BVG Trust

J. Sci. Trans. Environ. Technov. 2015, 9(1) : 1-4

Scientific Transactions in Environment and Technovation

Antimicrobial activity of non-volatile essential oils of certain medicinal plants against some enteric bacterial pathogens

https://doi.org/10.20894/STET.116.009.001.001
www.stetjournals.com

Sumit Kumar, Sangeeta Saini* and R.C. Dubey

Gurukul Kangri Vishwavidyalaya, Haridwar (U.K), India - 249 404.
*Indian Herbs Specialities Pvt. Ltd., Sharda Nagar,Saharanpur - 247 001.

Abstract

Antibacterial efficacy of essential oils (EOs) of *Ocimum sanctum*, *Mentha arvensis*, *Citrus lemon* and *Citrus maxima* were tested against some enteric pathogens viz., *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Bacillus subtilis* by filter paper disk-diffusion and tube-dilution methods. The essential oil of *O. sanctum* resulted in more inhibitory antibacterial activity against all enteropathogens than that of *M.arvensis* and *Citrus* spp. The minimum inhibitory concentration (MIC) of the non-volatile oil of the *O. sanctum*, *M. arvensis*, *C. maxima* and *C. lemon*was 600 ppm against all the enteric pathogens.

Keyword : Essential oils, antibacterial properties, enteric pathogens

INTRODUCTION

Enteric bacteria cause anumber of gastrointestinal disorders like diarrhoea, dysentery, typhoid fever, food poisoning, etc. where, diarrhoeal disease is a major concern of global public health. According to the World Health Organization (WHO) ~80% of the world's population relies on traditional medicines due to better cultural acceptability, least side effects and better compatibility with the human body (Pawar and Arumugam., 2011).Since, most human bacterial pathogens have developed antibiotic resistance therefore alternative to antibiotic therapy is theneed of the hours. The most ancient script Ayurvedadescribesa large reservoir of essential oil-bearing plants.Plant oils and extracts have been used for a wide variety of purposes for thousands of years. Essential oils are important, because they entail special premise for use as fumigant. Depending on their nature essential oils may have different effect such as analgesic, antispasmodic, hyperemic, antimicrobial, and antiviral or anti-helminthes effect (Leung, 1980). Plant essential oils (PEOs) and their constituents have attracted much interest because of their widespread use in cosmetics,as the principal antimicrobial in a variety of sanitary, pharmaceutical products, and food preservatives and additives (Averbeck and Idaomar, 2008).

Higher plants contain a wide spectrum of secondary metabolites such as phenol, flavonoids, tanins, quinones, essential oils, alkaloids, saponins and steroids. Antimicrobials of plant origin have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating side effects associated with synthetic antimicrobials (Iwu *et al.*, 1999). *O. sanctum*, *M. arvensis*,*C.lemon*and *C. maxima* are the medicinal plant commonly used in various oilments. In the present study an attempt was made to investigate the antimicrobial properties of non-volatile essential oil of *O. sanctum*, *M. arvensis*, *C. lemon* and *C. maxima* on someenteric bacterial pathogens.

*Corresponding Author :
email: sumitarya360@gmail.com

MATERIAL AND METHODS

Bacterial strains

Pure cultures of enteropathogenicbacteria used in this study were *Escherichia coli* (MTCC1687), *Staphylococcus aureus* (MTCC737) *Pseudomonas aeruginosa*(MTCC1688), *Bacillus subtilis* (MTCC441) and *Salmonella typhi* (MTCC1251) obtained from IMTECH (Chandigarh) and maintained in laboratory for further study.

Culture Media

Nutrient broth, nutrient agar andsoya casein digest agar media used in the study were prepared according to the manufacture's (HiMedia Laboratories) instructions.

Extraction of oils: Different plants parts were collected locally and brought to the laboratory. Essential oils were separately obtained by hydro-distillation method using Clevenger'sapparatus (Guenther, 1949). Leaves of *O.sanctum, M. arvensis*, and fruitepicarp of *C.lemon* and *C. maxima* were collected and put in the vessel of Clevenger's apparatus with water in a ratio of 1:2(w/v). The essential oils were separately dried over anhydrous sodium sulphate and kept at 4-5°C for further use.Different concentrations of essential oils were separately prepared by using sterile dimethyl-sulphoxide(DMSO). This solvent does not have any antimicrobial effect. The antibacterial properties of essential oil were tested by performing the following two methods.

