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Determination of Antibacterial Activity of Essential Oils Against Gram Negative Bacterial Pathogens

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Abstract

The present study was aimed to study the Minimum Inhibitory Concentration (MIC) and Percentage Growth Inhibition of Essential oils against three different Gram negative bacterial pathogens (*Escherichia coli, Salmonella typhi* and *Proteus mirabilis*). The selected Essential oil samples were collected from the Sidha Medicine Shop, Tirupattur, Vellore district, Tamil Nadu, India. The standard Broth dilution method was used for the determination of Minimum Inhibitory Concentration (MIC) of Essential oils against Gram negative bacteria. The Minimum Inhibitory Concentration (MIC) and Percentage Growth Inhibition were determined in various concentrations of Essential oils *viz*., 25 µl/ml, 50 µl/ml, 75 µl/ml and 100 µ/ml. The Essential oils exhibited the inhibitory activity against all the three Gram negative bacteria in all the concentrations. Among the seven Essential oils tested, Pungam oil has the capacity of inhibiting the Gram negative pathogenic coliform *Escherichia coli*. The Neem oil has the capacity of inhibiting the Typhoid casing bacteria *Salmonella typhi* and Swarming motility showing bacteria *Proteus mirabilis*. Finally, it was concluded that the Essential oils are the best source for treating the Gram negative bacterial diseases in human beings.

Keywords: Essential oils, Minimum inhibitory concentration (MIC), Percentage bacterial growth inhibition, Escherichia coli, Salmonella typhi and Proteus mirabilis

1 Introduction

Nature is a gifted source of pharmacological agents and an uncountable number of modern drugs have been isolated from the sources of many natural products. The presence of multiple number of biologically active constituents in the natural sources like plants is preparing the minds of the scientists to analyze the plants for their therapeutic uses in treating enormous amount of microbial infectious diseases. Since ancient times, the medicinal plants were act as a fundamental basis of remedies used in traditional medicinal system. The medicinal plants are acting as the vital source of plenty number of bioactive phytochemicals and it has contributed more for the discovery of novel therapeutic agents for microbial diseases. Increase in the incidence of new infectious diseases and antimicrobial drug resistance to various antibiotics induced the researchers to search and identify the new antimicrobial compounds, either in the natural form or synthetic form [1].

Since from the ancient times, the Volatile oils extracted from the natural sources, particularly medicinal plants have been widely used for their bactericidal, fungicidal and antiparasitic properties in medicine, food, agriculture, veterinary science, poultry, aquaculture and cosmetics. Today, their use in the microbial disease therapy is showing an increased interest in special interest of safety in drug administration, zero level side effects, easy and friendly acceptance by diseased patients and human health benefits. In addition to uses in drug industry, essential oils are also used as the functional ingredients for the food industries, all type of beverages and trending cosmetics. The antimicrobial potential of Essential oils may vary because its quality is depending on the type of species, chemical composition, nature of essential oil and pedoclimatic conditions [2].

Essential oils are the natural oils which are the essentially used for treating various infections. They are obtained from the distillation process and having a particular pleasant smell of the plant or others source from where it is extracted. They are widely distributed in the plastid of the plant leaf, vesingenous layer of cell wall or by the reaction of sure Glycosides. Several essential oils constituents of same plant are often extracted from the totally different elements of the plants with fully different properties. They are created exploitation totally different ways steam distillation could be a common technique whereas alternative ways like mechanical expression,

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solvent extraction or superficial fluid extraction are used [3].

Essential oils are odorous volatile viscous liquid which are stored in the special plant cells like glands, glandular hairs, oil ducts and resin ducts. They may also occur and extracted from its leaves, roots, wood, stem region, flowers, fruits, saps and stems bark. These essential oils are accountable for the distinctive nature of aroma which are associated with individual plant species. Various reports on Essential oils have also been shown to possess its pharmacological properties like antibacterial, antifungal, antiprotozoan, antihelminthic, antiviral, insecticidal, larvicidal, antiinflammatory, anticancer and antioxidant properties. Some of the essential oils extracted from the natural herbal plants have been used in treating various types of cancers. Some other oils have been used as food preservatives for food preservation, aromatherapy and cosmetics products producing industries. Essential oils are a rich source of plenty of biologically active phytochemical compounds. Nowadays, there has been an increasing interest in finding the antimicrobial properties of extracts from aromatic herbal plants based products, particularly essential oils. Therefore, it is highly reasonable and acceptable to expect a variety of biologically active compounds in the essential oils with specific as well as general antimicrobial and antibiotic potential [4].

