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A COMPARITIVE STUDY OF ANTIMICROBIAL ACTIVITY OF SOME HERBS AND THEIR SYNERGISTIC EFFECT

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ABSTRACT

Plants produce certain bioactive compounds which are naturally toxic to microorganisms and so have been investigated as therapeutic agents. The present study was carried out so as to evaluate the antimicrobial effect of essential oils of few aromatic medicinal plants namely, Lemongrass, Pongamia, Ocimum and Sandalwood. In vitro evaluation was done for individual oils as well as a combination of the oils, two at a time, in 1:1 ratio against bacterial pathogens. Test cultures used were namely *Staphylococcus aureus, Pseudomonas aeruginosa* and *E. coli.* The synergistic action was tested that is the combination formula with two assumptions, one that individual oil may show ineffectiveness if used repeatedly as seen in antibiotics and second the combined formulation may show the wider range of antimicrobial activity. In vitro assay was also carried out using commonly used antibiotics against these pathogens and compared with the synergistic effect of herbal extracts. The study showed promising results for the use of Lemongrass, Pongamia, Ocimum and Sandalwood in specific combination against sepsis causing *Staphylococcus aureus, Pseudomonas aeruginosa* as well as leading nosocomial agent *E. coli.*

KEYWORDS: Antibiotics, Antibacterial properties, Bacterial pathogens, Essential oils, Synergistic effect

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INTRODUCTION

Herbal medicine represents one of the most important fields of traditional medicine all over the world[1]. In herbal medicine, crude plant extracts in the form of infusion, decoction, tincture or herbal extract are traditionally used by the population for the treatment of diseases, including infectious diseases[2]. Plants produce certain bioactive compounds which are naturally toxic to microorganisms and so have been investigated as therapeutic agents[3]. To promote the proper use of herbal medicine and to determine their potential as sources for new drugs, it is essential to study medicinal plants, which have a folklore reputation in a more intensified way[4]. The present study was carried out so as to evaluate in vitro the antimicrobial effect of essential oils of few aromatic medicinal plants namely, Lemongrass (*Cymbopogon citratus*), Pongamia (*Millettia pinnata*), Ocimum (*Ocimum tenuiflorum*) and Sandalwood (*Santalum album*). The in vitro evaluation was done for individual oils and combination of the oils, two at a time, in 1:1 ratio against bacterial pathogens of nosocomial infections namely *Staphylococcus aureus, Pseudomonas aeruginosa* and *E. coli.* The synergistic action was tested. In vitro assay was also carried out for commonly used antibiotics against these pathogens and compared with the synergistic effect of herbal extracts.

MATERIALS AND METHODS

1. Materials required

1.1. Plant extracts

Pure essential oils of Lemongrass, Pongamia, Ocimum and Sandalwood were obtained from a local outlet in Hyderabad and Pune, India, which sells the plant extracts as therapeutic agents with GMP and ISO certifications.

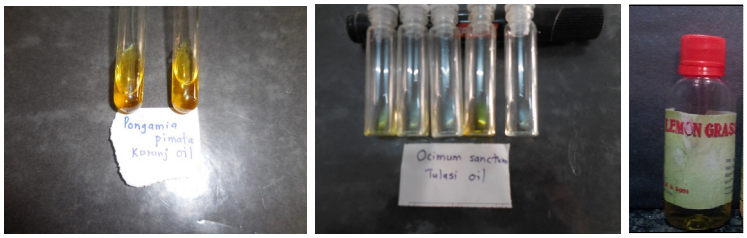

Figure 1
Plant essential oils

1.2. Bacterial test cultures

Pure cultures of the following Microorganisms were used for screening the antimicrobial properties of plant essential oils.These pure cultures of the test bacteria were obtained from the National Collection of Industrial Microorganisms (NCIM), NCL, India. The cultures were maintained on Nutrient Agar (HI Media, India) slopes at 4^0C and sub-cultured before use.

a) *Staphylococcus aureus* - NCIM 2079
b) *Pseudomonas aeruginosa* -NCIM 2037
c) *Escherichia coli*-NCIM 2803

