chloroform	0	16	14	12	12	16	26	28
Acetone	0	12	18	14	10	14	15	24
Diethyl ether	0	12	12	14	10	14	0	12



Graph 1: The graphical representation of Zone of inhibition by using various solvent extracts of *Ocimum basillicum*

The *Ocimum gratissimum* has shown very effective zone of inhibition against *Serratia marcescens* whereas *Streptococcus* species

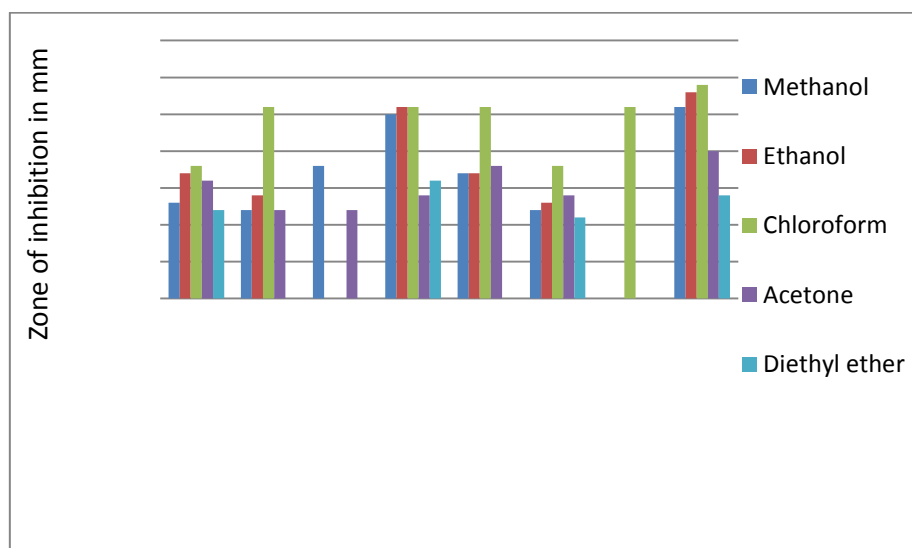
showed resistance in the Chloroform extract (Table 2 and Graph 2). Okigbo et al. (2 May 2006). have studied the antimicrobial activity

of *Ocimum gratissimum* on *Proteus mirabilis*, *E.coli*, *Staphylococcus aureus* and *Candida albicans*. The zone of inhibition against *E. coli*, *Proteus mirabilis* and *Staphylococcus aureus* was observed by using the ethanolic aqueous (cold) and aqueous (hot) extracts of *Ocimum gratissimum*. The zone of inhibition on *Staphylococcus aureus* and *Proteus mirabilis* were ranged 3mm than *E.coli* by

using the ethanolic extract of *Ocimum gratissimum*. The zone of inhibition ranging about 2-3mm was observed on *Staphylococcus aureus* and *Proteus mirabilis* by using the aqueous cold extract but the highest zone of inhibition on *Proteus mirabilis* ranging about 7mm was observed by using the aqueous hot extract.

Table 2: Evaluation of Antimicrobial activity of *Ocimum gratissimum* using various solvents against selected Human pathogens (The values represents the zone of inhibition in mm)

	<i>Staphylococcus aureus</i>	<i>E.coli</i>	<i>Streptococcus sps</i>	<i>Pseudomonas aeruginosa</i>	<i>Klebsiella pneumonia</i>	<i>Bacillus subtilis</i>	<i>Salmonella typhimurium</i>	<i>Serratia marcescens</i>
Methanol	13	12	18	25	17	12	0	26
Ethanol	17	14	0	26	17	13	0	28
Chloroform	18	26	0	26	26	18	26	29
Acetone	16	12	12	14	18	14	0	20
Diethyl ether	12	0	0	16	0	11	0	14



