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EVALUATION OF ANTIMICROBIAL EFFECT OF FORMULATED ESSENTIAL OIL BY DIRECT CONTACT AND VAPOUR PHASE METHOD

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ABSTRACT

Medicinal plants and their essential oils have been used for centuries in traditional system of medicine and they have been appreciated for multiple effects against a variety of infections. Bacterial infections and their complications are presently regarded as the most difficult and expensive to treat. Though the discovery of antibiotics has proven to be a major asset against bacteria, the extensive use of antibiotics since recent days has led to the emerging increase in resistant strains of bacteria. Therefore the present millennium is in search of safe and natural drugs to tackle these common pathogens and to prevent the optimum increase in mortality rates due to multi drug resistant bacteria. The purpose of this research was to estimate the antibacterial effect of the formulated essential oil against common and selected pathogens *E.coli*, *Proteus mirabilis*, *Staphylococcus aureus* and *Vibrio cholera* by means of Disc diffusion assay and Agar vapour assay. The zone of inhibition was compared in both states of direct contact and vapour phase methods at the concentrations of 10, 15, 20 and 25 micro litre/ml against the selected pathogens. The results were statistically analysed by paired T test and P Value <0.05 for *Proteus mirabilis*, *Staphylococcus aureus* and *Vibrio cholera* indicated that the formulated essential oil showed antimicrobial activity against both gram positive and gram negative bacteria. The formulation also portrayed considerable antimicrobial effect at vapour phase (25 micro litre/ml) than by direct contact method.

KEYWORDS: Antimicrobial Action, Inhalation Therapy, *Vedhu*, *Siddha*, Essential Oil, Herbal Medicine.

INTRODUCTION

Infections are as old as mankind. Worldwide there is an emerging challenge due to the increase in the infectious diseases and it has become the most important cause of death contributing to approximately one half of all deaths in tropical countries.[1] The discovery of antibiotics in medical fraternity and their extensive use as chemotherapeutic agents led to the belief of eventual eradication of infectious diseases. However in contrary overuse of antibiotics for the treatment of simple self resolving infections and prophylaxis has become the multifaceted problem of the emergence of multi drug resistant strains of several groups of organisms.[2] Rapid evolution of antibiotic resistance, increased side effects of antibiotics, loss of beneficial bacteria in antibiotic therapy continues to threaten the treatment of bacterial infections and requires the development of new alternative strategies.

Siddha system is a treasure house of herbal science with its boundless therapeutics. The *Siddha* Materia Medica ensures promising natural antimicrobials with incredible results. This system of medicine owes a perfect lifestyle to mankind through a variety of its internal and external therapeutics. Among the 64 varieties of therapeutics explained in *Siddha* system "*Vedhu*" (Inhalation therapy) is one among the external therapy indicated for common respiratory ailments and wound healing. *Vedhu* is an external therapy and facilitate perspiration through which the relief from ailments is obtained. According to literature, the essential oil or essential oil containing aromatic herbs will be mixed with water and boiled and the vapors are to be inhaled. *Vedhu* also involves a method of igniting threads made of a cloth coated with herbal powder. The present study was aimed to compare the antimicrobial effects of the formulated essential oil by direct contact and vapour phase method and the results were tabulated and discussed.[3]

MATERIALS AND METHODS

Preparation of essential oil formulation: Eucalyptus oil was collected from small factory in Nilgiris by double distillation of Eucalyptus leaves. All other essential oils for the preparation of the formulation were collected from local market of Chennai. The antimicrobial study was conducted at Gloris Biomed Research centre, Chennai during the year 2008.The ingredients of formulated essential oil are as follows

Eucalyptus oil -100 ml
Menthol - 10 gm
Thymol - 10 gm

Camphor - 10 gm

Clove oil - 20 ml

Bacterial culture

The standard pathogenic bacterial cultures of *E.coli*, *Proteus mirabilis*, *Staphylococcus aureus* and *Vibrio cholera* were procured from stock culture of Christian medical college, Vellore and used in the present study. The bacteria were rejuvenated in Muller Hinton Broth (Himedia laboratories, Mumbai, India) at 37^0c for 18 hours and stocked at 4^0C in Muller Hinton Agar. The pathogenic bacterial culture was inoculated into sterile nutrient broth and incubated until the turbidity of 0.5 Mcfarland unit is attained. Research work done at Glories Biomed Research Centre (p) Ltd. Saligramam Chennai.