1.3. Culture media

a) Nutrient broth
b) Nutrient agar
c) Mueller Hilton Agar

2. Methods followed

2.1. Agar well diffusion method for screening the antimicrobial activity of plant essential oils

In vitro antibacterial activity was studied against the bacterial strains using Agar well diffusion method. (4,13) In this method the antimicrobials present in the plant essential oil are allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. The Mueller-Hinton agar plates were seeded with the overnight broth culture of a test organism ($1.5x10^8$/ CFU/ml). Wells were prepared in seeded agar plates with 6mm diameter and 500 µl of each essential oil was introduced in each well. The solvent used for preparing essential oil solution was absolute alcohol. Solvent control well was run for every assay. All the inoculated plates were

incubated at 37^0C for 24 hours in the incubator. The antimicrobial spectrum of the extract was determined in terms of the diameter of inhibition zones. A zone of inhibition of 12mm (millimetre) or above was considered as sensitive and less than 12mm as resistant. The entire experiment was carried out under strict aseptic conditions. The samples were run in triplicates and each result is a mean of the three values.

2.2. Evaluation of the synergistic effect of the plant essential oils (phytochemicals)

This evaluation was done according to Agar well diffusion method on the three bacterial test cultures. The aliquots of 100µl of bacterial cultures grown in Nutrient broth for 18 hours ($1.5x10^8$/ CFU/ml) were spread plated on Mueller-Hinton agar medium. The wells were filled with 500µl of a combination of plant essential oils, (1:1). The methodology followed was same as above.

RESULTS

Table 1
Antimicrobial activity of antibiotics

Antibiotics	Zone of inhibition (mm)		
	Staphylococcus aureus	Pseudomonas aeruginosa	E. coli
Norfloxacin (10 mg)	27	22	21
Gentamicin (15mg)	20	20	10
Trimethoprim (300mg)	22	17	16
Tetracycline (30mg)	32	Resistant	Resistant
Sulpha (300mg)	32	Resistant	Resistant
Erythromycin (15mg)	Resistant	Resistant	Resistant

Table 2
Antimicrobial activity of plant essential oils

Plant essential oils	Zone of inhibition (mm)		
	Staphylococcus aureus	Pseudomonas aeruginosa	E.coli
Ocimum	16.3±0.94	40	16.3±0.94
Sandalwood	28±0.94	35	28±0.94
Pongamia	Resistant	Resistant	Resistant
Lemongrass	47	Resistant	50