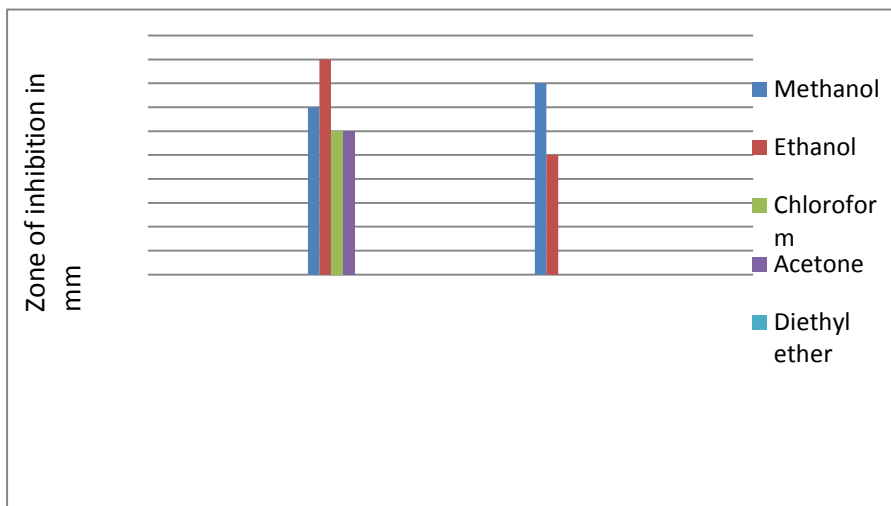
Graph 2: The graphical representation of Zone of inhibition by using various solvent extracts of *Ocimum gratissimum*

In *Ocimum kilimandscharicum*, pathogens like *Staphylococcus aureus*, *E.coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Salmonella*

typhimurium, *Serratia marcescens* except for *Streptococcus* species which got inhibited effectively (Table 3 and Graph 3).

Table 3: Evaluation of Antimicrobial activity of *Ocimum kilimandscharicum* using various solvents against selected Human pathogens (The values represents the zone of inhibition in mm)

	<i>Staphylococcus aureus</i>	<i>E.coli</i>	<i>Streptococcus sps</i>	<i>Pseudomonas aeruginosa</i>	<i>Klebsiella pneumonia</i>	<i>Bacillus subtilis</i>	<i>Salmonella typhimurium</i>	<i>Serratia marcescens</i>
Methanol	0	0	14	0	0	16	0	0
Ethanol	0	0	18	0	0	10	0	0
Chloroform	0	0	12	0	0	0	0	0
Acetone	0	0	12	0	0	0	0	0
Diethyl ether	0	0	0	0	0	0	0	0



Graph 3: The graphical representation of Zone of inhibition by using various solvent extracts of *Ocimum kilimandscharicum*