Disc diffusion assay

The disc diffusion assay was carried out by blotting 10, 15, 20, 25 mic litre/ml on 4 different paper disc followed by air drying at room temperature for 10 minutes. One plain disc for placebo has been used. All The 5 disc was placed at 5 places on the double layered agar medium in petri dish, sealed with vinyl tape and incubated at 32 degree Celsius for 24 hours. An inhibitory diameter was measured by means of a slide calliper. All the assays were done in triplicate and the mean values and standard deviate on SD were recorded.[4]

Agar vapour assay

The vapour activity of essential oils was determined according to the method adopted by Maruzzella et al.[5,6] and Kienholz[7] in 1959, using the inverted petridish technique.The technique in which a volatile compound placed in a cup or a paper disc was exposed to the inverted agar medium plate inoculated with test test strains at about 5mm distance was convenient and has been used by subsequent researchers.[8,9]

Five different paper disc containing 10, 15, 20, 25 micro litre of the test sample was fixed using a stapler at the centre of 5 filter paper each (75mm in diameter), which as placed inside the upper lid of separate dishes. Droplets of water were put around the perimeter of the filter paper to fix the paper on the lid. The Petri dish containing the double layered agar medium was inverted and placed on the upper lid containing filter paper. The surface of the disc was at a distance of about 4mm from the growth surface of the test organism. The dish was sealed with vinyl tape and was incubated as described above, and the diameter of inhibition zone was measured.

RESULTS

The diameter of inhibition zone was measured and tabulated for the selected strains of bacteria by Agar diffusion assay as well as Agar vapour assay.

Table. 1: Antibiotic activity of formulated oil against *Vibrio cholera.*

Concentration of formulated essential oil (micro litre/ml)	Measurement of Inhibitory zones of bacterial growth	
	Disc diffusion assay(mm)	Agar vapour assay(mm)
10	14	38
15	17	40
20	20	46
25	23	50
Mean	18.50	43.50
SD	3.87	5.51
P Value(Paired T test)	0.0001**	

Results were analysed using Paired T test, P Value <0.05 **Extremely statistically significant.

Table. 2: Antibiotic activity of formulated oil against *Staphylococcus aureus.*

Concentration of formulated essential oil (micro litre/ml)	Measurement of Inhibitory zones of bacterial growth	
	Disc diffusion assay	Agar vapour assay
10	15mm	46mm
15	18mm	58mm
20	20mm	65mm
25	23mm	70mm
Mean	19.00	59.75
SD	3.37	10.40
P Value(Paired T test)	0.0014**	

Results were analysed using Paired T test, P Value <0.05 * Extremely statistically significant.

Table. 3: Antibiotic activity of formulated oil against *Proteus mirabilis.*

Concentration of formulated essential oil (micro litre/ml)	Measurement of Inhibitory zones of bacterial growth	
	Disc diffusion assay	Agar vapour assay
10	8mm	42mm
15	11mm	53mm
20	13mm	55mm
25	16mm	60mm
Mean	12.00	52.50
SD	3.37	7.59
P Value(Paired T test)	0.0004**	

Results were analysed using Paired T test, P Value <0.05 **Extremely statistically significant.