Determination of minimum inhibitory concentration of essentials oils by tube dilution method: MIC of the essential oils was determined following the tube–dilution method by using nutrient broth medium (Srivastava, 1984). A loopful culture of three bacteria

Table 1.Effect of essential oil of *O.sanctum*, *M.arvensis*,*C.lemon* and*C.maxima* radial growth of different bacteria *in vitro* by disc diffusion method.

Oil concentration (ppm)			**Growth inhibition (%)**		
O.sanctum	*B.subtilis*	*E.coli*	*P.aeruginosa*	*S.aureus*	*S.typhi*
600	17.9	27.0	35.2	34.6	30.4
700	20.3	36.0	40.6	44.9	34.4
800	25.4	44.5	46.7	48.7	38.1
900	28.5	50.5	52.0	51.7	47.8
1000	34.7	57.0	62.2	53.0	65.2
M.arvensis					
500	0.0	0.0	0.0	0.0	30.0
600	23.0	30.3	34.4	29.2	38.5
780	29.5	35.4	41.8	38.4	44.4
800	34.1	41.7	48.3	45.6	55.1
900	39.5	47.6	56.1	59.3	63.7
1000	48.8	63.8	67.6	67.3	70.0
C.lemon					
00	23.8	0.0	31.1	25.1	25.9
700	30.3	29.5	36.0	33.1	29.6
800	35.2	34.6	42.2	40.7	34.8
900	38.7	39.7	47.1	47.5	39.2
1000	44.5	44.1	50.2	54.3	44.4
1100	50.6	49.6	NT	NT	NT
C.maxima					
400	0.0	0.0	0.0	0.0	0.0
500	19.5	0.0	25.4	0.0	0.0
600	26.3	41.4	31.1	28.1	25.2
700	32.0	48.4	37.3	34.6	30.4
800	33.9	55.9	43.0	39.5	35.2
900	45.5	60.6	48.7	44.9	44.8
1000	52.0	64.2	57.4	52.5	53.3

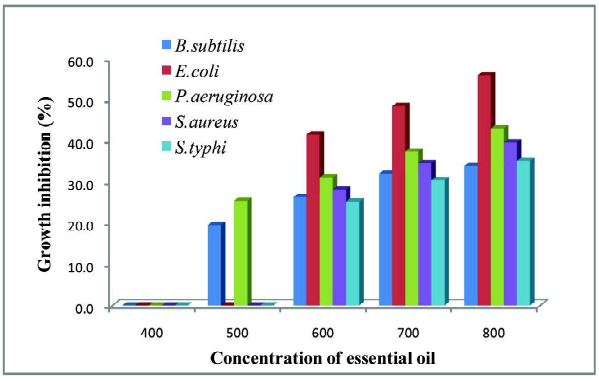

Fig.1. Effect of essential oil of *O. sanctum* on radial growth of pathogenic bacteria.

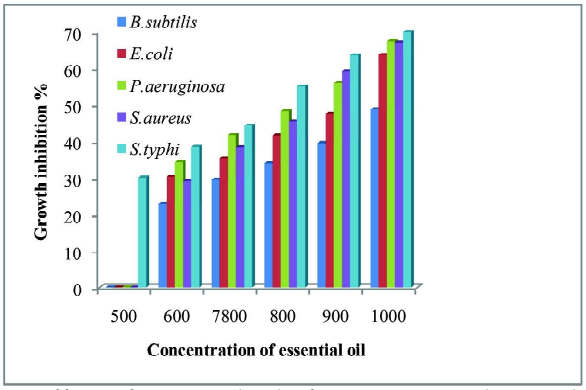

Fig.2. Effect of essential oil of *M. arvensis* oil on radial growth of pathogenic bacteria.

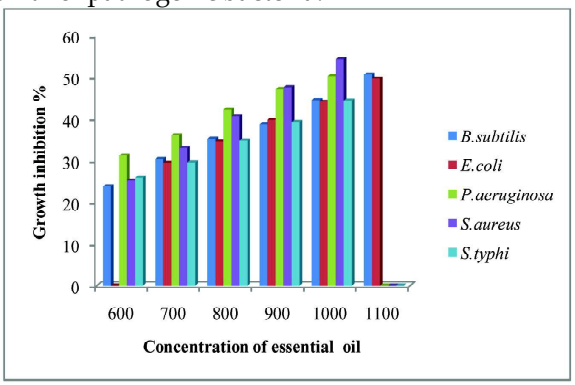

Fig.3. Effect of *C. lemon* oil on radial growth of pathogenic bacteria.

