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Antibacterial effect of essential oil of *Ocimum sanctum* L. by minimal bactericidal concentration, disc diffusion, and gaseous contact exposure methods over 18 bacteria

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Abstract
The leaves of *Ocimum sanctum* were hydro distilled in the Clevenger apparatus and the extracted *Ocimum sanctum* L. essential oil was investigated for its antibacterial effects by three methods. Minimal bactericidal concentration method showed that the 17 bacteria tested were killed by different concentrations of *Ocimum sanctum* essential oil. *Klebsiella pneumoniae* was killed by 2.81% concentration of *Ocimum sanctum* essential oil. *Salmonella typhimurium, Burkholderia cepacia, Vibrio cholerae, Salmonella typhi, Escherichia coli, Coagulase negative Staphylococcus*, and *Corynebacterium diphtheriae* were killed by 5.62% *Ocimum sanctum* essential oil. *Acinetobacter baumannii, Shigella boydii, Serratia marcescens, Proteus mirabilis, Enterobacter aerogenes, Pseudomonas aeruginosa, Staphylococcus aureus, MRSA* and *Enterococcus faecalis* were killed by 11.25% *Ocimum sanctum* essential oil. Disc diffusion method showed that the growths of 18 bacteria were inhibited by undiluted *Ocimum sanctum* essential oil. 4 bacteria (*Acinetobacter baumannii, Serratia marcescens, Escherichia coli,* and *Pseudomonas aeruginosa*) showed lesser diameter of growth inhibition (from 6.5 to 8 mm), whereas other 14 bacteria showed growth inhibition diameters from 10 to 14 mm. Undiluted *Ocimum sanctum* essential oil inhibited the growth of 3 (MRSA, *Staphylococcus aureus, and Haemophilus influenzae*) out of 18 bacteria by gaseous contact exposure method. The Gas Chromatogram-Mass Spectrum analysis of the *Ocimum sanctum* essential oil revealed the presence of 19 components and the major components were Eugenol, Caryophyllene oxide, and Isoaromadendrene epoxide.

Keywords: *Ocimum sanctum* essential oil, antibacterial, MBC, Disc diffusion, gaseous contact exposure, Eugenol, Caryophylline oxide, isoaromadendrene

Introduction
Infectious diseases are one of the most important causes of human death, worldwide. Due to the widespread use of antimicrobial agents, many organisms have developed drug resistance to many available antimicrobials. These multidrug resistant organisms are killed hardly by one or two antimicrobials available today and soon it is possible that resistance may develop to these few antimicrobials also. Therefore detection or development of newer antimicrobial agents is needed.

For centuries medicinal plants have been used to treat human diseases. During the last 20 to 30 years advances in photochemistry and identification of plant components have shown that plant components can be used as effective antimicrobial agents. Studies on oils from aromatic and medicinal plants are growing because they are known to have many biological activities such as antibacterial, antifungal, antioxidant and anticancer [1].

Plants belonging to the genus *Ocimum* are one of the important medicinal herbs in the world. Genus *Ocimum* belongs to the family Lamiaceae and is distributed in the tropical and subtropical regions of Asia, Africa, Central America, and South America [2] The chemical composition and antibacterial effects of *Osmium species* essential oils have been reported from different parts of the world [3, 4, 5, 6, 7]. The present work is designed to find the composition, and antibacterial properties of the *O. sanctum* L. species grown in Viluppuram district, Tamilnadu, India.

Many bioassays such as disc-diffusion, macro tube dilution, micro tube dilution, well-diffusion, agar dilution, flow cytometry and bioluminescent assay are available to assess the antibacterial effects. We used disc diffusion and micro tube dilution methods to assess the antibacterial effects of essential oil as these methods are widely used, simple and do not require sophisticated equipment. Apart from these two methods we also tried to find the anti

bacterial effect of *Osmium sanctum L.* Essential oil (OsEO*)* by gaseous contact exposure method.

Materials and methods

Essential oil extraction: The leaves of *O.sanctum* L.were collected and washed in clean water. 120 grams of leaves were hydro distilled with 400 ml of distilled water in a Clevenger-type apparatus for 6 hours. The collected OsEO was separated from the water, dried over anhydrous sodium sulphate and stored in dark at 4^{o}C.