Table. 4: Antibiotic activity of formulated oil against *Escherichia coli.*

Concentration of formulated essential oil (micro litre/ml)	Measurement of Inhibitory zones of bacterial growth	
	Disc diffusion assay	Agar vapour assay
10	21mm	25mm
15	25mm	30mm
20	30mm	55mm
25	33mm	65mm
Mean	27.25	43.75
SD	5.32	19.31
P Value(Paired T test)	0.1020	

Results were analysed using Paired T test, P Value > 0.05 Not statistically significant.

DISCUSSION

The multiplication of drug resistant pathogens is currently thought to be one of the most alarming threats to successful treatment of microbial diseases. Since age old times, essential oils from aromatic plants have provoked interest as sources of natural products to combat a multitude of microorganisms.[10] The antimicrobial activity of essential oil against viruses, mycoplasma and chlamydia, bacteria, fungi protozoan and harmful insects such as mites confers due to its activity of cell wall damage, cellular lyses and leakage of cell contents.[11,12] In medical practice, inhalation therapy of essential oils is being used to treat acute and chronic bronchitis and acute sinusitis and is reported to maintain the ventilation and drainage of the sinuses[13] and had an anti-inflammatory effect on the trachea[14] and to reduce asthma.[15]

In this study antimicrobial activity of the formulated essential oil both by direct contact and vapor phase was assessed qualitatively by the presence or absence of zone of inhibition. Table 1 to 4 compares the growth – inhibitory diameters of formulated essential oils against *Vibrio cholera, Staphylococcus aureus, Proteus mirabilis*, *Escherichia coli* by agar diffusion assay and agar vapour assay. The effectiveness of the essential oils was calculated by measured the diameter (in mm) of the zone of microorganism growth inhibition above the disc. The results reveal that the essential oil formulation showed antibacterial activity with higher zone of inhibition in Agar vapour assay method, when compared with direct contact by Disc diffusion method.

The zone of inhibition of the formulated essential oil against *Vibrio cholera, Staphylococcus aureus, Proteus mirabilis* and E.coli, at the highest tested concentration of 25 micro litre/ml was found to be 50mm, 70mm, 60mm and 65mm respectively. The results were analysed

statistically using paired T-test to compare the statistical difference between Agar vapour Assay and Disc diffusion assay. The results show that the formulated essential oil show extremely significant zone of inhibition against *Vibrio cholera, Staphylococcus aureus, Proteus mirabilis* (Table1-4) with P Value <0.05. Though the gram negative pathogen E.Coli showed a zone of inhibition (65mm) at 25 micro litre/ml, the difference was not found to be statistically significant.

Various essential oils have been evaluated for their impending use as natural antibiotics to have sensitivity against antibiotic resistant pathogens such as Methicillin resistant *Staphylococcus aureus* (MRSA), Penicillin resistant *Staphylococcus pneumonia* (PRSP), Vancomycin resistant *Enterococcus foecium* (VRE) flucanozole and itraconazole resistant candida.[16,17] The formulated essential oil used in this study was more effective against Staphylococcus aureus at a concentration of 25 micro litre/ml which is generally considered to be gram-positive bacteria.

