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Bioactivity of Essential Oils against Multi-Drug Resistant Bacteria from Clinical Samples

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Abstract
Spread of antibiotic resistant bacteria is most frequently seen in clinics leading to prolonged hospitalization, treatment failures and often high mortality rates. This has necessitated the search for alternative agents that may have antibacterial effect such as essential oils. These are complex natural mixtures isolated by steam distillation and have been traditionally used for treatment of acne inflammation. Aspirin, digoxin, morphine are few examples of drugs discovered from plant extracts.**Objectives**: To determine the bioactivity of essential oils against multidrug resistant bacteria from clinical samples. **Methods:** Nine clinical isolates were isolated from patients without duplicates. Bacterial isolates were identified and characterized by use of morphological, cultural, biochemical and antibiotic sensitivity patterns. **Results:** All nine different clinical isolates were multi drug resistant with 100%, 50% and 30% of isolate resistant to erythromycin, cloxacillin, ampicillin, neomycin, gentamycin and rifampicin respectively. Essential oils showed antibacterial activity against all 9 clinical isolates. **Conclusion**: Essential oils have proved to have antimicrobial potential against the multidrug resistant bacteria and this has led to their use in therapeutic treatment. High polar solvents are more effective.
Keywords: Wound isolates, multidrug resistant, essential oils, antibiotics

Introduction

Antimicrobial resistant bacteria are a major challenge for effective management of infections especially in hospital settings (Khan et al., 2004, Akram M et al., 2007). The spread of drug resistant pathogens is one of the most serious threats to successful treatment of microbial disease. Most common route of spread is through indirect transmission from the health care to their patients. Staff may carry the resistant bacteria on their hands or clothing and even the contaminated equipment in the hospitalcan be a source of infection. New alternative and effective therapies will therefore be required to treat infections caused by antibiotic resistant bacteria. One of the possible strategies towards this objective is the rational localization of bioactive phytochemicals. Plants have an almost limitless ability to synthesize aromatic substances. Most are secondary metabolites of which at least 12,000 have been isolated, a number estimated to be less than 10% of the total. In many cases, these substances serve as plant defense mechanisms against predation by microorganisms, insects and herbivores. Many of the herbs and spices used by humans to season food yield useful medicinal compounds including those having antimicrobial activity (Wallace, 2004; Thuille et al., 2003; Singh et al., 2002).

Essential oils have been traditionally used for treatment of infectious and diseases all over the world for centuries (Rio et al., 2005). Today the use of essential oils is a growing market and there are a considerable range of applications. The oils are used in the food and beverage industries, also as fragrances in perfumes and cosmetics. They also cover a broad spectrum of biological activity which has led to increased interest among researchers. In recent years there has been extensive research to explore and determine the antimicrobial activity of essential oils due to spread of antibiotic resistance. Essential oils and plant extracts have been screened for their potential uses as alternative treatment of many infectious diseases (Tepe et al., 2004). Majority of world's population depends on traditional medicine for primary health care (WHO-Traditional Medicine, 2003).

Essential oils have been shown to possess antibacterial, antifungal, antiviral, insecticidal and antioxidant properties (Burt et al., 2004). Some oils have been used in cancer treatment. These are aromatic oily liquids obtained from plant materials. They can be obtained by expression, fermentation or extraction by steam distillation (commercial production). Estimated 3,000 essential oils are known (Van de Brack Leijten et al., 1999). Chemically they are derived from terpenes and their oxygenated compounds. Generally Bacteria have the genetic ability to transmit and acquire resistance to drugs, which in turn may be utilized as therapeutic agents (Cohen 1992). Plant extract for the treatment of various ailments were highly regarded by the ancient civilizations. About 80% populations of the developed countries use traditional medicines derived from medicinal plants. Plants are also well known to be the risk sources of biologically active compounds. Therefore, one approach that has been used for the discovery of antimicrobial agents from natural sources is based on the evaluation of traditional plant extracts. Aspirin, astropine, ephedrine, digoxin, morphine, quinine, reserphine and tubocurarine are few examples of drugs which were originally discovered through the study of traditional cures and folk knowledge of indigenous people. Therefore these plants should be investigated thoroughly to determine

their structural and functional properties (Ellof, 1998).