The Essential oils are the rich source of bioactive compounds and pharmacologically active in nature. The most elements of those oils measures the presence of Terpenoids that square measure synthesized from Isoprene that square measure followed by Diterpenes and aromatic compounds. These oils square measure used for numerous functions. A number of them are: Lavender, Marjorere, Mellissa, etc. Essential oil extracted from the plants belongs to the family Lamiaceae has antibacterial activity, Black cumin (*Nigella sativa*) exhibits antifungal, antibacterial drug and inhibitor character. Plants utilized in Japanese scent sachets because of their sedative activity square measure Galangal, patchouli, cinnamon, Clove etc. [5]. Essential oils are having wide number of applications to the human beings that includes, used as perfumes, flavors for foods and beverages, or to heal each body and mind for thousands of years. Pepper mint, lavender, geranium, eucalyptus, rose, bergamot, wood and *Chamaemelum nobilis* essential oils square measure the foremost often listed ones [6]. The present study was aimed to study the Minimum Inhibitory Concentration (MIC) and Percentage Inhibition (%) of Essential oils against Gram negative bacterial pathogens.

2 Experimental

Essential oils selected for present research

a) Pungam oil – *Pongamia pinnata*
b) Mahualongif oil – *Madhuca longifolia*
c) Sesame oil – *Sesamum indicum*
d) Castor oil – *Ricinus communis*
e) Groundnut oil – *Arachis hypogaea*
f) Neem oil – *Azadirachta indica*
g) Coconut oil – *Cocos nucifera*

Collection of Essential oils

The Essential oils selected for the present study was collected from Sidha Medicine Shop, Tirupattur, Vellore district, Tamil Nadu, India.

Collection of test Bacterial cultures

Two different Gram positive bacterial cultures, *Escherichia coli, Salmonella typhi* and *Proteus mirabilis* were procured from Microbial Type Culture Collection (MTCC), Chandigarh, India.

Maintenance of bacterial cultures

The bacterial cultures were sub-cultured and maintained on Nutrient agar slants and stored in refrigerator at 4 °C.

Bacterial inoculum preparation

Bacterial inoculum was prepared by inoculating a loopful of bacteria in 5 ml of Nutrient broth and incubated at 37 °C for 12 hours till a moderate turbidity was developed. The turbidity was matched with 0.5 McFarland standards and then used for the determination of antibacterial activity.

Determination of Minimum Inhibitory Concentration (MIC) Broth Dilution Method

The Broth dilution method proposed by Ericsson and Sherris [7] was used for the determination of Minimum Inhibitory Concentration (MIC) of Essential oils against three selected Gram negative bacteria, *Escherichia coli, Salmonella typhi* and *Proteus mirabilis*. Essential oils were diluted into various concentrations *viz*., 25µg/ml, 50 µg/ml, 75 µg/ml and 100 µg/ml in a sterile Nutrient broth in test tubes. Using standard wire loop (Hi-media), a loopful of Gram positive bacterial culture was inoculated into test tubes containing various concentrations of Essential oils in Nutrient broth. The tubes were incubated at 37 °C for 24 hours and thereafter observed for growth or turbidity. The bacterial growth which was observed in the form of turbidity was measured by using UV Visible Spectrophotometer (SYSTRONICS - 108) at 600 nm and the Optical density was recorded. These experiments were repeated three times.

Determination of Percentage growth inhibition

The Percentage bacterial growth inhibition of bacteria was determined by using the formula:

% Growth Inhibition = Optical Density of Initial Bacterial growth (Nutrient broth inoculated with bacteria) – Optical Density of Inhibited Bacterial growth (after addition of Essential oils)/ Optical Density of Initial Bacterial growth × 100.