Composition of OsEO

A Shimdzu GC-2010 plus gas chromatograph was equipped with a straight deactivated 2 mm direct injector liner and a 15m Alltech EC-5 column (250 µl I.D., 0.25 µ film thickness). A split injection was used for sample introduction and the split ratio was set to 10:1. The oven temperature program was programmed to start at 35^{0}C, hold for 2 minutes, then ramp at 20^{0}C per minute to 450^{0}C and hold for 5 minutes. The helium carrier gas was set to 2 ml/ minute flow rate (constant flow mode). A direct connection with capillary column metal quadupole mass filter prerod mass spectrometer operating in electron ionization (EI) mode with software GCMS solution ver.2.6 was used for all analyses. Low-resolution mass spectra were acquired at a resolving power of 1000 (20% height definition) and scanning from m/z 25 to m/z 1000 at 0.3 seconds per scan with 0.2 second inter-scan delay. High resolution mass spectra were acquired at a resolving power of 5000 (20% height definition) and scanning the magnet from m/z 65 to m/z 1000 at 1 second per scan. Identification of components of the compound was matching their recorded spectra with the data bank mass spectra of NIST library V 11 provided by the instruments software. GC/MS metabolomics Database was used for the similarity search with retention index. (Figure 1, Table 1)

Antibacterial activity of OsEO

American type culture collection strains of bacteria [*Klebsiella pneumoniae (ATCC 13882), Acinetobacter baumannii (ATCC 19606), Proteus mirabilis (ATCC 43071), Shigella boydii (ATCC 9207), Serratia marcescens (ATCC 14041), Salmonella typhimurium (ATCC 25241), Burkholderia cepacia (ATCC 25416), Enterobacter aerogenes (ATCC 35029) and Haemophilus influenzae (ATCC 19418)]* were purchased from HIMEDIA Pvt. Ltd, Bombay, India (KWIKSTIC). Clinical isolates of bacteria (*Salmonella typhi*, *Vibrio cholerae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus, Methicillin resistant Staphylococcus aureus (MRSA), Coagulase negative Staphylococcus, Enterococcus faecalis,* and *Corynebacterium diphtheriae*) were obtained from the Microbiology department of Mahatma Gandhi Medical College and Research Institute, Puducherry, India. Imipenam(10mcg /disc) vancomycin (30 mcg / disc), Mueller Hinton broth (MHB) base, Dimethyl sulphoxide (DMSO), sterile susceptibility test discs and McFarland standard were purchased from HIMEDIA Pvt Ltd, Mumbai, India. Mueller Hinton agar (MHA) was purchased from MICROEXPRESS, Goa, India. Microtiter plates were purchased from TARSONS, Kolkata, India.

Minimal bactericidal concentration determination by micro tube dilution method: Minimal bactericidal concentration (MBC) is defined as the lowest concentration of antimicrobial agent needed to kill 99.9% of the final inoculum after incubation for 24 hours under a standardized set of conditions described in document M26-A of Clinical and Laboratory Standards Institute (CLSI).[8] 157.5 µl of OsEO was mixed with 17.5 µl of DMSO. Then 525 µl of MHB was added in 30 µl aliquots, with brief vortexing between each addition to get a 22.5% dilution of OsEO [9]. Then two fold dilutions of the OsEO mixture with MHB was carried out in the 96 well micro titer plate.[10] Briefly, 50 µl of MHB was added to well numbers 2 to 8 and 11 to 12 in a 96- well sterile U bottomed micro titer plate. 50 µl of EO mixture was added to each of the 1st and 2 nd wells. The content in 2nd well was mixed and 50 µl transferred to the 3 rd well. Likewise a serial double dilution was carried out up to the 8th well and 50 µl was discarded from the 8th well. 50 µl of 2.5%DMSO diluted in MHB was added to 9th well (diluent control), 100 µl of MHB was added to 10th well (medium control) and 50 µl of EO mixture was added to the 11 th well (test drug control) The final dilution of OsEO in the 1st well was 11.25% (equal volumes of 22.5% OsEO mixture and bacterial suspension). The final dilution in the 2nd, 3rd, 4th, 5th, 6th, 7th, and 8th wells were 5.62%, 2.81%, 1.42%, 0.71%. 0.36%, 0.18%, and 0.09% respectively.

