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In-vitro Efficacy Analysis of Few Essential Oils for their Antibacterial Activity against Methicillin Resistant *Staphylococcus Aureus*

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ABSTRACT

Hospital acquired infections are commonly associated with Methicillin Resistant *Staphylococcus aureus* infection and treating these infections with chemotherapy is very perilous. The augment of multidrug-resistant strains makes it necessary to discover new classes of antimicrobials and compounds that restrain their resistance mechanisms. In the past few decades, the search for new anti-infective agents has betrothed many research groups in the field of ethno pharmacology. Many focus on determining the antimicrobial activity of plant extracts found in folk medicine, essential oils or isolated compounds. There are a large number of species that have been and are still used as medicinal plants, particularly in folk medicine. In the present study, we analyse the possibility of essential oils as potential antimicrobial crude drugs as well as a source for natural compounds that act as new anti-infectious agents.

Keywords: multi drug resistance, antimicrobial, medicinal

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INTRODUCTION

Varieties of aromatic medicinal plants are tested for treating infectious diseases. These plants have been mentioned in different phototherapy manuals due to their availability, fewer side effects and reduced toxicity. The essential oils of these aromatic plants are responsible for their fragrances as well as biological properties¹. The term essential indicates that the oil carries distinctive scents (essences) of the plant, not that it is an especially important or fundamental substance. Essential oils do not as a group need to have any specific chemical properties in common, beyond conveying characteristic fragrances. Essential oils are complex mixtures of volatile secondary metabolites that mainly consist of mono & sesquiterpenes including carbohydrates, alcohols, aldehydes, ketones & ethers. These are responsible for both the fragrant and biological effects of aromatic medicinal plants²⁻⁴. An important characteristic of essential oils and their constituents is their hydrophobicity, which enables them to partition in the lipids of bacterial cell membranes & mitochondria, thus disturbing the structures & rendering them more permeable^{5,6}. Beside their antibacterial properties essential oils have also been shown to possess antifungal, antiviral, insecticidal and anti-oxidant properties^{7,8}. Some essential oils have also been used for cancer treatment⁹, food preservation¹⁰, aromatherapy¹¹ and fragrance industries. In the present scenario, interest of biologists to find antimicrobial properties in aromatic plants especially essential oils is continually increasing.

Increasing prevalence of MRSA infections coupled with social & economic implications, has led to constant striving to produce cost effective and safer substance for prevention & treatment of this disease. Natural products have recently been investigated vigorously as promising agents for prevention of MRSA infections. The increasing gain of resistance to available antimicrobials and side effects associated with these drugs have attracted the attention of scientific community towards the search and development of new cost effective drugs of natural & synthetic origin¹². Some studies demonstrated that plant derived essential oils may be an effective alternative to overcome microbial resistance to conventional drugs^{13,14}.

Present study was designed to investigate the antibacterial activity of essential oils against MRSA, since these bacteria are gaining worldwide attention due to increasing prevalence of infections. However, we also made an attempt to analyse the antibacterial activity of these essential oils in comparison with vancomycin drug, the most widely used antimicrobial against MRSA. This study also tried to straighten the traditional use of aromatic compounds in Indian civilization and its effect on MRSA occurrence.

MATERIALS AND METHODS

Essential oils obtained from Department of Pharmaceutical Science, Jiwaji University, Gwalior, India were used in this study. These oils were selected on the basis of literature survey and their use in traditional and conventional medicine.

Test organism

Micro-organisms used in this study were isolated from patients suffering from the MRSA infections. Test bacteria were characterized on the basis of their biochemical characteristics described earlier. More emphasis was placed to analyse antibacterial activity of plant essential oils against MRSA, so the reference strain of *Staphylococcus aureus* (ATCC No. 29213 and ATCC No. 25923) were also procured from HiMedia Pvt. Ltd. Mumbai, India. The cultures of bacteria were maintained in the Brain Heart Infusion Agar slants at 4°C throughout the study and used as stock culture.