There are many published reports on the effectiveness of traditional herbs against Gram positive and Gram negative bacteria microorganisms and as a result, plants are still recognized as the bedrock for modern medicine to treat infectious diseases (Evans et al ., 2002). Infectious disease physicians are alarmed by the prospect that effective antibiotics may not be available to treat seriously ill patients in the future.

Most previous studies on plants for antibacterial activity were mainly performed with the extract of aerial part e.g. leaves, stem, flowers, roots and rhizomes but meager with essential oil. Hence the following oils, *Eucalyptus tareticornis*, *Syzygium aromaticum*, *Azadirachta indica*, *Brassica juncea*, *L. czern*, *Helianthus annus*, *Zingiber officinale*, *Citrus Limon*, *Mentha piperita*, *Melaleuca alternifolia*, *Pipernigrum* were selected and screened against nine different bacterial cultures.

Materials and Methods

Collection of clinical samples: Fifty five wound swab samples were collected from 90 patients and analyzed for identification of microorganisms and for the determination of antibiotic susceptibility from PSG hospital. Nine species of wound isolates were obtained from PSG hospital. No duplicate isolates from a single patient were included. (Table 1)

Biochemical characterization of bacteria: The identity of the isolates was confirmed by use of gram stain, biochemical tests and serotyping. The isolates were tested for their sensitivity against the antibiotics (Ampicillin, Chloramphenicol, Neomycin, Ofloxacin, Gentamicin, Cloxacillin, Erythromycin, Polymyxin-B, Rifampicin and Tetracycline) respectively by disc diffusion method. Cultures were enriched in sterile nutrient broth for 6-8hrs at 37^{o}C. After incubation, the cultures were aseptically swabbed on the surface of sterilized Nutrient Agar plates using sterile cotton swabs. Antibiotic discs were aseptically placed over the seeded plates sufficiently separated from each other to avoid overlapping of the inhibition zones. Plates were incubated at 37^{o}C for 24hrs and the diameter of the zone of inhibition was measured in mm.**Collection of essential oil samples:** The essential oils collected from commercial outlets were, Eucalyptus, Clove, Neem, Mustard, Sunflower, Lemon, Ginger, Black pepper, Peppermint and Tea tree. **Well diffusion method (Vinoth Kumar et al, 2010)**: The organisms were cultured in nutrient broth and the test carried out on Muller-Hinton agar plates. The Inoculum of the microbial strains was prepared from 24hrs broth cultures and suspensions adjusted to optical density of 1-600nm turbid. After solidification of agar, 0.1 ml of strains was inoculated in the media separately. After medium solidification, a well was made in the plate with sterile borer (6mm). The oil samples (50µl) were introduced into the well and plates incubated at 37^{o}C for 24hrs. Zone inhibition was measured correctly.