The bacteria to be tested was inoculated on MHA plate and incubated overnight at 37^{o}C. Few colonies of the growth were picked up and mixed with 1 ml of MHB and incubated at 37^{o}C for 2 hours. From this suspension. 100µl was transferred to another tube containing 1 ml of MHB and the density was adjusted to 0.5McFarland with MHB to get 1x10^{8} CFU (colony forming units)/ml suspension. 100 µl of this suspension was transferred to another tube containing 10,000 µl of MHB to get 1x10^{6} CFU/ml suspension.[10] (When 50µl of this bacterial suspension was mixed with 50µl of OsEO dilutions in the microtiter plate wells, the final mixture in the wells had 5x10^{5} CFU/ml). 50 µl of bacterial suspension was added to all the wells except 10th (MHB media control) and 11th (tested drug control) wells. For each bacterium, the experiment was carried out in triplicate. The plates were incubated for 24 hours at 37^{0}C. From each well 10 µl suspension was aspirated and inoculated on MHA plates. The plate was incubated at 37^{0}C for 24 hours and growth or no growth of bacteria was observed and recorded. There should not be growth in 9, 10, and 11th wells (DMSO control, MHB medium control and test drug control, respectively), but 12 th well should have bacterial growth (bacterial growth control). Among wells 1 to 8, the well with least OsEO concentration up to which there was no growth was taken as the MBC. Of the 3 experiments for each bacterium, the highest MBC is takes as the MBC for that particular bacterium. (Table 2).

Disc diffusion method to find the antibacterial effect of OsEO: A sterile swab was dipped into the bacterial suspension adjusted to 0.5McFarland density (as explained above) and the swab was pressed along the sides of the tube to remove excess fluid. The swab was streaked in three directions on MHA plate to get a lawn of bacterial growth [11]. The agar plate was left at room temperature for 15 minutes. Sterile 6mm susceptibility test discs were placed on the agar surface. 15 µl each of undiluted (neat) OsEO, 45% OsEO mixture (45 µl OsEO+ 2.5 µl DMSO + 52.5 µl MHB) and 2.5% DMSO in MHB (diluent control) mixture were dropped on different discs on the agar plate. Vancomycin disc (for Gram positive bacteria) or imipenam disc (for Gram negative bacteria) were placed on the agar plate as positive bacterial growth inhibitors. The plate was incubated for 24 hours at 37^{0}C and the diameters of inhibition of growth around discs were measured with a scale and recorded. Of the 3

experiments for each bacterium, the least diameter of growth inhibition was taken as the diameter of growth inhibition for that particular bacteria (Figure 2, Table 3).

Gaseous contact exposure method to find the antibacterial effect of OsEO

25 µl of neat (undiluted) OsEO was placed on the middle of the inner aspect of the agar plate cover. Bacterial suspension containing $1x10^6$ CFU/ml (as explained above) was inoculated onto MHA plate on three directions, left at room temperature for 15 minutes and then inverted over the petri dish cover containing 25µl of OsEO. The plate was incubated at 37^0C for 24 hrs and the maximum growth inhibition diameters in two directions of the oval growth inhibited area were measured and recorded. Out of 18 bacteria tested growth of 3 bacteria were inhibited by 25 µl of undiluted OsEO. The three bacteria whose growth was inhibited by 25µl of undiluted OsEO were further tested for the dose dependent inhibition of growth with different volumes (5 µl, 10 µl, and 20 µl) of undiluted OsEO. (Figure 2; Table 4).

Results

The extraction yield was calculated as the volume of OsEO (ml) per leaves weight (g) and multiplied by 100. 0.4 ml OsEO was collected from 120 gm of leaves and the yield was 0.33%. Gas Chromatogram-Mass Spectrum (GC-MS) analysis of OsEO revealed the presence of 19 components. The major components in the OsEO were Eugenol, Caryophyllene oxide, and isoaromadendrene epoxide (Figure 1, Table 1)

Table 1: Chemical composition of essential oil from leaves of *Ocimum sanctum* L. by Gas Chromatogram-Mass Spectrum (GC-MS) analysis