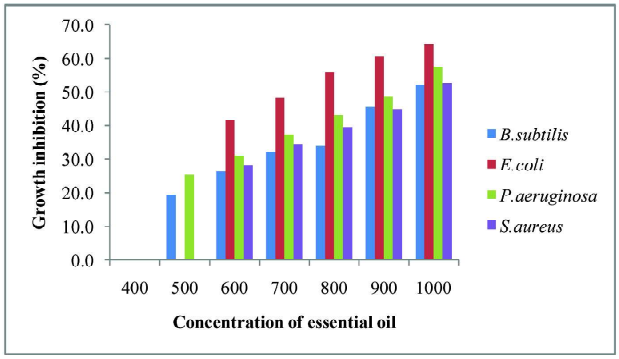

Fig.4. Effect of essential oil of *Citrus maxima* on radial growth of pathogenic bacteria.

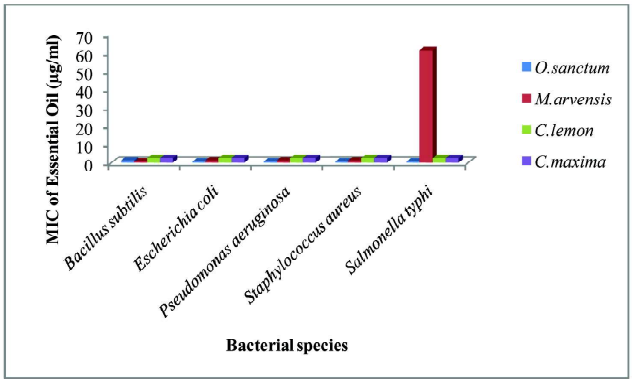

Fig.5.Minimum inhibitory concentration of essential oils of *O. sanctum*, *M. arvensis*, *C. lemon* and *C. maxima* on growth of pathogenic bacteria by tube dilutionmethod.

Table 2: Minimum inhibitory concentration of essential oils of *O. sanctum*, *M.arvensis*, *C. lemon* and *C. maxima* on growth of some bacteria by tube dilution method.

Organisms	Essential oils MIC (ppm)			
	O.sanctum	*M.arvensis*	*C.lemon*	*C.maxima*
Bacillus subtilis	1.25	0.625	2.5	2.5
Escherichia coli	0.625	1.25	2.5	2.5
Pseudomonas aeruginosa	0.625	1.25	2.5	2.5
Staphylococcus aureus	0.625	1.25	2.5	2.5
Salmonella typhi	0.625	61.25	2.5	2.5

was separately inoculated in nutrient broth and kept at 37°C for 24 hours. Fresh nutrient broth (20ml) was seeded with 0.25 ml of 24 hour old culture broth. Known amount of essential oil of each plant was separately dissolved in DMSO to obtain a known concentration of each oil followed by serial dilution of oil to get the lowest concentration. A set of tubes containing only seeded broth was kept as control. The bacterial species were incubated at 37°C for 18-48 hours.The last tube with no visible growth of bacteria was considered as MIC (mg/ml) of essential oils.

Filter Paper Disk Diffusion Method: Filter paper disk-diffusion method (Vincent and Vincent, 1944) was followed to measure the antibiotic sensitivity of essential oils against the test pathogens. The inoculum of test bacteria was prepared. A loopful culture from the stock was transferred in sterile broth and incubated at 37°C for 24 hours. Thereafter, 20 ml sterilised base agar was poured aseptically in sterile Petri dishes and allowed to set uniformly. Then 0.2 ml of old broth (fresh 5 ml) was spread uniformly in each Petri dish. Sterile filter paper discs (Whatman filter paper No. 44, 6mmdiam) thoroughly moistened with the oil of different concentrations were placed on the seeded agar plates.

A control was set simultaneously by placing a blank disc moistened with DMSO seeded agar medium. The inhibitory effect of the oil was noted against test bacteria after proper incubation of each microorganism. The diameter of the zone of inhibition was measured with the help of the divider. The zones of inhibition were compared with standard drug of under identical condition. All the tests were conducted in triplicates and the average of six reading was recorded. Growth inhibition was recorded by using the following formula

% inhibition =

$$\frac{\text{Zone of inhibition in test plates}}{\text{Zone of inhibition in standard plates}} \times 100$$

RESULTS

Antimicrobial Activity of *Ocimum sanctum* oils: Growth of *B. subtilis*, *E. coli*, *P. aeruginosa*,*S. aureus* and *S.typhi* was inhibited by oils of *Ocimum* spp at different concentrations (Fig.1,Table 1). *O. sanctum* oil at600 ppm caused minimum inhibition of *B.subtilis* and maximum of *P.aeruginosa*. *O. sanctum* oilat 1000 ppm was highly effective on *S.typhi*but not on *B. subtilis*. At this concentration colony growth of*E. coli*, *P.aeruginosa* and *S.aureus* was inhibited more than 50% followed by *B.pumilus* (45.5%).