Disc diffusion method: Sterile paper discs were loaded with 50µl of the sample oil and were left to dry for 30mins at 37^{o}C. Paper discs treated with oil were used as negative control. All discs were applied on the nutrient agar medium inoculated with 100µl of bacteria suspension and plates were incubated for 37^{o}C for 24hrs. Zone of inhibition around the disc was measured after incubation period and recorded. **Checker board assay:** Microtitre plate wells from each column in row 1 were marked and 100µl of oils were added. Later, 50µl of sterile distilled water was added to row 2-11. Two fold serial dilutions were performed by transferring 50µl of solution from row 1 to row 2. This was repeated down to row 12. 40µl of double strength nutrient broth and 10µl of bacterial solution were added to all the wells, so the final concentration on inoculum in all the wells is 100µl. Plates were covered by plastic cover and incubated at 37^{O}C overnight. The bacterial growth was determined after addition of 40µl of Tetra-zolium red (0.2mg/ml). The MIC of isolates was taken as the lowest concentration of the antibiotic of which the bacterial tested did not show visible growths.**Time killing assay**: A standardized bacterial suspensionwas added into nutrient broth containing oils (75µl). The mixture was then incubated for 37^{O}C for 24hrs at 200rpm. An oil free control was included. Viable counts were performed at 0,2,4,6 incubated. Bacteria were counted after 24-48hrs. The essential oils were considered to be bactericidal at the lowest concentration which reduced the original inoculum (99.9% reduction in bacterial population) in 4hrs. The multiple antibiotic resistant (MAR) index were calculated using the following formula. [MAR index for isolates = Number of antibiotics to which the isolates is resistance / number of antibiotics tested. While MAR index for antibiotic = number of antibiotics resistant to the isolates / (Number of antibiotics x Number of isolates).

Bioautography: Pre coated silica gel plates were used for thin layer chromatography. Later, 5µl of essential oils were loaded on the TLC plates. It was air dried overnight, each of the plates were placed in a humid chamber and overlaid with 10ml molten nutrient agar seeded with 0.2ml of methicillin resistant bacteria. These were left for 30mins then incubated at 37^{O}C for 24hrs and sprayed with 1mg/ml TTC. Plates were incubated at 35^{O}C in the dark for color development. The R_f value was also obtained. Extraction of tannins: 500µl of oil samples was taken in a glass beaker. 10ml of aqueous acetone (70%) was added and the beaker was suspended in water bath for 20mins at 37^{O}C. contents were transferred to centrifuge tubes and centrifuged at 3000rpm for 10mins. Collected the supernatant and kept it on ice. **Analysis of total phenolic compound**: Collect the aliquots of the tannin-containing extract in test tubes, make up the volume to 0.5ml with distilled water. Add 0.25ml of the Folin-Ciocalteu reagent. Add 1.25ml of Sodium Carbonate solution. The appearance of color was observed.

Results and discussion

In total 9 clinical wound isolates were isolated from the hospital samples. Isolates showed 100%, 50% and 30% of the isolates resistant to Erythromycin, Cloxacillin, Ampicillin, Neomycin, Gentamycin and Rifampicin, respectively (Table III). The MAR index of isolated bacteria was greater than 0.1, which implies that the strains of such bacteria originate from an environment where several antibiotics were used. When the essential oils were assayed against 9 isolates, by agar disc diffusion assay, the 100µl concentrations of the extract were found to be similar or better compared to tested antibiotics (Table IVA, B, and C). The antimicrobial screening of all the oils were more effective against all 9 isolates than antibiotics. The results obtained were promising especially, the activity of high polar *Melaleuca alternifolia* (20 to 24 mm zone) *and Eucalyptus tareticornis* (35 to 40 mm zone) against clinical isolates. (Table V). Minimum zone was observed against some oils except *Juncea, L. Czern, Helianthus annus, Mentha piperita* etc. (Table VI-A, VI-B). Our data agreed with Abdulla et al., (2007) who reported (after the investigation using lemon , ginger oil under disc and well diffusion assay against target oil isolates. A positive correlation was found between antibacterial activity and the concentration of oil. Checkerboard assay method was used to assess antibacterial potential of plant extracts. (Table VII). Lowest MBC concentration was defined when inoculated microorganisms were completely killed.