3 Results and Discussion

The effect of Essential oils on Percentage bacterial growth inhibition was studied in the present research against the Gram negative coliform bacilli (rod) *Escherichia coli* and results were showed in Table – 1. The Minimum Inhibitory Concentration (MIC) studies were conducted by using various concentrations of Essential oils *viz*., 25 µl/ml, 50 µl/ml, 75 µl/ml and 100 µ/ml. It was

showed that the growth inhibition was observed in all the concentrations and the increase in concentration of essential oils increase the percentage growth inhibition of *Escherichia coli*. Among the oils tested, maximum growth inhibition percentage was observed in Pungam oil (35.0 %) followed by Coconut oil (25 %), Castor oil (24.4 %), Mahualongif oil (23.9 %) and Sesame oil (16.3 %), Groundnut oil (16 %). Lowest activity was observed against Neem oil (12.7 %).

The effect of Essential oils on Percentage bacterial growth inhibition was studied in the present research against the Gram negative Typhoid fever bacilli *Salmonella typhi* and results were showed in Table – 2. The Minimum Inhibitory Concentration (MIC) studies were conducted by using various concentrations of Essential oils *viz*., 25 µl/ml, 50 µl/ml, 75 µl/ml and 100 µ/ml. It was showed that the growth inhibition was observed in all the concentrations and the increase in concentration of essential oils increase the percentage growth inhibition of *Salmonella typhi*. Among the oils tested, maximum growth inhibition percentage was observed in Neem oil (32.6 %) followed by Groundnut oil (31.3 %), Mahualongif oil (30.0 %), Coconut (26.5 %), Castor oil (26.4 %) and Pungam oil (18.8 %). Lowest activity was observed against Sesame oil (14.8 %).
The effect of Essential oils on Percentage bacterial growth inhibition was studied in the present research against the Gram negative bacteria *Proteus mirabilis* and results were showed in Table – 3. The Minimum Inhibitory Concentration (MIC) studies were conducted by using various concentrations of Essential oils *viz*., 25 µl/ml, 50 µl/ml, 75 µl/ml and 100 µ/ml. It was showed that the growth inhibition was observed in all the concentrations and the increase in concentration of essential oils increase the percentage growth inhibition of *Proteus mirabilis*. Among the oils tested, maximum growth inhibition percentage was observed in Neem oil (32.2 %) followed by Groundnut oil (30.0 %), Coconut oil (27.2 %), Castor oil (23.4 %), Sesame oil (18.7 %) and Mahualongif oil (17.6 %). Lowest activity was observed against Pungam oil (17.5 %).

Venturi *et al*. [8] screened the selected the antibacterial activity of Essential oils against four Gram negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Proteus vulgaris*) and two Gram positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*). The Minimum Inhibitory Concentration (MIC) of the active Essential oils were tested using two fold Agar dilution method at concentrations ranging from 0.2 to 25 mg/m. Out of 21 Essential oils tested, 19 oils has showed antibacterial activity against pathogenic bacteria. Out of many oils tested, Cinnamon oil has showed promising inhibitory activity against bacteria even at low concentration, whereas Eucalyptus oil and camphor oil has exhibited least active against the tested bacteria. In general, the Gram positive bacilli *Bacillus subtilis* was the most susceptible. On the other hand, the Gram negative Bacilli *Klebsiella pneumoniae* exhibited low degree of sensitivity. However, Cinnamon, Clove and Lime oils were reported to be inhibiting both Gram positive and Gram negative bacteria and Cinnamon oil can be concluded as a good source of antibacterial agents. Amal *et al*. [9] examined the Minimal inhibitory concentration (MIC) values of the Essential oil extracted from *Cyclotrichium niveum*. The essential oil showed remarkable antibacterial activity against *Klebsiella pneumoniae* and *Staphylococcus aureus*. In our present study, we observed that the *Escherichia coli* were highly inhibited by the Pungam oil and the Neem oil have the ability of inhibiting the growth of *Salmonella typhi* and *Proteus mirabilis*.