Peak	R. time	Name	Area%	Height%
1	7.019	Eugenol	21.89	18.35
2	7.330	Copaene	2.87	3.92
3	9.372	2-Hydroxy-2,4,4-trimethyl-3-(3-methlbuta-1,3-dienyl)cyclohexanone	1.88	2.74
4	9.430	Naphthalene,1,2,3,5,6,8a-hexahydro-4,7-dimethyl -1-(1-methylethyl)-(1s-cis)-	1.76	2.61
5	10.738	Caryophyllene oxide	50.50	43.99
6	11.669	Isoaromadendrene epoxide	8.43	8.66
7	11.787	(-)-Spathulenol	4.29	4.76
8	12.955	1-Ethyl-4,4-dimethyl-cyclohex-2-en-1-ol	0.67	1.04
9,	13.783	cis-7,10,13,16-Docosatetraenoic acid, methyl ester	0.32	0.67
10	14.140	7-Isopropenyl-1,4a-dimethyl-4,4a,5,6,7,8-hexahydro-3H-naphthalen-2-one	0.70	1.03
11	14.300	1,4-Methanoazulen-7(1H)-one,octahydro-4,8,8,9-tetramethyl-,(+)-	2.74	5.72
12	14.541	Methyl dihydroisosteviol	0.58	0.46
13	14.666	Andrographolide	0.49	0.66
14	14.489	Phosphonous dichloride,(1,7,7-trimethylbicyclo[2.2.1]hept-2-yl)-	0.19	0.41
15	15.249	2H-2,4a,Ethanonaphthalene,1,3,4,5,6,7-hexahydro-2,5,5-trimethyl-	0.10	0.24
16	17.800	1-Chloroundecane	0.04	0.12
17	17.981	1-Iodo-2-methylnonane	0.08	0.20
18	23.143	Phytol	2.41	4.27
19	26.014	Citronellyl isobutyrate	0.06	0.15

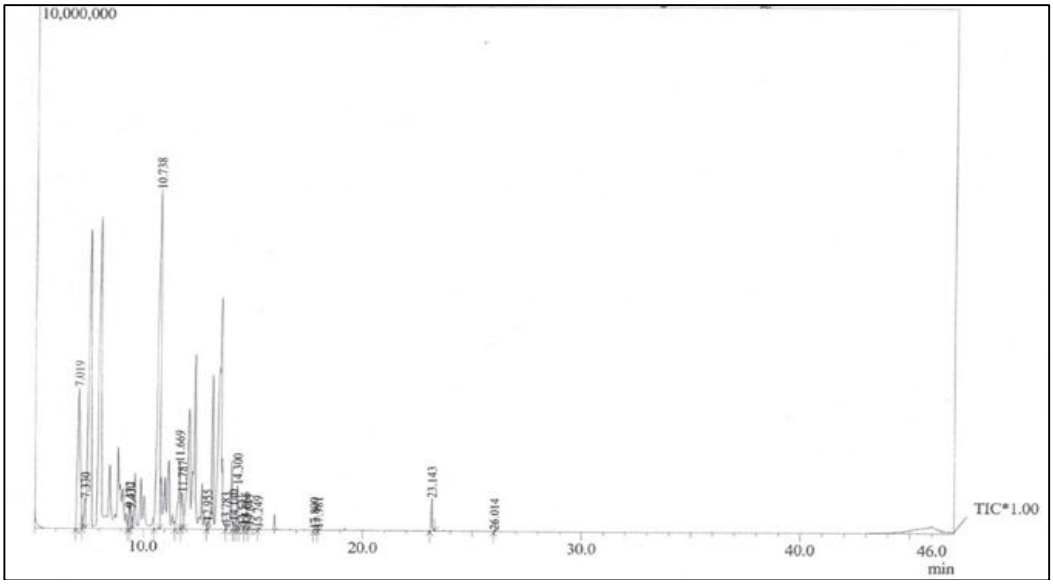

Fig 1: Gas Chromatogram- Mass Spectrometric analysis of OsEO

Minimal bactericidal concentration of OsEO: All the 17 bacteria tested were killed by different concentrations of OsEO (Table 2). *Klebsiella pneumoniae* was killed by 2.81% concentration of OsEO. *Salmonella typhimurium, Burkholderia cepacia, Vibrio cholerae, Salmonella typhi, Escherichia coli, Coagulase negative Staphylococcus*, and *Corynebacterium diphtheriae* were killed by 5.62% OsEO. *Acinetobacter baumannii, Shigella boydii, Serratia marcescens, Proteus mirabilis, Enterobacter aerogenes, Pseudomonas aeruginosa, Staphylococcus aureus, MRSA* and *Enterococcus faecalis* were killed by 11.25% OsEO.