Phytochemical analysis of the M. alternifolia showed the presence of alkaloids, phenols, amines and tannins. All the compounds are biologically active. All oils were bactericidal (99.9%) against the 9 isolates after 12 and 24hrs at 3,5x MIC. 90% killing of all strains after 12hrs at 3x MIC concentration in all the 10 essential oils. In high polar oils, minimum zone was observed in acetone and petroleum ether extracts. In MIC checker board assay, the ethanol, methanol and chloroform extract of *Mentha piperita, Melaleuca alternifolia*, and *Piper nigrum* showed between 100mg/ml to 0.91mg/ml against 9 isolates. *Eucalyptus tareticornis* and *Syzygium aromaticum* showed MIC of 50mg/ml. In time killing assay of 10 essential oils showed 99.9% bactericidal for 9 isolates after 24hrs at 3xMIC concentration. The 9 strains did not respond in 0.5hrs even at 5xMIC concentration in all five extracts. The results show that the killing assay by use of essential oils depends on both the time and concentration. *P. nigrum* was fractionated by Bioautography on Silica gel eluted with ethanol and ethyl acetate concentration. Fractions were pooled according to their physical similarity and R_F value and antimicrobial activity was tested by checkerboard assay. This research has triggered the subsequent research work aims at evaluating the antimicrobial potential of the essential oils.

Bioautography showed that Secondary metabolites like tannins and Phenolic compounds were present in trace amounts in oil sample. (Table VIII). When oils react with Folin- Ciocalteu reagent, blue color was observed. No color was seen in *Azadirachta indica* and *Piper nigrum* indicating absence of tannins and phenolic. Clear bands were observed in TLC plate which indicates the presence of Aromatic compounds, Phenols, Amines, Carboxylic acids and Alcohols (Table IX). These contribute to effective management of plant disease and microbial contamination in several agricultural conditions. Phytochemicals are highly concentrated on the leaves, stems and roots rather than fruits and flowers.

Conclusion:

Out of 10 oils, *Eucalyptus tareticornis*, *Zingiber officinale*, *Mentha piperita*, *Melaleuca alternifolia* and *Piper nigrum* showed promising results against clinical isolates. More research work is required to evaluate effectiveness of these products against a wider range of bacterial pathogens.

Competing interests: The authors declare no conflicting of interest.

Author's contributions

Edna developed the idea, took a lead role in proposal development, supervised data management and analysis, manuscript writing and funded the studies. ESK participated in design of study. STK participated in data analysis, drugs and oils sensitivity and manuscript supervision. PO assisted in data collection and literature review. OL assisted supervision on overall manuscript preparation. All authors read and approved the final manuscript.

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References

Akram, M.; Shahid, M.; Khan, A. U. (2007). Etiology and Antibiotics Resistance Pattern of Community Acquired Urinary Infections in J N M C Hospital Aligarh India.*Ann. Clin.Microbiol. Antimicrob, 6*, 4.

Abdulla, A. E. M**.,** Darwish, S. M. Ayad, E. H. E. and El-Hamahmy. R. M. (2007). Egyptian mango byproduct 2: Antioxidant and antimicrobial activities of extract and oil from mango seed kernel. Food Chem. (103): 1141-1152.

Agaoglu, S., Dostbil, N. and Alemder. S. (2005). Antimicrobial effect of seed extract of cardamom. Yyu Vet Ft Derg. 16(2): 99-101.

Ahmad I, AZ Bog (2001) Antimicrobial and phytochemical studies on 45 Indian Medicinal plants against multidrufg resistant human pathogens. J. Ethnopharmacol. 74: 113-123.

Akinpelu D. A., and Onakoya. T. M. (2006). Antimicrobial activities of medicinal plants used in folklore remedies in South-Western. J. Biotech. 5(11): 1078- 1081.

Ann Hagerman and Irene Mullar-Harvey. (2000). Quantification of Tannins in Tree. Foliage. J. Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. pp: 3-5.

Arias BA, L. Ramon-Laca: (2005). Pharmacological properties of citrus and their ancient and medical uses in the Mediterranean region. J Ethnopharmacol, 97:89-95.

Aruoma OI, JP. Spencer, Rossi R, Aeschbach R, khan A, Mahmood N, Munoz A, Murcia A, Buteler J, Halliwell B. (1996). An evaluation of the antioxidant and antiviral action of extracts of rosemary and provencal herbs. Food ChemToxicol, 34:449-456.

