EFFECT OF ESSENTIAL OILS ON MDR PATHOGENS: A COMPARATIVE STUDY

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ABSTRACT

Comparison of the antimicrobial activity of eucalyptus (Eucalyptus citriodora), clove (Syzygium aromaticum) and pine oils (Pinus sylvestris) against eleven Multiple Drug Resistant (MDR) pathogens was studied. Maximum antibacterial activity was shown by clove oil (Syzygium aromaticum), followed by eucalyptus oil (Eucalyptus citriodora). Pseudomonas aeruginosa was found to be inhibited maximum by clove oil. Eucalyptus and clove oils were found to be most effective against MDR pathogens, except Salmonella typhi. A comparative study of effect of fourteen essential oils on different species of same genera was performed and it was observed that the most pathogenic and most common species (Klebsiella pneumoniae and Proteus vulgaris) were inhibited the least by the oils. Rare and less pathogenic species are found to be more sensitive towards the essential oils. Citrobacter diversus and Citrobacter freundii had shown almost similar sensitivity patterns.

Key Words: Essential oils, Multiple drug resistant, Antibiotics, Pathogens, Antibacterial activity

INTRODUCTION

Many microorganisms, which cause damage to human health, exhibit drug resistance due to inadequate use of antibiotics. Thus, there is a need for the search of new substances from natural sources including plants. Essential oils of many medicinal plants have been used for evaluation of their antibacterial and antifungal activities by many workers 1-3. The oils as well as their major components i.e. thymol and eugenol are responsible for antimicrobial activity. The essential oils of four Ocimum sp. grown in Rwanda were found to display antimicrobial activity as demonstrated earlier4. It has been reported that the volatile oil of this plant contains mostly phenols, particularly thymol and that these are probably responsible for its reported antimicrobial action. The antibacterial activity of different extracts from the leaves of Ocimum gratissimum against Staphylococcus aureus, E. coli, Salmonella typhi and Salmonella typhimurium was studied earlier5. Multidrug resistant bacteria and fungi of clinical origin were found to be inhibited by five herbal extracts6. Some workers used essential oils and plant extracts to control multidrug resistant bacteria infecting upper respiratory system in human population7, also some demonstrated the antibacterial activity of clove against gram negative bacteria8. The chemical composition of clove oil and its correlation with antimicrobial activity against fish systemic bacteria isolated from aquaculture sites was established earlier9.

AIMS AND OBJECTIVES

The aim of the present investigation was to evaluate the effects of commonly available essential oils on multi drug resistant enterobacterial pathogens.

MATERIAL AND METHODS

The susceptibility of MDR pathogens to essential oils was tested by disc diffusion
method on chlorephenicol and gentamycin supplemented Muller Hinton Agar following the method described earlier\textsuperscript{10-12}. Twenty four hour grown culture (0.5 ml) was inoculated in 10 ml sterile molten Muller Hinton Agar (seed agar) and poured on Muller Hinton Agar plates (base agar). Sterile disc of 5 mm diameter of whatmann paper no.2 loaded with 5µl of test oil were placed on solidified medium and the plates were incubated at 37 °C for 48 hours\textsuperscript{13,11}. Diameter of zone of inhibition of growth was measured in mm by zone reader. Each test was performed in triplicate. Essential oils purchased from local market and botanical nomenclature of source plants are presented in Table 1. MDR isolates used in the study were \textit{E. coli}, \textit{Klebsiella pneumoniae}, \textit{Citrobacter diversus}, \textit{Shigella flexneri}, \textit{Salmonella typhi}, \textit{Klebsiella ozaenae}, \textit{Proteus vulgaris}, \textit{Citrobacter freundii}, \textit{Pseudomonas aeruginosa}, \textit{Klebsiella oxytoca} and \textit{Proteus myxofaciens}.

\textbf{RESULTS AND DISCUSSION}

The antimicrobial activity of eucalyptus (\textit{Eucalyptus citriodora}), clove (\textit{Syzygium aromaticum}) and pine oils (\textit{Pinus sylvestris}) against 11 MDR pathogens was evaluated (Fig. 1) Maximum antibacterial activity was shown by clove oil (\textit{Syzygium aromaticum}), followed by eucalyptus oil (\textit{Eucalyptus citriodora}). Eucalyptus and clove oils were found to be most effective against all pathogens except \textit{Salmonella typhi}. Effect of eucalyptus oil against \textit{Salmonella typhi} has also been previously demonstrated with similar results\textsuperscript{14}. Clove oil had shown maximum zone of inhibition against most of the isolates, followed by eucalyptus oil. Number of isolates inhibited by both the oils is same, but clove oil gave larger zone diameters. Results obtained for clove oil