Table 2: Minimal bactericidal concentration (MBC) of *Ocimum sanctum* L. essential oil (OsEO)

Bacteria	Minimal bactericidal concentration	
	Highest bactericidal concentration out of 3 experiments%	Mean ± Standard deviation of 3 experiments%
Klebsiella pneumonia	2.81	2.81± 0
Acinetobacter baumannii	11.25	7.5± 3.25
Proteus mirabilis	11.25	7.5±3.25
Shigella boydii	11.25	7.5± 3.25
Serratia marcescenes	11.25	11.25± 0
Salmonella typhimurium	5.62	5.62± 0
Salmonella typhi	5.62	5.62± 0
Vibrio cholera	5.62	5.62± 0
Escherichia coli	5.62	5.62± 0
Pseudomonas aeruginosa	11.25	7.5± 3.25
Burkholderia cepacia	5.62	5.62± 0
Enterobacter aerogenes	11.25	7.5± 3.25
Staphylococcus aureus	11.25	9.37±3.25
MRSA	11.25	11.25± 0
Coagulase negative Staphylococcus	5.62	5.62± 0
Enterococcus faecalis	11.25	11.25± 0
Corynebacterium diphtheriae	5.62	5.62± 0
Haemophilus influenzae	Not done	Not done

Disc diffusion method to find the bacterial growth inhibition by OsEO (Table 3): Growth of all the 18 bacteria tested was inhibited by undiluted OsEO. 4 bacteria (*Acinetobacter baumannii, Serratia marcescens, Escherichia coli*, and *Pseudomonas aeruginosa*) showed lesser diameter of growth inhibition (from 6.5 to 8 mm). Other 14 bacteria showed growth inhibition diameters from 10 to 14 mm.

(Figure 2, Table 3)

45% concentration of OsEO inhibited the growth 8 bacteria only and the diameter of growth inhibition ranged from 7 to 11 mm. The diameters of growth inhibition by 45% OsEO were lesser than those of the diameters of growth inhibition of the corresponding bacteria with undiluted OsEO.

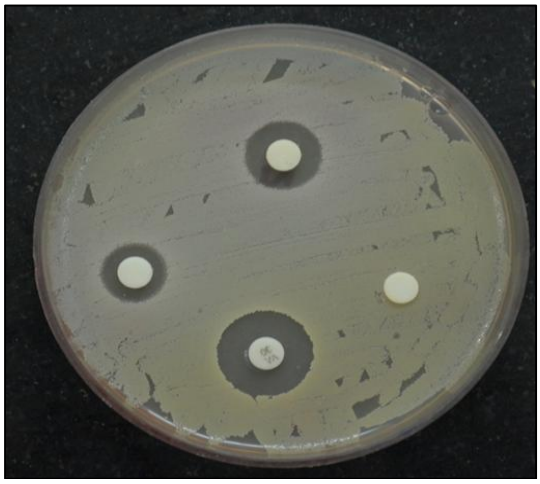

Fig 2: Inhibition of growth of MRSA by *Ocimum sanctum* essential oil (OsEO) by disc diffusion method. Top: 15 µl of undiluted OsEO; Left: 15 µl of 45% diluted OsEO; Right: 15 µl of 2.5% DMSO in MHB (diluent control); Bottom: Vancomycin disc (Positive growth inhibition control).

Table 3: Diameter of growth inhibition by *Ocimum sanctum* L. essential oil (OsEO) by Disc diffusion method

Bacteria	Neat Os EO		45% Os EO	
	Least diameter of 3 experiments- mm	Mean ± Standard deviation of 3 experiments-mm	Least diameter of 3 experiments-mm	Mean ± Standard deviation of 3 experiments-mm
Klebsiella pneumonia	14	14.7±0.6	-	-
Acinetobacter baumannii	6.5	6.5± 0	-	-
Proteus mirabilis	10	10 ± 0	7	7.7±0.6
Shigella boydii	12	13± 1	-	-
Serratia marcescenes	8	8± 0	-	-
Salmonella typhimurium	12	12.3± 0.6	10	10± 0
Salmonella typhi	12	12.7±0.6	-	-
Vibrio cholera	13	13.3±0.6	7	7± 0
Escherichia coli	7	7± 0	-	-
Pseudomonas aeruginosa	6.5	6.5± 0	-	-
Burkholderia cepacia	11	11± 0	9	9± 0
Enterobacter aerogenes	11	11.7±0.6	-	-