Balch, Phyllis and Balch, James. Prescription for nutritional Healing, 3rd ed., Avery Publishing, ©2000, pg. 94.

Barrow G. I., and Feltham. R. K. A. (1993). Cowan and Steel's manual for identification of medical bacteria. Cambridge University Press. New York. USA, pp 52.

Bauer, KIRBY, A. W., W. M. M. J.C Sherris and M.Turck. (1996) Antibiotic susceptibility testing by standardized single disk method. Am. J. Clin. Pathol. 45: 493-496.

Benabssaini H, Benabderrahmane, M. Chikhi K. (2003). Contribution a l' evaluation de bl' active antise de l' oleoresine et des huliesessentielles du pistachier de l' atlas surcertaines sources microbiennes: Candida albicans (ATC 20027), (ATCC 20032) ET saccharomyces cerevisae. Ethnopharmacologie. Fev. (30): 38-46. Proprieties.

Benayache S, Benayache F. Benyahia S. (2001). Leaf oils of some Eucalyptus Species Growing in Algeria. J. Essent. Oil Res. 13: 210-213.

Benjilali B, A. Tantaoui-Elaraki, M. Ismaili-Alaoui, A.Ayadi. (1986): Plant medicinal Phytotherapy. 20: 155-167

Brooker, M.I.H.; D.A Kleinig. (2006). Field Guide to Eucalyptus. Melbourne: Bloomings. Third edition. ISBN 1-876473-52-5 vol. 1. South-eastern Australia.

Burnneton J. (1995). In; Pharmacognosy, Phytochemistry of Medicinal plants. Pairs: Lavoisies Publishing.

Burt SA: Essential oils: their antimicrobial properties and potential applications in foods: a review.

Dahanukar S. A., R. A. Kulkarni and N. N. Rege. (2002). Pharmacology of medicinal plants and natural products. Indian J. Pharmacol. 32: S81-S118.

Kumar A, Samarth RM, Yasmeen S, Sharma A, Sugahara T, Terado T, Terado T, Kimurah. (2004): Anticancer and radio protective potentials of *Menthapiperita* Biofactors, 22:87-91.

Khan, A.U.; Musharraf, A. (2004). Plasmid Mediated Multiple Antibiotic Resistances in Proteus mirabilis isolated from Patients with Urinary Tract Infection. *Med. Sci. Mont.*, *10*,598-602.

WHO Media Centre: **Traditional Medicine.**[http://www.who.int/mediacentre/factsheets/fs134/en/] website. (2003). *Fact sheet N°134*.

Wallace R. J. (2004). Antimicrobial properties of plant secondary metabolites. Proc .Nutr. Soc. 63 (4): 621-9.

Tables

Table I: Biochemical characterization of bacteria

S. No	Clinical isolates	Gram's stain	Indole test	MR test	VP test	citrate test	urease test	motility	Triple sugar iron test		
									sucrose	Lactose	H_2S Production
1.	*Escherichia coli*	-	+	+	-	-	-	+	+/-	**AG**	-
2.	*Shigella dysenteriae*	-	+	+	-	-	-	-	+/-	-	-
3.	*Staphylococcus aureus*	+	-	+	+/-	-	-	-	+/-	-	-
4.	*Klebsiella pneumoniae*	-	-	-	+/-	+	+	-	**AG**	**AG**	-
5.	*Salmonella typhi*	-	-	+	-	+	-	+	+/-	-	+
6.	*Proteus vulgaris*	-	+	+	-	-	+	-	+/-	-	+
7.	*Pseudomonas aeruginosa*	-	-	-	-	+	-	+	-	-	-
8.	*Bacillus cereus*	+	-	-	+/-	-	-	+	+	-	-
9.	*Enterobacter aerogenes*	+	-	-	+	+	-	+	+/-	**AG**	-

MR-Methyl Red, **VP**- Vogues Proskauer, **AG** - Acid Gas.