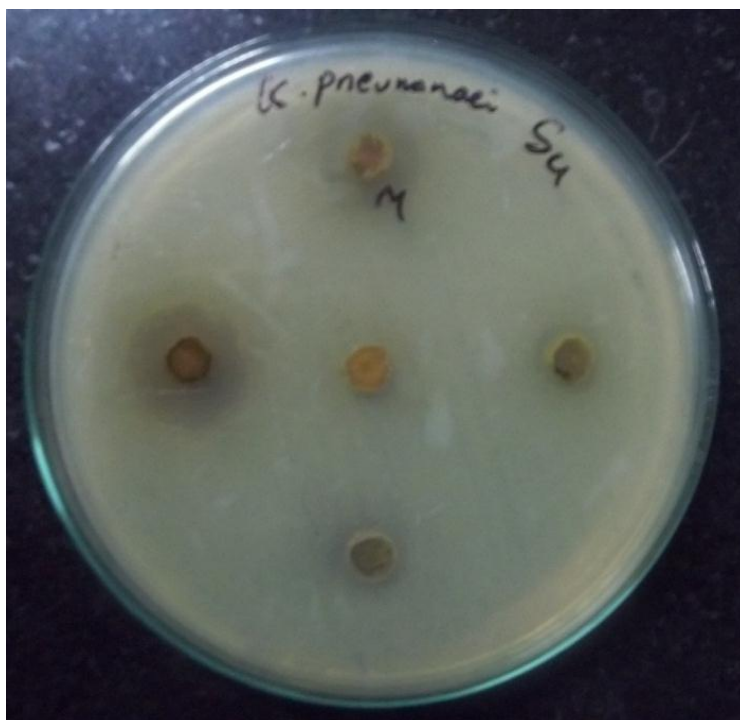


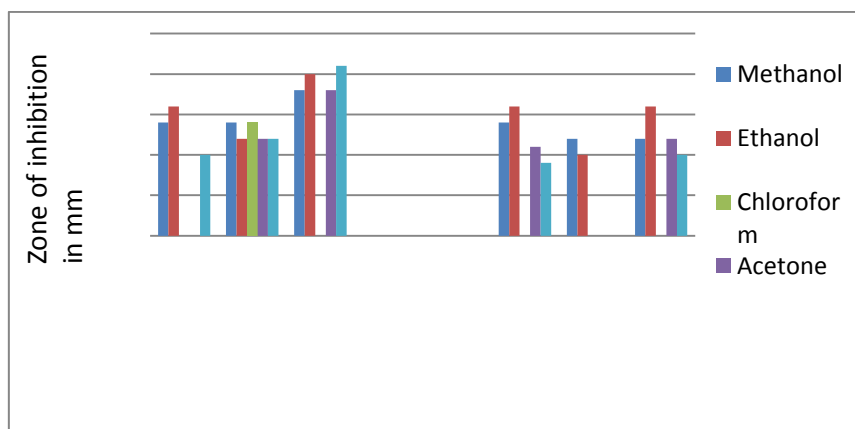
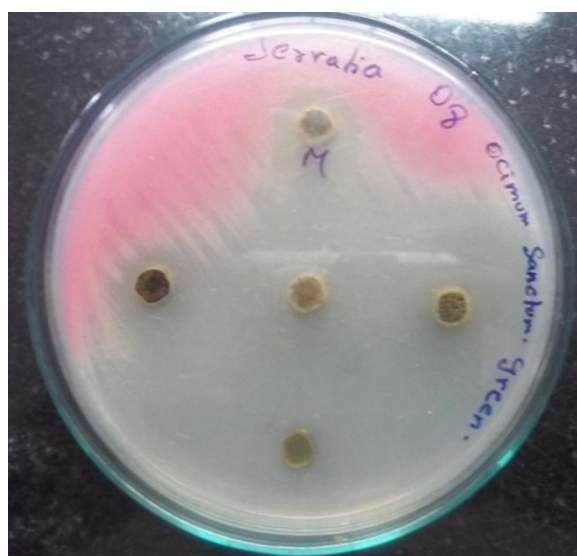
Fig. 1: Muller-Hinton agar plate showing zone of inhibition by using various solvent extracts of *Ocimum kilimandscharicum* against *Klebsiella pneumoniae*

In *Ocimum sanctum* green, *Streptococcus species* is effectively inhibited whereas *Pseudomonas aeruginosa* showed resistance (Table 4, Graph 4 and Figure 2). In *Ocimum sanctum* purple, *E.coli* and *Serratia*

marcescens got inhibited effectively whereas *Klebsiella pneumoniae*, *Salmonella Typhimurium* showed resistance (Table 5, Graph 5 and Figure 3).

Table 4: Evaluation of Antimicrobial activity of *Ocimum sanctum* green using various solvents against selected Human pathogens (The values represents the zone of inhibition in mm)

	<i>Staphylococcus aureus</i>	<i>E.coli</i>	<i>Streptococcus sps</i>	<i>Pseudomonas aeruginosa</i>	<i>Klebsiella pneumonia</i>	<i>Bacillus subtilis</i>	<i>Salmonella typhimurium</i>	<i>Serratia marcescens</i>
Methanol	14	14	18	0	0	14	12	12
Ethanol	16	12	20	0	0	16	10	16
Chloroform	0	14	0	0	0	0	0	0
Acetone	0	12	18	0	0	11	0	12
Diethyl ether	10	12	21	0	0	9	0	10

**Graph 4: The graphical representation of Zone of inhibition by using various solvent extracts of *Ocimum sanctum* green****Fig. 2: Muller-Hinton agar plate showing zone of inhibition by using various solvent extracts of *Ocimum sanctum* green against *Serratia marcescens***

Gomathinayagam Subramanian et al. (2014) has studied the antimicrobial activity of *Ocimum tenuiflorum*, also known as *Ocimum sanctum* by using ethanol, methanol, ethyl acetate, chloroform against three human pathogens like *E.coli*, *Staphylococcus aureus*,