Recently, Nagalakshmi *et al*. [10] determined the Minimum Inhibitory Concentration (MIC) and Percentage Growth Inhibition of Essential oils against two Gram positive bacterial pathogens, *Staphylococcus aureus* and *Bacillus subtilis*. The Broth dilution method was used for the determination of Minimum Inhibitory Concentration (MIC) of Essential oils. The Minimum Inhibitory Concentration (MIC) was determined in different concentrations *viz*., 25 µl/ml, 50 µl/ml, 75 µl/ml and 100 µ/ml. Among the seven Essential oils tested, Mahualongif oil has showed maximum percentage bacterial growth inhibition when compared to other Essential oils. In the present study, Pungam oil has the capacity of inhibiting the growth of *Escherichia coli*. Neem oil has the capacity of inhibiting the Typhoid casing bacteria *Salmonella typhi* and Swarming motility showing bacteria *Proteus mirabilis*.

Table – 1: Minimum Inhibitory Concentration (MIC) and Percentage bacterial growth inhibition of Essential oils against *Escherichia coli*

S. No	Essential oils	Nutrient broth	Optical Density at 600 nm and Percentage bacterial growth inhibition								
			Control	25 µl/ml	% inhibition	50 µl/ml	% inhibition	75 µl/ml	% inhibition	100 µl/ml	% inhibition
1.	Pungam oil	0.10	0.57	0.46	5.6 %	0.53	7.0 %	0.41	28.0 %	0.37	35.0 %
2.	Mahualongif oil	0.10	0.46	0.42	8.6 %	0.52	11.5 %	0.39	15.2 %	0.35	23.9 %
3.	Sesame oil	0.10	0.49	0.47	4.0 %	0.43	12.2 %	0.49	12.5 %	0.41	16.3 %
4.	Castor oil	0.10	0.49	0.51	0 %	0.46	6.1 %	0.42	15.2 %	0.37	24.4 %
5.	Groundnut oil	0.10	0.50	0.48	4.0 %	0.48	4.0 %	0.46	8.0 %	0.42	16.0 %
6.	Neem oil	0.10	0.48	0.47	2.0 %	0.45	6.25 %	0.44	8.33 %	0.55	12.7 %
7.	Coconut oil	0.10	0.52	0.55	5.45 %	0.44	15.3 %	0.40	23.0 %	0.39	25.0 %

*Control – *Escherichia coli* inoculated in Nutrient broth

Table – 2: Minimum Inhibitory Concentration (MIC) and Percentage bacterial growth inhibition of Essential oils against *Salmonella typhi*

S. No	Essential oils	Nutrient broth	Optical Density at 600 nm and Percentage bacterial growth inhibition								
			Control	25 µl/ml	% inhibition	50 µl/ml	% inhibition	75 µl/ml	% inhibition	100 µl/ml	% inhibition
1.	Pungam oil	0.10	0.53	0.47	5.2 %	0.60	5.9 %	0.47	11.3 %	0.43	18.8 %
2.	Mahualongif oil	0.10	0.57	0.57	0 %	0.53	7.0 %	0.50	12.2 %	0.47	21.2 %
3.	Sesame oil	0.10	0.47	0.45	4.2 %	0.45	4.2 %	0.43	8.5 %	0.40	14.8 %
4.	Castor oil	0.10	0.53	0.49	7.5 %	0.48	9.4 %	0.44	16.9 %	0.39	26.4 %
5.	Groundnut oil	0.10	0.51	0.49	3.9 %	0.45	11.7 %	0.42	17.6 %	0.35	31.3 %
6.	Neem oil	0.10	0.49	0.44	10.2 %	0.42	14.2 %	0.41	16.3 %	0.33	32.6 %
7.	Coconut oil	0.10	0.52	0.52	0 %	0.47	9.6 %	0.42	9.6 %	0.39	26.5 %

*Control – *Salmonella typhi* inoculated in Nutrient broth

Table – 3: Minimum Inhibitory Concentration (MIC) and Percentage bacterial growth inhibition of Essential oils against *Proteus mirabilis*