Inoculum Preparation

Each culture to be tested was streaked onto a non-inhibitory agar medium (BHIA) to obtain isolated colonies. After incubation at 37°C overnight, few well isolated colonies were selected with an inoculating loop and the bacteria were inoculated in to a tube of non-selective broth (BHI broth) at 37°C. Then the turbidity of bacterial suspension was compared to 0.5 McFarland standards.

Inoculation Preparation

Within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab was dipped into the suspension. The swab was streaked over the entire surface of the medium three times, rotating the plate approximately 60° after each application to ensure an even distribution of the inoculum.

Antimicrobial analysis of essential oils

Screening of essential oils for antimicrobial activity was performed by disc diffusion method, which is normally used as rapid method to assess antimicrobial activity of essential oils⁷. The antimicrobial discs were prepared from Whatman filter paper no. 1. These discs were sterilized in hot air oven and stored in the refrigerator (4°C) for use. Upon removal of the discs from the refrigerator, the package containing the cartridges was left unopened at room temperature for approximately an hour to allow the temperature to equilibrate. This reduces the amount of condensation on the discs.

Under aseptic conditions, empty sterilized discs were impregnated with 25µl of respective

essential oil and placed on agar surface¹⁵. Antimicrobial discs were applied to the plates within 15 minutes after inoculation. Discs were placed individually with sterile forceps and then gently pressed down on to the agar. Diffusion of the oil in the disc begun immediately, therefore, once a disc contacted the agar surface, the disc was not moved.

Recording & Interpreting Results

After the inoculation of bacterial culture over media, plates were inverted and incubated at 37⁰ C for 16 to 18 hours. After incubation, the diameter of the zones of complete inhibition was measured and recorded in millimetres. The measurements were made with the help of antibiotic zone scale (HiMediaPvt. Ltd., Mumbai, India) on the under surface of the plate without opening the lid (Fig.1). With few plant essential oils, a slight amount of growth occurred within the inhibition zone. In this instance, slight growth (80% inhibition) was ignored and the zone diameter was measured to the margin of heavy growth¹⁶. Antibiotic (0.2% w/v) solution was used as a control during the study. Each experiment was performed in triplicate and the diameter of each of the plate was measured. The average diameter of zone of inhibition was recorded. The zones of inhibition were compared with each other and with antibiotic control.

RESULTS AND DISCUSSION

Antimicrobial activity was performed in triplicate and the mean zone of inhibition was recorded and analysed in triplicates. Plant essential oils and extracts have been used for many thousands of years¹⁷, in food preservation, pharmaceuticals, alternative medicine and natural therapies^{18,19}. It is necessary to investigate those plants scientifically which have been used in traditional medicine and improve the quality of healthcare. Essential oils are potential sources of novel antimicrobial compounds²⁰ especially against bacterial pathogens. In vitro studies in this work showed that the essential oils inhibited bacterial growth but their effectiveness varied. The antimicrobial activity of many essential oils have been previously reviewed and classified as strong, medium or weak²¹. Thus, the discovery of essential oils or components of such oils that possess biological properties has been the subject of many investigations: antiviral, antimycotic, antitoxigenic, and insecticidal.

In this study, we found highest activity of cinnamon oil and peppermint oil against selected bacteria (Table 2). Olive oil, Mustard oil, Rasna oil, Ajwain oil Tulsi oil, clove oil and Neem Oil have also shown very good activity against the test isolates. Present study is in concordance with the earlier studies showing the antimicrobial activity of these natural products. As explained in the earlier part of this study, the plants like Neem and Tulsi have antimicrobial activity in their

methanolic extracts as well besides in their oils. The antimicrobial properties of essential oils have been well recognized for many years²², and preparations of them have found applications as naturally occurring antimicrobial agents in pharmacology, pharmaceutical botany, phytopathology, medical and clinical microbiology and food preservation.