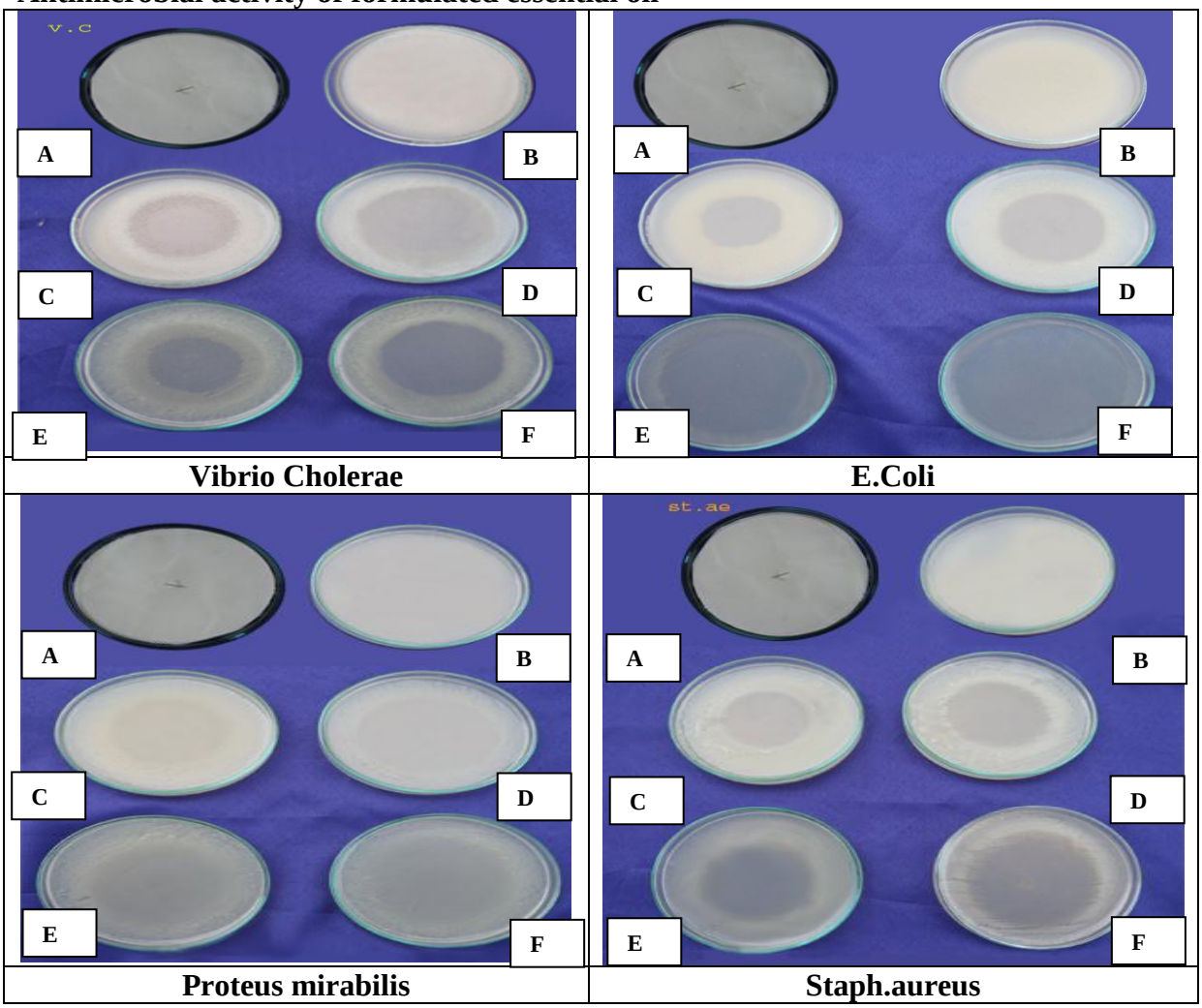
Generally gram-positive bacteria have a hydrophobic peptidoglycan cell wall that can allow the phenolic compounds of essential oil to penetrate and act on the cell wall and within the cytoplasm. The cell wall of gram negative bacteria has a thick peptidoglycan layer of 2-3nm thick and therefore resistant to essential oils when compared to gram-positive bacteria. The phenolic compounds present in essential oil show antibacterial effect against gram positive bacteria which at lower concentration interfere with energy production and denatures the protein at higher concentration. Though the formulated essential oil showed the highest zone of inhibition against gram positive bacteria *Staphylococcus aureus* (Mean-59.75), it has also shown significant inhibitory zones against the gram negative pathogens *Vibrio cholera* and *Proteus mirabilis*. Hence the study results indicate the efficacy of formulated essential oil against both gram positive and gram negative strains of bacteria.[18,19]