Antimicrobial Activity of *Menthaarvensis* oil: The radial growth of test bacteria was inhibited at different concentration of *M.arvensis* oil. *M. arvensis* oilat 500 ppm was effective only against *S.typhi* (30%) but not the other bacteria. Growth of all bacteria was inhibited at 600 ppm. Growth of *B.subtilis* was inhibited by 23%,*S. aureus*by 29.2%, *E. coli*by 30.3%, and *P.aeruginosa*by34.4% at this concentration. However, the rate of inhibition increased with increasing the oil concentration. The oil posed the maximum inhibition of *S.typhi*(70.0%) followed by *P.aeruginosa*(67.6%)*S.aureus*(67.3%) and *E. coli*(63.8%).*B.subtilis* 48.8% showed < 50% inhibition in comparison to the inhibition caused by ciprofloxacin (Fig.2).

Antimicrobial Activity of *Citrus lemon* oil: Bacterial growth inhibition caused by *Citrus lemon* oil revealed a good antibacterial property against pathogenic bacteria. As compared tociprofloxacin, growth inhibition of *B.subtilis*(23.8%)*P.aeruginosa*(31.1%)*S.aureus*(25.1%) and *S.typhi*(25.9%)by *C. lemon* oil was recorded at 600 ppm significantly. But *E. coli* had no inhibition at 600 ppm. At higher concentration, *C. lemon* oil showed toxicity by causing inhibition>50%, *E. coli* showed the maximum inhibition by 64.2 %(Fig.3).

Antimicrobial Activity of *Citrus maxima* oil: Growth of different bacteria was effectively inhibited at different concentrations of*C. maxima* oil. At 1000 ppm concentration growth of was declined of *B.subtilis*, *E. coli*, *P.aeruginosa*, *S.aureus* and *S.typhi*by 52.0, 64.2, 57.4, 52.5 and 53.3%, respectively. *E. coli* showed the maximum inhibition by 64.2%. (Fig. 4).

Minimum Inhibitory Concentration (MIC) of Essential Oils: MIC of *O. sanctum* and *M.arvensis* oil was 600 ppm against all tested bacteria except *Salmonella typhi* (500 ppm). MIC of *C. lemon* oil was 600 ppm against *B. Subtilis*, *S.aureus*, *P.aeruginosa* and *S.typhi*. MIC of *C. maxima* oil was 500 ppm against *B.subtilis*, *P.aeruginosa*, and 600 ppm against *E. coli, S.aureusand S.typhi.(Table 2)*

MIC of *O. sanctum* oil ranged from 0.625 to 1.25 mg/ml by using tube dilution method.These values were much higher than the MIC values of other essential oils. The MIC values of *M.arvensis*oil ranged from 0.625 to 2.5 mg/

ml. MIC values of both *C. lemon* and *C. maxima* were 2.5 mg/ml for all test bacteria.

DISCUSSION

In the present investigation inhibitory properties of the essential oils of *O. sanctum*, *M.arvensis*, *C. lemon* and *C. maxima* was recorded against five test bacteria. Essential oils were most effective due to the presence of several compounds. Shukla *et al*. (2000) found the prominent antibacterial activity of *Oenotherabiennis* and its constituent against *E. coli*, *Klebsiella pneumonia* (MTCC109), *S. mutans* and *S.epidermidis*.We have found that essential oil of *O. sanctum*had the maximum antibacterial activity than the other essential oils viz., *M. arvensis*, *C. lemon* and *C. maxima*. Growth of different bacteria was inhibited by *O. sanctum* oil forming a wider halo zone around wells containing aliquot of essential oil. Effect of *O. sanctum* oil on growth of pathogenic bacteria (Fig.1) is in conformitywith that of Prasad *et al*. (1994) who studied the antimicrobial activity of *O. sanctum* against Gram-positive and Gram-negative bacteria and fungi. Khan *et al*. (2015) also evaluated the antibacterial activity of essential oil of *O. sanctum*.