Table 1: List of selected essential oils used in the study

Code	Common Name	Botanical Name	Family
EO-1	Rasna oil	<i>Pluchelanceolata</i>	Compositae
EO-2	Ajwain oil	<i>Carumcopticum</i>	Umbelliferae
EO-3	Tulsi oil	<i>Ocimum sanctum</i>	Labiatae
EO-4	Peppermint oil	<i>Menthapiperata</i>	Alliaceae
EO-5	Garlic oil	<i>Allium sativum L.</i>	Alliaceae
EO-6	Til oil	<i>Tagetesminuta L.</i>	Alliaceae
EO-7	Cinnamon oil	<i>Cinnamomumzeylanicum</i>	Lauraceae
EO-8	Turpentine oil	<i>Pinuspinaster</i>	Pinaceae
EO-9	Eucalyptus oil	<i>Eucalyptus globulus</i>	Pinaceae
EO-10	Clove oil	<i>Syzygiumaromaticum</i>	Myrtaceae
EO-11	Mentha oil	<i>Menthaarvensis</i>	Lamiaceae
EO-12	Olive oil	<i>Oleaeuropea</i>	Oleaceae
EO-13	Mustard oil	<i>Brassica campestris</i>	Poaceae
EO-14	Castor oil	<i>Ricinuscommunis</i>	Euphorbiaceae
EO-15	Coconut oil	<i>Coccusnucifera</i>	Pamae
EO-16	Camphor oil	<i>Cinnamomumcamphora</i>	Lauraceae
EO-17	Amla oil	<i>Emblicaofficinalis</i>	Euphorbiaceae
EO-18	Neem oil	<i>Azadirachtaindica</i>	Meliaceae

Table 2: Antimicrobial resistance pattern of test bacterial organism against the selected plant essential oils included in the study

S. No.	Plant essential oils	Resistant	Sensitive
1	Rasna oil	17 (18%)	77 (82%)
2	Ajwain oil	17 (18%)	77 (82%)
3	Tulsi oil	17 (18%)	77 (82%)
4	Peppermint oil	0 (0%)	94 (100%)
5	Garlic oil	77 (82%)	17 (18%)
6	Til oil	77 (82%)	17 (18%)
7	Cinnamon oil	0 (0%)	94 (100%)
8	Turpentine oil	77 (82%)	17 (18%)
9	Eucalyptus oil	42 (45%)	52 (55%)
10	Clove oil	19 (20%)	75 (80%)
11	Mentha oil	42 (45%)	52 (55%)
12	Olive oil	17 (18%)	77 (82%)
13	Mustard oil	17 (18%)	77 (82%)
14	Castor oil	77 (82%)	17 (18%)
15	Coconut oil	77 (82%)	17 (18%)
16	Camphor oil	94 (100%)	0 (0%)
17	Amla oil	58 (55%)	36 (45%)
18	Neem oil	19 (20%)	75 (80%)
	Control (Vancomycin 40µl)	9 (9.57%)	85 (90.43%)

Several studies have shown that cinnamon and clove oil had strong and consistent inhibitory effect against various pathogens^{23,24}. Rasna oil also has many important medicinal applications: it is used as laxative, analgesic, antipyretic and nervine tonic²⁵, we also found activity with Rasna oil against MRSA. Camphor oil was not inhibitory for MRSA while amla oil was fairly active against the test bacteria. In opposite to this, Garlic oil was less active. This can be correlated with the differential properties and pathogenic capabilities of the test organism. The activity of cinnamon oil and peppermint oil were found to be more than vancomycin drug control. So these can be better inhibitory agents for inhibition of MRSA after suitable toxicological analysis. All other oils showed moderate activity.