Agar diffusion testing developed in 1940, is the official method used in many clinical microbiology laboratories for routine antimicrobial susceptibility testing. Chao et al evaluated the antimicrobial activity of 45 essential oils on 8 bacteria and 3 fungi using the agar diffusion assay.[20,21] However kubo et al stated that the diffusion method was inappropriate for essential oils, as poorly soluble compounds did not diffuse well in the agar medium.[22] Griffin et al[23] mentioned that water soluble components of oils either remained on the disc or evaporated and only water soluble components diffused in the agar from the disc leading to underestimation of the activity of the oils.[24]

A previous study that assessed the contribution of vapours to the antimicrobial effect in the direct disc diffusion method, reported that only the water-soluble components diffused across the agar whilst the redeposition of the vaporized components on the surface of the agar accounted for the remainder of the inhibition. For oils containing alcohols, ketones, esters, oxides and hydrocarbons, the major inhibition is achieved from the vapours, whereas for oils containing greater volumes of aldehydes, inhibition is achieved from diffusion.[25]

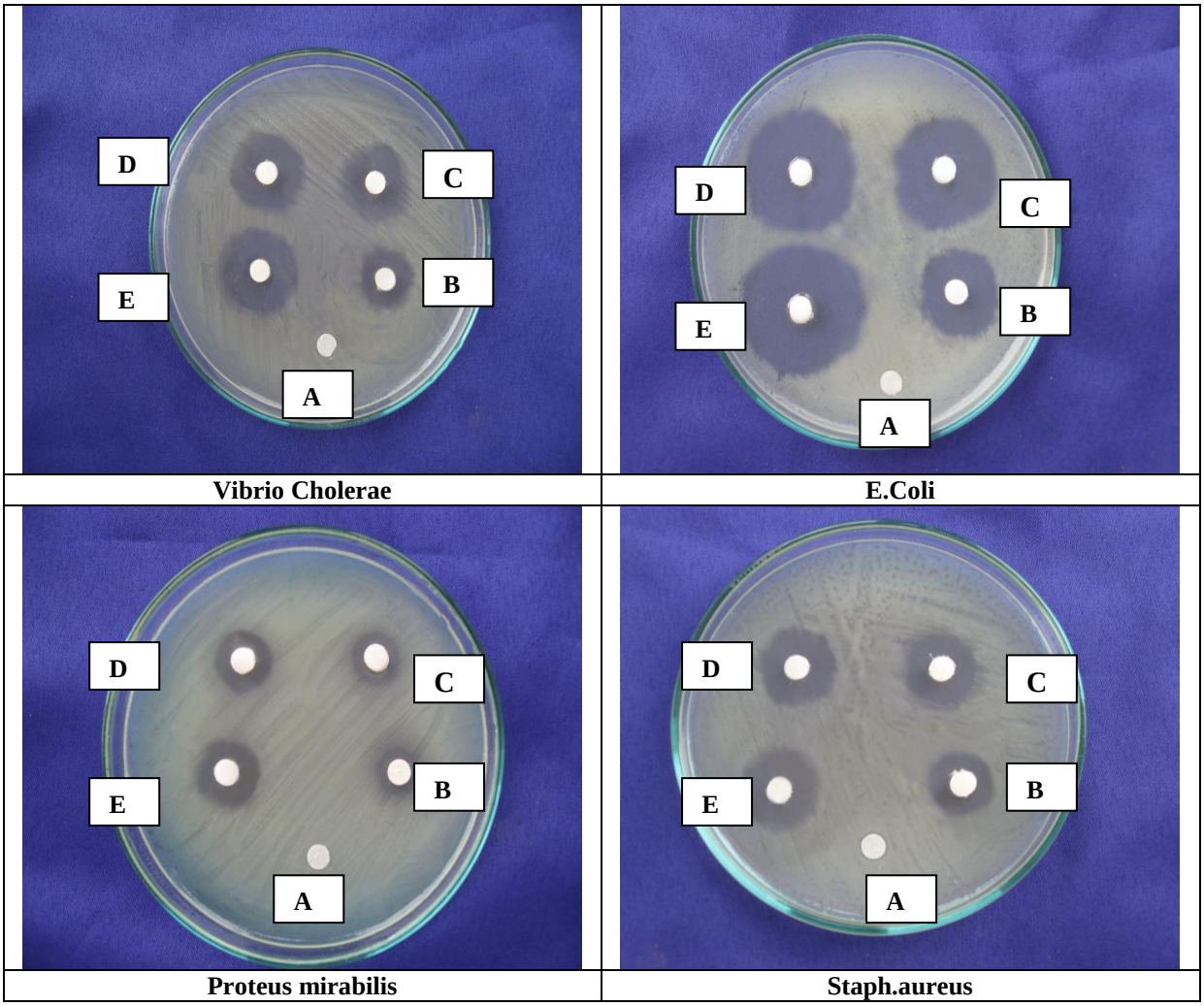
In the light of the above facts and previous reports, the present study clearly indicates that the vapour activity of essential oils contributed considerably to the increased diameter of inhibitory zone than by direct contact method. The results suggest that the Agar vapor assay provides a valuable assay for the determination of their antimicrobial activity in which the poor diffusivity of oil was compensated by the high volatility to exhibit a large inhibition zone.[26,27]