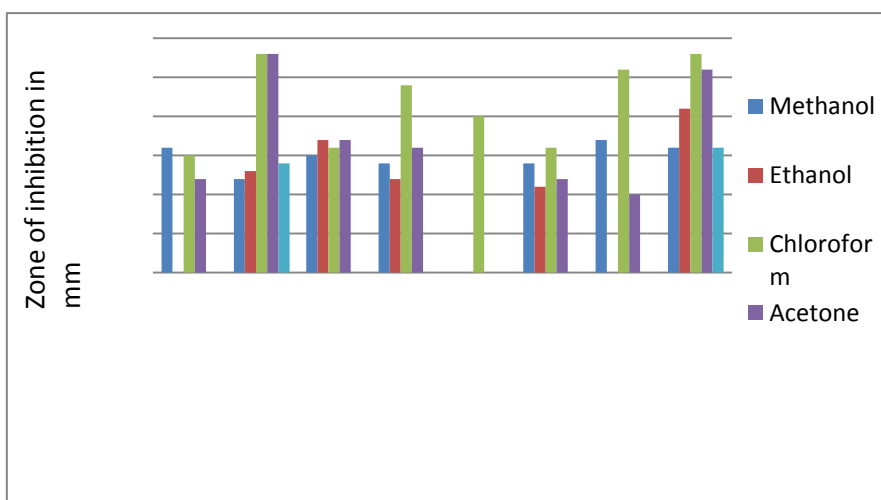
Candida albicans through the good diffusion method. The strongest Minimum inhibition concentration (MIC) was observed in methanol extract. The *Staphylococcus aureus* (Gram-positive bacteria) has shown a greater zone of inhibition than *E.coli* and *Candida*

albicans. The most effective result was found in the ethanol leaf extract against *E.coli* in well diffusion method. Prasad et al., (2012) studied the antibacterial activity of *Ocimum sanctum* purple, *Ocimum sanctum* green, *Ocimum gratissimum*, *Ocimum basilicum* and *Camphor basil* against pathogens using various solvents like ethanol, methanol, propanol, chloroform, petroleum ether by agar diffusion method. The greater zone of inhibition was observed in the Isoamyl extract of *Ocimum*. The *Ocimum sanctum* purple

exhibited 24mm zone of inhibition and *Ocimum sanctum* green exhibited 32mm zone of inhibition against *Bacillus subtilis*. Isoamyl extract of *Ocimum gratissimum* exhibited 26mm zone of inhibition, *Ocimum basilicum* exhibited 28mm zone of inhibition and *Camphor basil* exhibited 22mm zone of inhibition against *Salmonella typhi*. Their study showed *Bacillus subtilis* and *Salmonella typhi* were more susceptible for *Ocimum* extract among the tested pathogens in the antimicrobial assay.

Table 5: Evaluation of Antimicrobial activity of *Ocimum sanctum* purple using various solvents against selected Human pathogens (The values represents the zone of inhibition in mm)

	<i>Staphylococcus aureus</i>	<i>E.coli</i>	<i>Streptococcus sps</i>	<i>Pseudomonas aeruginosa</i>	<i>Klebsiella pneumonia</i>	<i>Bacillus subtilis</i>	<i>Salmonella typhimurium</i>	<i>Serratia marcescens</i>
Methanol	16	12	15	14	0	14	17	16
Ethanol	0	13	17	12	0	11	0	21
Chloroform	15	28	16	24	20	16	26	28
Acetone	12	28	17	16	0	12	10	26
Diethyl ether	0	14	0	0	0	0	0	16



Graph 5: The graphical representation of Zone of inhibition by using various solvent extracts of *Ocimum sanctum* purple

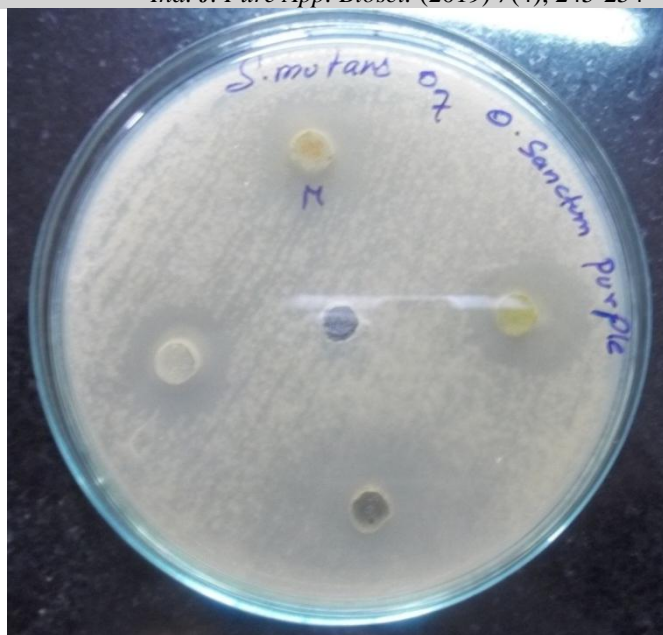


Fig. 3: Muller-Hinton agar plate showing zone of inhibition by using various solvent extracts of *Ocimum sanctum purple* against *Streptococcus mutans*

In the research paper of Selvamohan et al. (213) the methanolic extract of *Phyllanthus niruri* exhibited maximum inhibitory zone (30mm) against *Staphylococcus* species whereas *Murryakoenigi*, *Cynodondachylon*, *Lawsoniainermis*, and *Adha-Thoda Vasica* from ethanol and aqueous extracts exhibited least inhibitory zone. The medicinal plants extracted by using methanol exhibited maximum antimicrobial activity done by agar well diffusion method.