Cinnamon oil & clove oil are very ancient ingredients of food in Indian system; their antimicrobial activity further supports the traditional natural medicinal system of India. Previous GC-MS study of cinnamon oil has shown various components of this oil. Cinnamaldehyde was the predominant active component found in cinnamon oil^{26,27}. Earlier studies suggested that the antibacterial activity of cinnamon oil was probably due to their major component, cinnamaldehyde and their properties could be multiple. Cinnamaldehyde is a natural anti-oxidant and the animal studies suggest that an extract of cinnamon bark taken orally may help to prevent stomach ulcer. Cinnamaldehyde was completely inhibiting both sensitive & resistant strain of *Helicobacter pylori*²⁸. Cinnamon oil was not harmful when consumed in food products and it inhibited the growth of molds, yeast & bacteria²³. The use of commercial cinnamon preparation produced an improvement of oral candidiasis of HIV infected patients²⁹. Cinnamon oil is locally applied with much benefit in neuralgia and headache. As an antiseptic, it is used internally in typhoid fever. This oil is also used in the treatment of cancer & other microbial diseases³⁰.

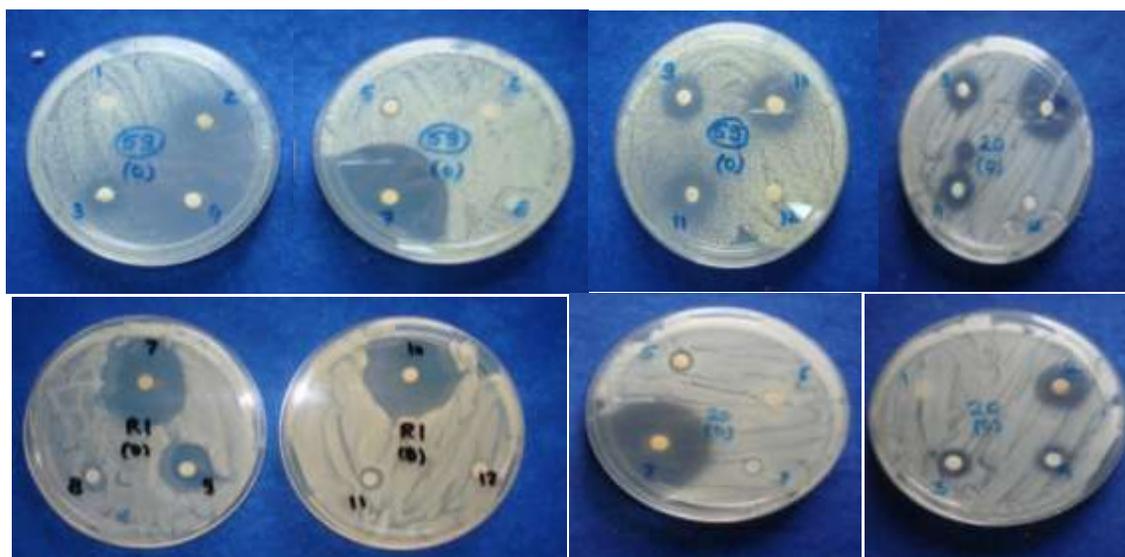


Figure 1. Antibacterial activity of plant essential oils against the test bacteria

Zone diameter measured with the antibiotic susceptibility zone diameter measurement scale (HiMedia Pvt. Ltd., Mumbai). The clear zone (absence of growth) indicates sensitivity of the test isolate against the essential oil. Zone diameter less than 10mm is considered to be resistant and more than 10mm is sensitive.

Clove oil has also been shown as potent antimicrobial agent against various pathogenic strains of bacteria and fungi including *Aspergillus*, *Penicillium* & yeast etc.²³. Methanolic extract of clove has shown to inhibit many pathogenic bacteria. The main constituents of the essential oil are phenyl propanoids such as carvacrol, thymol, eugenol & cinnamaldehyde³¹. The characteristic aroma of clove is due to the compound eugenol. It is the main component of clove essential oil, comprising 72-90%³².

CONCLUSION

This study confirms that many essential oils possess in vitro antibacterial activity. We believe that the present investigation together with previous studies provide support to the antibacterial properties of peppermint oil and cinnamon oil. These can be used as antibacterial supplement in the developing countries towards the development of new therapeutic agents. This can be helpful in designing antimicrobial agents for topical & oral applications also.

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