Table III: Antibiotic resistant profile of clinical isolates

S. No	Clinical isolates	A pattern of antibiotic phenotypic	Pattern	No. of Antibiotics	Types of Resistant
1.	*Escherichia coli*	Cx-E-Pb-R-T-A-N-Of-G	#1	9	MDR
2.	*Shigella dysenteriae*	Cx-E-Pb-R	#2	4	MDR
3.	*Staphylococcus aureus*	E-Cx-Pb-A-C-N-Of-G	#3	8	MDR
4.	*Klebsiella pneumoniae*	Cx-E-G	#4	3	MDR
5.	*Salmonella typhi*	E	#5	1	NMDR
6.	*Proteus vulgaris*	E-R-Cx-A-C-N-Of-G	#6	8	MDR
7.	*Pseudomonas aeruginosa*	E-R-T-Cx-A-C-N	#7	7	MDR
8.	*Bacillus cereus*	R-E	#8	2	MDR
9.	*Enterobacter aerogenes*	Cx-E-R-T-A-C-Of-G	#9	8	MDR

Cx: Cloxacillin, E: Erythromycin, Pb: Polymyxin-B, R: Rifampicin, T: Tetracycline, A: Ampicillin, C: Chloramphenicol, N: Neomycin, Of- Ofloxacin, G: Gentamicin.

Table IV-A: MAR Index of clinical isolates against antibiotics

S.No	Clinical isolates	No. of resistant isolates	No. of sensitivity isolates	MAR Index	% Of Frequency
1.	*E. coli*	9	1	0.9	90
2.	*S. dysenteriae*	4	6	0.4	40
3.	*S. aureus*	8	2	0.8	80
4.	*K. pneumonia*	3	7	0.3	30
5.	*S. typhi*	1	9	0.1	10
6.	*P. vulgaris*	8	2	0.8	80
7.	*P. aeruginosa*	7	3	0.7	70
8.	*B. cereus*	2	8	0.2	20
9.	*E. aerogenes*	8	2	0.8	80

Table IV-B&C: Antibiotic resistant and MAR index against Gram positive and negative Bacteria.

S. No	Antibiotics	No. of resistant isolates (+ve)	No. of resistant isolates (–ve)	No. of sensitive isolates (+ve)	No. of sensitive isolates (-ve)	MAR Index (+ve)	MAR Index (-ve)	% (-ve)	% +ve
1.	Cloxacillin	2	5	1	1	0.06	0.08	83	66
2.	Erythromycin	2	6	0	0	0.10	0.10	100	100
3.	Polymyxin-B	1	2	2	4	0.03	0.03	33	33
4.	Rifampicin	2	4	1	2	0.06	0.06	66	66
5.	Tetracycline	1	2	2	4	0.03	0.03	33	33
6.	Ampicillin	2	3	1	3	0.06	0.05	50	66
7.	Chloramphenicol	2	2	1	4	0.06	0.03	33	66
8.	Neomycin	1	3	2	3	0.03	0.05	50	33
9.	Ofloxacin	2	2	1	4	0.06	0.03	33	66
10.	Gentamicin	2	3	1	3	0.06	0.05	50	66

Table V: Antimicrobial activity of essential oils by disc diffusion method

S.No	Isolates	Zone of inhibition (mm)						
		E	C	B	G	L	P	T
1.	*E Coli*	20	11	15	8	26	25	23
2.	*S dysenteriae*	16	12	13	23	18	15	34
3.	*S aureus*	28	12	12	21	14	13	22
4.	*K pneumonia*	10	10	14	21	16	21	27
5.	*S typhi*	14	14	9	12	12	13	15
6.	*P vulgaris*	16	10	14	22	20	23	28
7.	*P aeruginosa*	15	10	14	12	9	13	11
8.	*Bacillus cereus*	12	15	20	29	40	32	40
9.	*E aerogenes*	16	10	-	12	10	11	8

E: Eucalyptus oil, C: Clove, B: Black pepper oil, G: Ginger oil, L: Lemon oil, P: Pepper-mint oil, T: Tea tree oil

Table VI-A&B: Antimicrobial activity of essential oils by well diffusion method.