Antimicrobial activity of formulated essential oil



A-Control Upper lid, **B**-Control, **C**-10 µl/Ml, **D**-15 µl/Ml, **E**-20 µl/Ml, **F**-25 µl/Ml

Fig. 1: Growth inhibition of Formulated essential oil by Vapour assay method.



A-Control **B**- -10 µl/Ml, **C** -15 µl/Ml, **D**-20 µl/Ml, **E**-25 µl/Ml.

Fig. 2: Growth inhibition of Formulated essential oil by Disc diffusion method.

CONCLUSION

The purpose of this preliminary in vitro study was to evaluate the antimicrobial activity of formulated essential oil both by direct method and vapour phase through Agar diffusion assay and Agar vapour assay. Through this effort it can be revealed that the antimicrobial effect of formulated essential oil is superior in vapour phase. The results reveal that the formulated essential oil is effective for inhalation therapy against bacterial infections. Hence more preclinical and clinical trials may be warranted to confirm the efficacy of the vaporized state of the formulated essential oil for the management of infectious diseases.

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REFERENCES

1. Josephin Sheeba.B, and Selva Mohan. T, Antimicrobial activity of Adhatoda vasica against clinical pathogens. Asian Journal of Plant Science and Research, 2012; 2(2): 83-88.
2. Sharma A. Antimicrobial resistance: no action today, no cure tomorrow. Indian J Med Microbiol, 2011; 29: 91-2.
3. Thyagarajan.R, Gunapadam Thaathu jeeva vaguppu, 4th edn., Chennai; Dept of Indian medicine and Homeopathy publication, 2004.
4. Abbas SZ, Hussain K, Hussain Z, Ali R, Abbas T, Anti-Bacterial Activity of Different Soaps Available in Local Market of Rawalpindi (Pakistan) against Daily Encountered Bacteria. Pharm Anal Acta, 2016; 7: 522.
5. Maruzzella JC, Baler J, Katza: the action of perfume –oil vapours on fungi. American perfumer and Aromatics, 1960; 74: 21-22.
6. Maruzzella JC, SicurellaNA: Antibacterial activity of essential oil vapors. J of American Pharmaceutical Associaton, Scientific edition, 1960; 49: 692-694.
7. Kienhols M. Studies on the antibacterial action of ethereal oils. Arzneimittel-Forchung Drug research, 1959; 9: 519-521.
8. Pandey DK, Tripathi NN, Tripathi RD, Dixit SN: Fungi toxic and phytotoxic properties of the essential oils of Hyptissauveolens. J of Plant Diseases and Protection, 1982; 89: 344-349.
9. Gocho S: Antibacterial action of aroma compound in vapour state. J of antibacterial and antifungal agents, 1991; 19: 324-329.
10. Hammer K, Carson C, Riley T. Antimicrobial activity of essential oils and other plant extracts. J. Appl. Microbiol, 1999; 86: 985–990.
11. Burt S. Essential oils: their antibacterial properties and potential applications in foods—a review. Int. J. Food Microbiol, 2004; 94: 223–253.
12. Burrow a, Eccles r, Jones AS: The effect of camphor, eucalyptus and menthol vapors on nasal resistance airflow and nasal sensation. Acta otalaryngologica, 1983; 96: 157-161.
13. Shubina LP, Siurin SA, Savchenko M: Inhalation of essential oils in the combined treatment of patients with chronic bronchitis. VrachebnoeDelo (Kiev) Part 5, 1990; 66-67.
14. Frohlich, E: Lavender oil; review of clinical, pharmacological and bacteriological studies. Contribution to clarification of the mechanism of action. Wiener Medizinische Wochensochrift, 1968; 118: 345-350.