Staphylococcus aureus	10	10.7±0.6	7	7.7±0.6
MRSA	13	13± 0	11	11± 0
Coagulase negative *Staphylococcus*	15	15± 0	11	11 ± 0
Enterococcus faecalis	13	13.7± 0.6	-	-
Corynebacterium diphtheriae	10	10.6±0.6	-	-
Haemophilus influenzae	15	15 ± 0	10	10.3±0.6

: No growth inhibition
Mm: Diameter of growth inhibition in millimeter

Gaseous contact exposure method to find the bacterial growth inhibition by OsEO: Of the 18 bacteria tested with 25 µl of undiluted OsEO, 3 bacteria (*MRSA, Staphylococcus aureus, and Haemophilus influenzae*) showed growth inhibition. These 3 bacteria were retested with 5 µl, 10 µl, and 20 µl of undiluted OsEO and all the 3 bacteria showed increase in the area of growth inhibition with increase in the volume of the OsEO. (Figure 3; Table 4)

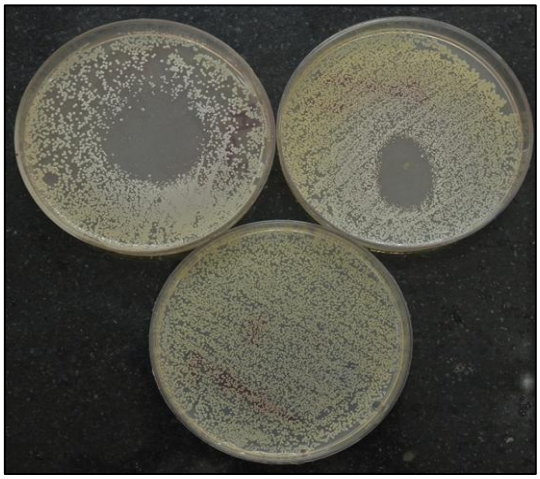

Fig 3: Inhibition of *Staphylococcus aureus* growth by undiluted *Ocimum sanctum* L. essential oil (OsEO) through Gaseous contact exposure method. Left: 20 µl of OsEO; right- 10 µl of OsEO, bottom-5 µl of OsEO

Table 4: Bacterial growth inhibition by undiluted *Ocimum sanctum L.* essential oil (OsEO) through gaseous contact exposure method

Bacteria	**Area of growth inhibition by OsEO [@]-millimeter^2 Mean ± Standard deviation of 3 experiments**		
	5 µl OsEO	**10 µl OsEO**	**20 µl OsEO**
MRSA	-	703±46	1475±65
Staphylococcus aureus	-	407±7	852±12
Haemophilus influenzae	-	32±5	128±7

[@]Area calculated by multiplying the two maximum growth inhibition diameters measured in two directions of the oval growth inhibition area.
-: No growth inhibition

Discussion

Essential oils extracted from plants are a complex mixture of terpenes, sesquiterpenes, oxygenated derivatives and other aromatic compounds. These components are characteristic for basil aroma, which are precursors to the presence of 1, 8-cineole, methylcinnamate, methyl cavicol and linalool. In general these substances are volatile and present in at low concentrations. The chemical composition of essential oils is known to vary with the local climate, harvest period, and environmental conditions [12]. The chemical composition of essential oils of different *Ocimum* species shows a large interspecies variability and, within the same species, it seems to depend on the genetic characteristics of the plant and environmental conditions under which they grow [13]. The differences between the composition and quantity of essential oils from different species of *Ocimum* may be due to the different environmental conditions, genetic factors, different chemo types, and the nutritional status of the plants as well as other factors than can influence the oil compositions [14] GC-MS analysis of the OsEO in the present study revealed the presence of 19 components and the major components of OsEO were Eugenol, Caryophyllene oxide, and isoaromadendrene epoxide.