Essential oil of *M.arvensis* inhibited the growth of pathogenic bacteria at minimum concentration. Rasooli and Rezaei(2002) also reported the antibacterial effect of essential oil of *M.longifolia* and *Zatariamultiflora* against *E. coli*, *Staph. aureus* by disc diffusion method. Iscan *et al*. (2002) found the antimicrobial activity essential oils of *Menthapiperita* against 21 human and plant pathogenic microorganisms. The peppermint oil moderately inhibited human pathogens.

Essential oils of *C. lemon* and *C. maxima* had better antibacterial properties than control. These works are similar to those reported by Delia and Lourds(1994) who found inhibitory effecton growth of *S.aureus*, *E. coli*, *P.aeruginosa*, *Bacillus* sp. and *Trichophyton rubrum*. Rasooli and Rezaei (2002) reported the MIC and MBC of *Zatariamult Iflora* and *Mentha longifolia* essential oil against *E. coli* and *S.aureus*.Borah *et al*.(2013) also reported the antibacterial activity of *C. maxima*on *E. coli* and *P.aeruginosa.* It may be concluded that the essential oils of *M. arvensis*, *C. lemon* and *C. maxima* possess the significant antibacterial activity as compared to ciprofloxacin antibiotic. The inhibitory activity recorded as above proved its utility in pharmaceutical industry and therapeutically useful against disease caused by the human pathogens.

REFERENCES

Al Abbasy, D. W., Pathare, N., Al-Sabahi, J. N., and Khan, S. A. (2015). Chemical composition and antibacterial activity of essential oil isolated from Omani basil (*Ocimumbasilicum* Linn.). *Asian Pacific J. of Trop. Dise.*, 5(8): 645-649.

Bakkali, F., Averbeck, S., Averbeck, D., and Idaomar, M. (2008). Biological effects of essential oils–a rev. *Food and Chem. Toxicol.,* 46(2): 446-475.

Borah, M., Das, S., and Ahmed, S. (2013). Antibacterial activity of the ethanolic extract of leaves of *Citrus maxima* (Burm.)Merr.on*Escherichia coli* and *Pseudomonas aeruginosa*. *Asian J. Pharm. Clin. Res.,* 6: 4.

Delia, CO., Lourdes, L.D.(1994). *In vitro* antibacterial activity of rind essential oil of *Citrus grandis*.Abst.8th Asian Symp. Med. PL, Spices Nat Prod, 12-16th June. Melaka, Malaysia. Abstr, No. CPIOD.

Guenther, E.(1949).The essential oils: Individual oils of the plant families Rutaceae and Labiatae*D. Nostrand. Princeton.N.J.,*3: 619-34.

Iscan, G., Kirimer, N., Kurkcuogly, M., Bser, K.H.C. and Demirci, F.(2002).Antimicrobial screening of *Menthapiperita* essential oils.*J.Agri. Food Chemi*., 50(15): 3943-3946.

Iwu, M. W., Duncan, A. R.and Okunji, C. O. (1999).New antimicrobials of plant origin. *Perspectives on New Crops and New Uses. ASHS Press, Alexandria,* VA: 457-462.

Leung, A.Y. (1980). *Encyclopedia of Common Natural Ingredients.* Used in Food Drungs and cosmetics, John. *Wiley and Sons Inc*. New York.

Pawar, N.K. and Arumugam, N. (2011). Leaf extract of *Centratherumpunctatum* exhibits antimicrobial, antioxidant and anti-proliferative properties. *Asian J. Pharm. Clin. Res*., 4(3): 71-76.

Prasad, Y.R., Alankararora, G.S.J.G. and Baby, P.(1994).*In vitro* antimicrobial screening of indigenous essential oil part –I*Ocimum* spp. *J. Sci. Res*., 9(1): 7-9.

Rasooli, I. and Rezaei, M.B. (2002) Bioactivity and chemical properties of essential oils from *Zatariamultiflora*Boiss and *Mentalongifolia* (L.)Huds.*J.EssentialOil Res*., 14(2): 141-146.

Shukla, N., Srivastava, A., Kumar, S.T.R., Khanuja,S.P.S. and Kumar,S.(2001) Antibacterial activity of *Oenotherabiennis* and one of its constituent. *Indian Drug*, 37(1): 60-61.

Srivastva, O.P.(1984). Techniques for the evaluation of antimicrobial properties of natural of products.The use of pharmacological techniques for the evalution of natural product. UNESCO: 72-79.

Vincent,J.G., Vincent, H. W. and Morton,J.(1944). Filter paper disc modification of the Oxford cup penicillin determination. *Exp. Biol. and Medi*.,55(3): 162-164.