S.No		Zone of inhibition (mm)									
		G	B	P	L	T	N	C	S	E	M
1.	*E Coli*	16	19	17	24	27	7	8	7	28	-
2.	*S dysenteriae*	17	15	11	15	19	-	-	-	18	7
3.	*S aureus*	19	18	19	15	25	8	24	8	20	-
4.	*K pneumonia*	22	18	19	17	17	-	25	-	35	-
5.	*S typhi*	26	16	16	14	28	9	30	-	35	9
6.	*P vulgaris*	21	12	18	18	23	-	-	9	25	-
7.	*P aeruginosa*	21	15	20	17	26	7	12	-	21	7
8.	*Bacillus cereus*	18	13	18	17	24	15	25	7	43	8
9.	*E aerogenes*	16	18	11	13	27	-	25	-	31	-

G: Ginger oil, B: Black pepper oil, P: Pepper-mint oil, L: Lemon oil, T: Tea tree oil.
N: Neem oil, C: Clove, S: Sunflower oil, E: Eucalyptus oil, M: Mustard.

Table VII: Time killing assay for clinical isolates using different essential oils.

S.No	Bacterial strains	Essential oil	Extraction concentration	Time (Hrs)					
				0	2	4	6	12	24
1.	*S. aureus*	Eucalyptus	3x	$220x10^{-6}$	$180x10^{-6}$	$70x10^{-4}$	$30x10^{-4}$	NIL	NIL
			5x	$190x10^{-6}$	$152x10^{-6}$	$20x10^{-4}$	$10x10^{-2}$	NIL	NIL
2.	*B. cereus*	Clove	3x	TNTC	$244x10^{-4}$	$152x10^{-4}$	$50x10^{-2}$	NIL	NIL
			5x	$544x10^{-6}$	$296x10^{-6}$	$65x10^{-4}$	$20x10^{-2}$	NIL	NIL
3.	*S. dysenteriae*	Ginger	3x	$119x10^{-6}$	$120x10^{-4}$	$64x10^{-2}$	$20x10^{-2}$	NIL	NIL
			5x	$195x10^{-6}$	$165x10^{-4}$	$54x10^{-4}$	$32x10^{-2}$	NIL	NIL
4.	*S. dysenteriae*	Black-pepper	3x	$322x10^{-6}$	$220x10^{-4}$	$46x10^{-2}$	$38x10^{-2}$	NIL	NIL
			5x	$560x10^{-6}$	$480x10^{-4}$	$280x10^{-2}$	$80x10^{-2}$	NIL	NIL
5.	*S. typhi*	Tea-tree	3x	TNTC	TNTC	$380x10^{-6}$	$230x10^{-4}$	$100x10^{-2}$	$35x10^{-2}$
			5x	TNTC	TNTC	$280x10^{-4}$	$140x10^{-4}$	$70x10^{-2}$	$15x10^{-2}$

Table VIII: Preliminary phytochemical analysis of essential oils.

S.No	plant extract	Aromatic compound	Amine	Phenol	Carboxylic acid	Alkaloid	Tannins	Phenolic compound
1.	Eucalyptus	+	+	+	+	+	+	+
2.	Clove	+	+	+	+	+	+	+
3.	Ginger	+	+	+	+	+	+	+
4.	Tea-tree	+	+	+	+	+	+	+
5.	Lemon	+	+	+	+	+	+	+
6.	Black pepper	+	+	+	+	+	+	+
7.	Peppermint	+	+	+	+	+	+	+

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