By micro tube dilution method, the present study finds that all the 17 tested bacteria were killed by different concentrations of OsEO (2.81%, 5.62% and 11.25%). By disc diffusion method, the study finds that the growth of all the 18 bacteria tested were inhibited by undiluted OsEO. Many authors have also reported that the essential oils of *Ocimum* species have antibacterial effect against different bacteria [9, 14, 15, 16, 17]. Joshi has reported that the essential oil of *Ocimum gratissimum* showed significant antimicrobial activity against *E. coli, S. marcescens and K. pneumoniae* and the essential oil of *Ocimum sanctum* showed significant antimicrobial activity against *Staphylococcus epidermidis, Pseudomonas aeruginosa, Aspergillous niger, and Streptococcus faecalis* [15]. Joshi tested the minimum bactericidal concentrations of essential oil of flowering parts of *Ocimum basilicum*. This study reports that more susceptible organisms are *Staphylococcus aureus, Bacillus subtilis, Aspergillous fumigatus, Streptococcus faecalis, Staphylococcus epidermidis, and Penicillium chrysogenum*, moderately susceptible bacteria are *Micrococcus flavus, Micrococcus luteus, Proteus mirabilis, Proteus vulgaris, and Pseudomonas aeruginosa*, while less susceptible bacteria are *Enterobacter aerogenes, Serratia marcescens, Salmonella typhimurium, Escherichia coli, and Klebsiella pneumoniae* [16]. Hanaa Yamani *et al* have reported that the essential oil extracted from *Ocimum tenuiflorum* showed antimicrobial activity against *S. aureus* (including MRSA) and *Escherichia coli*, but less activity against *Psedomonas aeruginosa*.[9] Mbata *et al* have reported that steam distilled and petroleum ether extracted essential oil from *Ocimum gratissimum* showed antibacterial activity against *Listeria monocytogenes* type 4a (food origin).[17] Soumen Saha *et al* have reported the antimicrobial effects of essential oils from five *Ocimum* species by micro dilution assay (Minimal inhibitory concentration) and disc diffusion method. They have reported that the essential oils of *Ocimum basilicum L., Ocimum kilimandscharicum Guerke, and Ocimum gratissimum L.* strongly inhibited the growth of *Bacillus subtilis, Micrococcus luteus, Pseudomonas aeruginosa, Shigella dysentriae, Escherichia coli, Vibrio cholerae, and Shigella Flexneri* [14]

Traditionally many plant extracts are used as an inhalant medicine to treat respiratory illnesses. In the present study, the gaseous contact exposure method showed that the

undiluted OsEO inhibited the growth of 3 (*MRSA, Staphylococcus aureus, and Haemophilus influenzae*,) out of 18 bacteria tested and the inhibition of growth was dose dependent. Mazrulla *et al* have reported that out of 133 essential oil vapors screened, 105 essential oil vapors had antibacterial activity and Gram positive bacteria were more susceptible than Gram negative bacteria [18]. Shigeharu Inouye *et al* have evaluated the antibacterial activity of 14 essential oils and their major constituents in the gaseous state against four bacteria. For most essential oils examined, *Haemophilus influenzae* was most susceptible, followed by, *Streptococcus pneumoniae*, and *Streptococcus pyogenes*, and then *Staphylococcus aureus* [19]

Conclusion

The essential oils from plants are complex mixtures of a wide diversity of components and their antimicrobial activity is therefore related to their composition, amount and their possible interaction. The interaction between different components can be additive or synergetic or antagonistic. The GC-MS analysis of the *O. sanctum* used in the present study revealed the presence of 19 Chemicals and the major components were Eugenol, Caryophyllene oxide, and isoaromadendrene epoxide. Other studies have reported the GC-MS analysis of few *Ocimum species and Ocimum sanctum*. There are more variables with respect to the nature of chemicals and their quantity in the different studies even within the same species.

In the present study, OsEO showed antibacterial effects on both gram positive and Gram negative bacteria by MBC method and Disc diffusion method. Furthermore the growth of 3 bacteria (*MRSA, Staphylococcus aureus,* and *Haemophilus influenzae)* was also inhibited by gaseous exposure method in a dose dependent manner and it implies that OsEO should be investigated for its use as an inhalant medicine to treat respiratory tract infections.

It is very difficult to attribute the antibacterial effect of a total essential oil to one or few active principles in the essential oil. Apart from the major components, other minor components in the essential oil may also significantly contribute to the antibacterial activity. Furthermore, the mechanisms of action of each phenolic compound against various bacteria are also very complicated [20]. Mallappa Kumara Swamy *et al* in their updated review on antimicrobial properties of plant essential oils have stated that the reactivity of essential oils depends upon the nature of their functional groups and orientation [21].

Therefore, it is essential to investigate and understand the relationship between antibacterial activity and chemical structure of each component in the essential oil. The ultimate goal of these research activities should be extended to find the individual antibacterial chemical or chemicals in the essential oil and use them for human or animal use. Further research is needed to find the active chemical components of the OsEO and their mechanisms of action and it should be taken to the level of animal experiments and ultimately to human clinical trials.

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