In the current study, *Ocimum species* have been tested for antimicrobial activity by agar well diffusion method. *Staphylococcus aureus* has shown resistance in all the solvents in *Ocimum basilicum* species, chloroform extract of *Ocimum basilicum* has shown a broad spectrum of the zone of inhibition (28mm) against *Serratia marcescens* (Table 1).

In *Ocimum gratissimum*, the chloroform extract has shown 29mm of the zone of inhibition, methanol showed 26mm of the zone of inhibition, Ethanol extract showed 28mm of the zone of inhibition against *Serratia marcescens* (Table2). *Staphylococcus aureus*, *E.coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Salmonella*

typhimurium, *Serratia marcescens* has shown resistance in all the extracts of *Ocimum kilimandscharicum* (Table 3). Diethyl ether extract of *Ocimum sanctum green* has shown the highest antimicrobial activity against *Streptococcus* species (Table 4). The chloroform extract of *Ocimum sanctum purple* exhibited the highest antimicrobial zone against *E. coli* (28mm), *Serratia marcescens* (28mm) of the inhibitory zone (Table 5). Therefore, the zone of inhibition indicates the efficiency of the medicinal plants acting against the pathogens which help in determining the antimicrobial activity of plants.

From the above study, it is clear that methanol, ethanol and chloroform extracts of *Ocimum gratissimum* (Table 2) has shown highest zone of inhibition against *Pseudomonas aeruginosa* and *Serratia marcescens* because of the antibacterial properties of *Ocimum gratissimum* evaluated by disc diffusion method.

The *Ocimum kilimandscharicum* has shown the least zone of inhibition against all the pathogens indicating these pathogens are resistant to the leaf extract of *Ocimum kilimandscharicum* (Table 3). Therefore, the organisms like *Pseudomonas aeruginosa*,

Serratia marcescens were sensitive thereby got inhibited against the leaf extracts of *Ocimum gratissimum* (Table 2).

Disc diffusion method is used to study the sensitivity of organisms and differentiate the organisms based on the zone of inhibition found around the discs which have thrown limelight in using these plants for medicinal purpose.

CONCLUSION

The naturally occurring organic compounds present in the plant extracts can be extracted depending upon the property of the solubility of the compound in the solvent. The method of extraction process differs between various compounds and the product. The plant crude extracts exhibit a strong antimicrobial activity. The antimicrobial compounds from higher plants have led to the pathway of combating against the pathogens resistant to multiple drugs and thus leading to the success of the phytomedicine fighting against the pathogens.