\begin{table}
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\begin{tabular}{|c|c|c|}
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\textbf{S/N} & \textbf{Essential oils} & \textbf{Botanical nomenclature of source plant} \\
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1. & Eucalyptus oil & \textit{Eucalyptus citriodora} \\
2. & Palmarosa oil & \textit{Cymbopogon martini} \\
3. & Clove oil & \textit{Syzygium aromaticum} \\
4. & Peppermint oil & \textit{Mentha piperita} \\
5. & Tulsi oil & \textit{Ocimum sanctum} \\
6. & Geranium oil & \textit{Pelargonium graveoleus} \\
7. & Sweet basil oil & \textit{Ocimum gratissimum} \\
8. & Black pepper oil & \textit{Piper nigrum} \\
9. & Lemon grass oil & \textit{Cymbopogon citratus} \\
10. & Thyme oil & \textit{Thymus vulgaris} \\
11. & Amla oil & \textit{Emblica officinalis} \\
12. & Citronella oil & \textit{Citronella spp.} \\
13. & Pine oil & \textit{Pinus sylvestris} \\
14. & Orange oil & \textit{Citrus aurantium} \\
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\end{tabular}
\caption{Essential oils and botanical nomenclature of source plants}
\end{table}
against *Pseudomonas aeruginosa*, *Shigella flexneri*, *Proteus vulgaris* in the present study were found to be similar as also reported by several workers\(^{10,15}\). Pine oil gave varied zone of inhibition ranging from 4 mm to 14 mm. Of the three oils, *Salmonella* was sensitive towards pine oil with a moderate zone of inhibition and was resistant towards eucalyptus and clove oils\(^{16}\). A maximum zone of inhibition (19.5 mm) was observed against *Proteus vulgaris* for clove oil. *E. coli* had shown maximum sensitivity towards clove oil followed by eucalyptus oil. It was found to be least sensitive towards sweet basil oil. *Pseudomonas aeruginosa* was found to be inhibited maximum by clove oil.

Comparison of sensitivity patterns of *Klebsiella pneumoniae*, *Klebsiella oxytoca* and *Klebsiella pneumoniae* sub sp *ozaenae* against 14 essential oils was performed (Fig 2). It was observed that all the three species (*K. pneumoniae*, *K. ozaenae* and *K. oxytoca*) gave almost similar zone diameters against eucalyptus, clove, geranium and thyme oils. *Klebsiella oxytoca* was found to be most sensitive (towards 9 oils) followed by *Klebsiella pneumoniae* sub sp *ozaenae* (towards 7 oils). Least sensitive was *Klebsiella pneumoniae*, the most pathogenic strain (towards 5 oils). Maximum zone of inhibition was observed for clove oils as also demonstrated earlier\(^{16}\), followed by pine oil. Similarly, sensitivity pattern of *Proteus vulgaris* and *Proteus myxofaciens* was studied (Fig 3). *P. vulgaris* was found to be inhibited by eucalyptus, palmarosa and clove oils with larger zone diameter. The above three oils have shown greater potency against *P. vulgaris*. Rest of the oils has shown less or no activity against *P. vulgaris*. *Proteus myxofaciens* was found to be sensitive towards most of the essential oils whereas *Proteus vulgaris* was found to be sensitive towards 8 oils only. Maximum zone diameter was observed for *Proteus myxofaciens* towards sweet basil oil (*Ocimum gratissimum*). Clove oil and eucalyptus oil had shown good activity against *Proteus vulgaris*. Thyme oil (*Thymus vulgaris*) had shown least activity. Comparison of *Citrobacter diversus* and *Citrobacter*
freundii towards essential oils (Fig. 4) has shown that both the pathogens showed almost similar sensitivity pattern against all the oils. These were moderately inhibited by eucalyptus, clove and thyme oil. Rest all the oils had shown low or no activity against C. diversus and C. freundii.

CONCLUSION

The antimicrobial effects of all the oils were found to be different for different species of same genera of pathogens. Among various species of the genera Klebsiella and Proteus, it was observed that the most pathogenic and most common species (Klebsiella pneumoniae and Proteus vulgaris) are inhibited the least by the oils. Rare and less pathogenic species are found to be more sensitive towards the essential oils. But Citrobacter diversus and Citrobacter freundii had shown almost similar sensitivity patterns. The reason is not known. Eucalyptus and clove oils were found to be most effective against MDR pathogens. Use of essential oils is suggested to overcome the problem of antibiotic resistance among pathogens.

REFERENCES

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