15. Nelson RRS: In vitro activities of five plant essential oils against methicillin resisant staphylococcus aueus and vancomycin resistant Enterococcus faecium. J.Antimicrob Chemother, 1997; 40: 05-306.
16. Inouye S, Yamaguchi H.Takizawa T: Screening of the antibacterial effects of a variety of essential oils on respiratory tract pahogens, using a modified dilution assay method. J Infect Chemother, 2001; 7: 251-254.
17. Vazquez JA, ZawawiAa: Efficacy of alcohol based and alcohol free melaleuca oral solution for the treatment of fluconazole–refractory oropharyngeal candidiasis in patients with AIDS. HIV Clin Trials, 2002; 3: 379-385.
18. MondelloF, DeBernardis F, Girolamo A, Salvatore G, Cassone A: In vitro and in vivo activity of tea tree oil against azole-susceptibe and resistant human pathogenic yeasts. J Antimicrob Chemother, 2003; 51: 1223-1229.
19. Trombetta, D.; Castelli, F.; Sarpietro, M.G.; Venuti, V.; Cristani, M.; Daniele, C.; Saija, A.;Mazzanti, G.; Bisignano, G. Mechanisms of antibacterial action of three monoterpenes. Antimicrob. Agents Chemother. 2005; 49: 2474–2478.
20. Tiwari, B.K.; Valdramidis, V.P.; O'Donnel, C.P.; Muthukumarappan, K.; Bourke, P.; Cullen, P.J.Application of natural antimicrobials for food preservation. J. Agric. Food Chem. 2009; 57: 5987–6000.
21. Chao Sc. young DG, Craig CJ.: Screening for inhibitory activity of essential oils on selected bacteria, fungi and viruses. J Essent Oil Res, 2000; 12: 639-649.
22. Kuo A Lunde CS, Kubo I: Antimicrobial activity of the olive oil flavour compounds. J Agric Food Chem, 1995; 43: 1629-1633.
23. Griffin SG, Markham JL, Lach DN: an agar dilution method for the determination of the minimum inhibitory concentration of essential oils. J Essent Oil Res, 2000; 12: 249-255.
24. N.G. Heatley, A method for the assay of penicillin. Biochem. J., 1944; 71–79 77 61–65.
25. Inouye S, Uchida, K., Maruyama, N., Yamaguchi, H. and Abe S. A novel method to estimate the contribution of the vapour activity of essential oils in agar diffusion assay. Jpn J Med Mycol. 2006; 47: 91–98.
26. Moleyar V, Narasimham P. Antifungal activity of some essential oil components. Food microbiology, 1986; 3: 331-336.
27. Gocho s. Antibacterial mechanism of aroma chemical vapors. J of antibacterial and anti fungal agents, 1991; 19: 511-513.