REFERENCES

- Burt, S. (2004). Essential oils: their antibacterial properties and potential applications in foods – a review. *International Journal of Food Microbiology*, 94, 223–253.
- Bassolé, I.H.N., & Juliani, H.R. (2012). Essential oils in combination and their antimicrobial properties. *Molecules*, 17, 3989–4006.
- Chaterjee, A., Sukul, N.C., Laskal, S., & Ghoshmajumdar, S. (1982). Nematicidal principles from two species of Lamiaceae. *J. Nematol.*, 14, 118-120.
- Deshpande, R.S., & Tipnis, H.P. (1997). Insecticidal activity of *Ocimum basilicum* L. *Pesticides*, 12, 21-28.
- Gallucci, M.N., Oliva, M., Casero, C., Dambolena, J., Luna, A., Zygadlo, J., & Demo, M. (2009). Antimicrobial combined action of terpenes against the food-borne microorganisms *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus*. *Flavour and Fragrance Journal*, 24, 348–354.
- Gomathinayagam, S., Brij, B., Tewari, R., Gomathinayagam, (2014). studies of Antimicrobial properties of different leaf extract of Tulsi (*Ocimum tenuiflorum*) against human pathogens, *American International Journal of Contemporary Research*, 4(8), 149-155.
- Joshi, B., Lekhak, S., & Sharma, A. (2009). Antimicrobial property of different medicinal plants: *Ocimum santum*, *Cinnamomum zeylanicum*, *Xanthorylum armatum*, and *Origanum majorana*. *Kathnandu Univ. J. Sci. Eng. Technol.*, 5, 143-150.
- Mann, C.M., Cox, S.D., & Markham, J.L. (2000). The outer membrane of *Pseudomonas aeruginosa* NCTC 6749 contributes to its tolerance to the essential oil of *Melaleuca alternifolia* (tea tree oil). *Lett. Applied Microbiol.*, 30, 294-297.
- Mirheidar, H. (1990) Herbal Knowledge. IstEdn., *Islamic Culture Publication, USA.*
- Mukherjee, S.P. (1987). *Ocimum-A Cheap Source of Eugenol*. CSIR., New Delhi.
- Nakamura, C.V., Ueda-Nakamura, T., Bando, E., Melo, A.F.N., Cortez, D.A.G., & Dias, F.B.P. (1999). Antibacterial activity of *Ocimum gratissimum* L. essential oil. *Mem. Inst. Oswaldo Cruz.*, 94, 675-678.
- Okazaki, K., Nakayama, S., Kawazoe, K., & Takaishi, Y. (1998). Antiaggregant effects on human platelets of culinary herbs. *Phytother. Res.*, 12, 603-605.
- Mishra, P., & Mishra, S. (2011). Study of antibacterial activity of *Ocimum sanctum* extract against Gram-positive and Gram-negative

- bacteria. *American Journal of Food Technology*, 6, 336-341.
- Prasad, M.P., Jayalakshmi, K., & Rindhe, G.G. (2012). Antibacterial activity of *Ocimum* species and their phytochemical and antioxidant potential, *International Journal of Microbiology Research*, 4(8), 302-307.
- Okigbo, R.N., & Ogonnaya, U.O. (2 May 2006). Antifungal effects of two tropical plant leaf extracts (*Ocimum gratissimum* and *Aframomum melegueta*) on post-harvest yam (*Dioscorea* spp) rot, *African Journal of Biotechnology* 5(9), 727-731.
- Rajeshwari, S. (1992). *Ocimum sanctum*: The Indian Home Remedy. In: Current Medicinal Scene, Rajeshwari, S. (Ed.). Rajeshwari Foundations Limited, Bombay, India.
- Reuveni, R., Fleisher, A., & Putievsky, E. (1984). Fungistatic activity of essential oils from *Ocimum basilicum* chemotypes, *Phytopathol. J.*, 110, 20-22.
- Saha, S., Tarak Nath, D., Sengupta, C., & Ghosh, P.D. (2013). Biological activities of essential oils and Methanol extracts of five *Ocimum* species against pathogenic bacteria, *Czech Journal of Food Science*, 31(2), 194-202.
- Selvamohan, T., & Ramadasetal, V. (2013). Antimicrobial activity of selected medicinal plants against some selected human pathogenic bacteria, *Advances in Applied science research*, 3(5), 3374-3381.
- Sen, P. (1993). Therapeutic potentials of Tulsi: From experience to facts. *Drugs News Views*, 1, 15-21.
- Shukla, R., & Sunadaram, S. (2002) Screening and in vitro evaluation of *Ocimum* for antibacterial activity, *Project Thesis, University of Allahabad*.
- Sikmon, J.E., Morales, M.R., Phippen, W.B., Vieira, R.F., & Hao, Z. (1990). Perspectives on New Crops and New Uses. In: A Source of Aroma Compounds and a Popular Culinary and Ornamental Herbs, Janick, J. (Ed.). ASHS Press, Alexandria, VA., 495-505.
- Volak, J., & Jiri, S. (1997). *Plants Medicinales Interpreter*, 3rd Edn., Tehran University Publications, Tehran, 20,
- Wannissorn, B., Jarikasem, S., Siriwangehaiand, T., & Thubthimthed, S. (2005). Antibacterial properties of essential oils from Thai medicinal plants. *Fitoterapia*, 76, 233-236.
- Yamasaki, K., Nakano, M., Kawahata, T., Mori H., & Otake T. (1998). et al., Anti HIV-1 activity of herbs in Labiatae, *Biol. Pharm. Bull.*, 21, 829-833.
- Zargari, A. (1990). *Medicinal Plants*. 4th Edn., Tehran University Press, Tehran, 1-57.