S. No	Essential oils	Nutrient broth	Optical Density at 600 nm and Percentage bacterial growth inhibition								
			Control	25 µl/ml	% inhibition	50 µl/ml	% inhibition	75 µl/ml	% inhibition	100 µl/ml	% inhibition
1.	Pungam oil	0.10	0.56	0.53	5.37 %	0.50	10.7 %	0.42	12.9 %	0.47	17.5 %
2.	Mahualongif oil	0.10	0.45	0.43	4.44 %	0.43	4.44 %	0.38	15.5 %	0.32	17.6 %
3.	Sesame oil	0.10	0.55	0.52	5.45 %	0.51	7.84 %	0.48	14.5 %	0.46	18.7 %
4.	Castor oil	0.10	0.47	0.42	10.6 %	0.56	17.3 %	0.38	19.1 %	0.36	23.4 %
5.	Groundnut oil	0.10	0.50	0.49	4.0 %	0.47	6.0 %	0.39	22.0 %	0.35	30.0 %
6.	Neem oil	0.10	0.59	0.57	3.38 %	0.45	16.0 %	0.41	30.5 %	0.40	32.2 %
7.	Coconut oil	0.10	0.55	0.52	10.5 %	0.48	12.7 %	0.44	20.0 %	0.40	27.2 %

*Control – *Proteus mirabilis* inoculated in Nutrient broth.

4 Conclusions

From this present research, we concluded that the Essential oils are the good source for the inhibition of growth of bacterial pathogens. It was also concluded that the Essential oils also have the capacity of inhibiting the bacteria in low concentration (25 µl/L) itself. Pungam oil has the capacity of inhibiting the water borne pathogenic coliform *Escherichia coli*. Neem oil has the capacity of inhibiting the Typhoid casing bacteria *Salmonella typhi* and Swarming motility showing bacteria *Proteus mirabilis*. Finally, it was concluded that the Essential oils are the best source for treating the bacterial diseases caused by Gram negative pathogens in human beings.

References

[1] Lin, J., Nishino, K., Roberts, M. C., Tolmasky, M., Aminov, R. I. and Zhang, L. (2015). Mechanisms of Antibiotic Resistance, *Frontiers Microbiology*, 6: 1 - 3.

[2] Bakkali, F., Averbeck, S., Averbeck, D. and Idaomar, M., (2008). Biological Effects of Essential Oils – A Review. *Food Chemistry and Toxicology,* 46: 446 - 475.

[3] Durga Devi, V., Kalpana, G and Saranraj, P. 2017. Antibacterial activity of Essential oils against human pathogenic bacteria. *Advances in Biological Research*, 11(6): 357 – 364.

[4] Saranraj, P and Durga Devi, V. 2017. Essential oils and its antimicrobial properties – A Review. *Life Science Archives*, 3(2): 994 - 1011.

[5] Bajpai, V.K., Baek, H.K. and Kang, S.C. 2012. Control of Salmonella in foods by using essential oils: A review. *Food Research International,* 45: 722–734.

[6] Cavaleiro, C., Salgueiro, L., Goncalves, M. J., Hrimplpeng, K., Pinto, J. and Pinto, E., (2015). Antifungal activity of the essential oil of Angelica major against *Candida, Cryptococcus, Aspergillus* and dermatophyte species, *Journal of Natural Medicines*, 69: 241 - 248.

[7] Ericsson, H. M. and J.C. Sherris. 1971. Antibiotic sensitivity testing. Report of an International Collaborative Study. Acta Pathology and Microbiology, Scand., Sec. B, Suppl. No.217.

[8] Venturi, C. R., Danielli, L. J., Klein, F., Apel, M. A, Montanha, J. A, Bordignon, S. A. L., Roehe, P. M, Fuentefria, A. M. and Henriques, A, T., (2015). Chemical analysis and *in vitro* antiviral and antifungal activities of essential oils from *Glechon spathulata* and *Glechon marifolia*, *Pharmaceutical Biology*, 53(5): 682 - 688.

[9] Amal K., Mitra, Oluwabunmi Ogungbe, Nusrat Kabir. (2019). Association of HCV Infection with C-Reactive Protein: National Health and Nutrition Examination Survey (NHANES), 2009–2010 *Department of Epidemiology and Biostatistics,

School of Public Health, Jackson State University, Jackson, MS 39213, USA.

[10] Nagalakshmi, S., Saranraj, P and Sivasakthivelan, P. 2019. Determination of Minimum Inhibitory Concentration (MIC) and Percentage Bacterial Growth Inhibition of Essential oils against Gram Positive Bacterial pathogens. *Journal of Drug Delivery and Therapeutics*, 9(3): 33 – 35.