Graph 1
Antimicrobial activity of plant essential oils

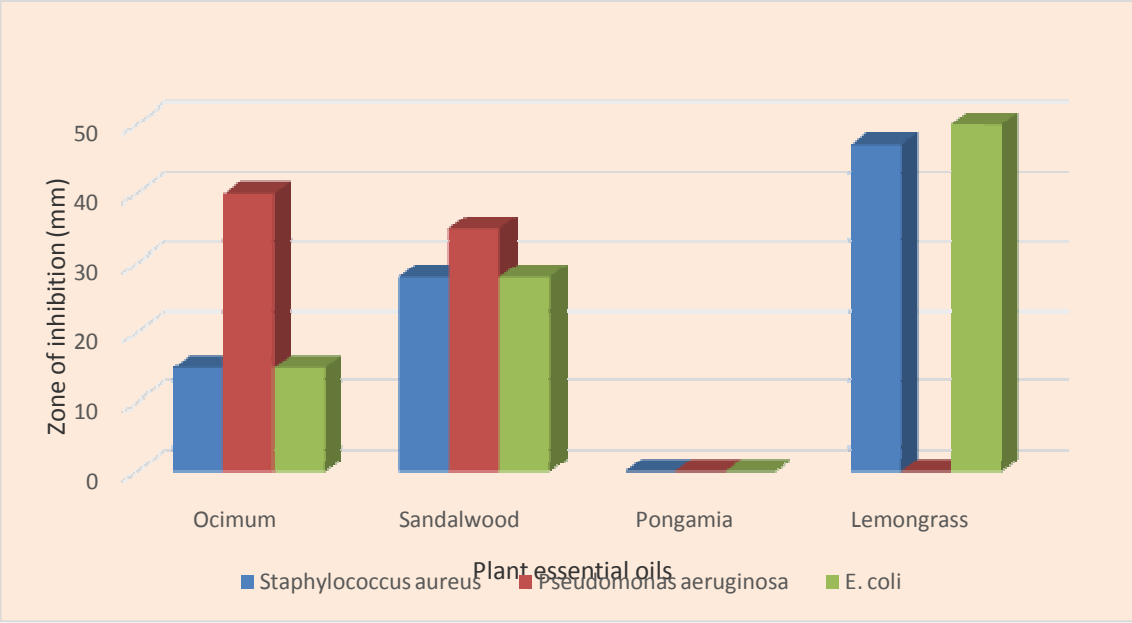

Table 3
Synergistic action of plant essential oils

Combinations of plant essential oils	Zone of inhibition (mm)		
	Staphylococcus aureus	Pseudomonas aeruginosa	E.coli
Ocimum+ Sandalwood	16.3±0.94	27	15±0.94
Ocimum+Pongamia	29±0.82	30	28±0.94
Ocimum+Lemongrass	Resistant	resistant	Resistant
Sandalwood+ Pongamia	Resistant	resistant	50±0.94
Sandalwood+ Lemongrass	52	35±0.94	52
Pongamia+lemongrass	28	resistant	Resistant

Results given in the form of (mean±standard deviation).

Graph 2
Synergistic effect of plant essential oils

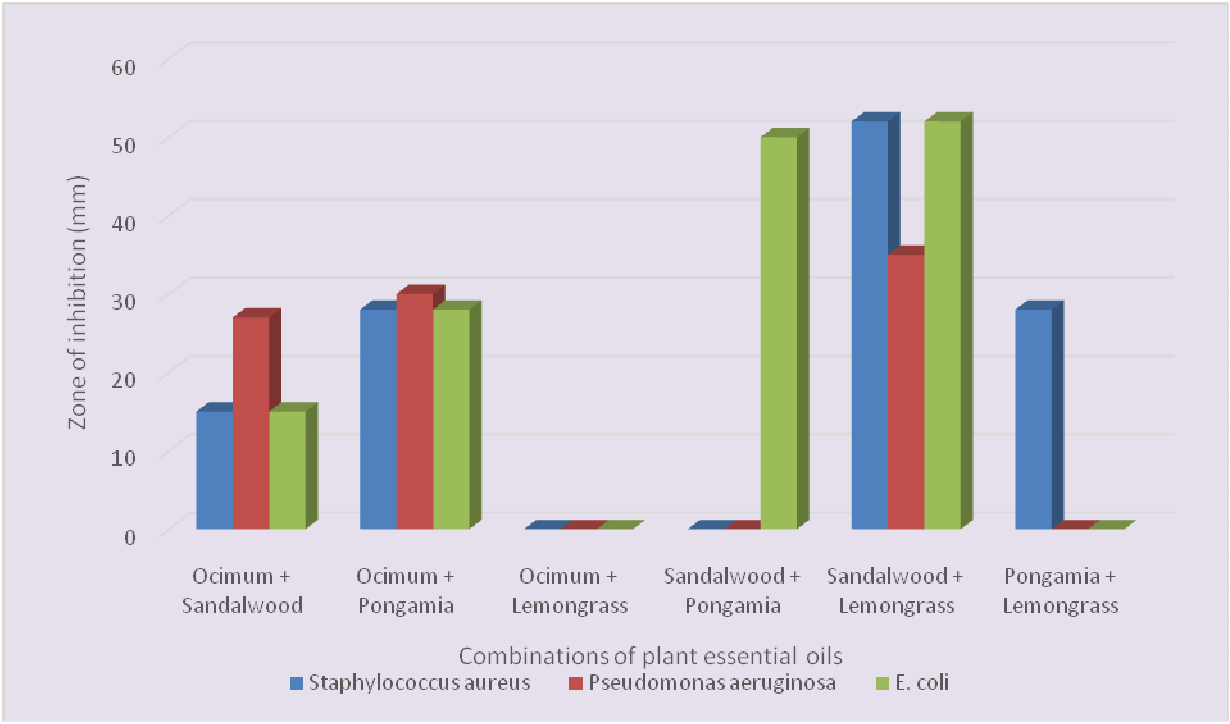

Figure 2
Zones of inhibition

DISCUSSION

The pathogens used like, *Staphylococcus aureus, Pseudomonas aeruginosa* and *E.coli* are the leading nosocomial infective agents[5]. The routes of pathogen transmission are many and varied. Spread is by direct or indirect contact with animate or inanimate

objects and may be horizontal or vertical[6]. Wide scale use of antibiotics led to microbial drug resistance, an adaptive response in which microorganisms are able to tolerate any amount of drug that would ordinarily be inhibitory[5]. Therefore, the use of essential oils from plants seems to be the practical alternative or a supportive treatment process[7,9,10]. Among the three bacterial strains tested, one was Gram positive bacterium namely, *Staphylococcus aureus* and others were Gram negative bacteria *Pseudomonas aeruginosa* and *E.coli*. *Staphylococcus aureus*, coagulase positive strain, is a leading nosocomial pathogen which causes sepsis[8]. *Pseudomonas aeruginosa* is another leading nosocomial pathogen and it shows drug resistance[2]. It causes wound and burn infections. *E. coli* is a part of the normal flora of the body and is an opportunistic pathogen. Gram positive and Gram negative bacteria show difference in their sensitivity towards antibiotics which are used as therapeutic agents .This difference is attributed to differences in their cell wall composition. Gram negative bacteria have a thin layer of peptidoglycan and has an outer membrane made up of lipoproteins and lipopolysaccharides. Gram positive cell wall has thick peptidoglycan layer and lacks outer membrane[6]. The oil extracts showed much higher zone of inhibition against *Staphylococcus aureus*. When used individually, Lemongrass oil was found to be the most effective against *Staphylococcus aureus* and *E. coli*. (Table 2, Graph 1). Ocimum and Sandalwood were effective against *Pseudomonas aeruginosa* but Pongamia didn't show considerable antibacterial activity. (Table 2, Graph 1) Lemongrass, Ocimum and Sandalwood exhibited better antibacterial action than most antibiotics. (Table 1, Table 2, Graph 1). Ocimum and Pongamia individually showed lesser inhibition but higher zones of inhibition when used in combination. (Graph 2).Their synergistic action is significant and superior to the antibiotics. However, the synergistic effect of Ocimum and Lemongrass and Pongamia and Lemongrass was negligible. The combination of Sandalwood and Pongamia was effective only against *E. coil* but was much better than the antibiotics. Sandalwood and Lemongrass together exhibited maximum antibacterial activity and highly significant synergistic effect. The combinations of Ocimum and Sandalwood, Ocimum and Pongamia and Sandalwood and Lemongrass showed promising inhibitory activity, more effective than antibiotics, against *Pseudomonas aeruginosa*. It's a Gram negative bacteria and has the cell wall composition such that it resists the entry of the antibiotic in the cell and thus they offer high resistance[5]. The synergistic action of these oils could be explained as these oils might have gained access through the cell wall and so the bacterial culture showed sensitivity. Antibiotics are one of our most important weapons in fighting bacterial infections and have greatly benefited the health-related quality of human life since their introduction. However, over the past few decades, these health benefits are under threat as many commonly used antibiotics have become less effective against certain illnesses due to the emergence of drug-resistant bacteria. Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanisms of action. The combined therapy may become useful in blocking the ways of microbes to develop resistance to antimicrobial agents.

CONCLUSION

Our study showed very significant results for the use of plant essential oils as antibacterial agents. The comparative study with antibiotics revealed the superior antibacterial action of the plant formulations. The synergistic action of the oils was highly promising. These plants can thus provide alternative medications.

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CONFLICT OF INTEREST

Conflict of interest declared none

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