

REVIEW ARTICLE

USE OF ESSENTIAL OILS AGAINST GRAM NEGATIVE PATHOGENS

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ABSTRACT:

The emergence of resistance to conventional antimicrobials has remained a serious problem for physicians. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plants. These products are known by their active substances like essential oils containing phenolic ingredients. The volatile oil of *Ocimum* contains mostly phenols, particularly thymol, those are probably responsible for its reported antimicrobial action. The antibacterial properties of *P. regnellii* (black pepper) justify its use in traditional medicine for treatment of wounds contaminated through bacterial infections. Thyme oil is widely used for inhibiting gram negative pathogens, carvacrol to be a predominant compound in thyme oil. Disk diffusion method has been commonly used to test the antimicrobial activity of essential oils. Use of essential oils is suggested to overcome the problem of antibiotic resistance.

Key Words: antibiotic resistance, antimicrobial activity, essential oils

INTRODUCTION

Multiple Drug Resistant Enterobacterial pathogens have always been known as etiological agents for causing diseases like typhoid, pneumonia, UTI, colitis, dysentery and salmonellosis. The main problem arises for the treatment of such infections due to their drug resistant nature. The emergence of resistance to conventional antimicrobials has remained a serious problem for physicians. The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. The need of the day is to continue studies to develop new drugs, either synthetic or natural. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient.

World Health Organization has suggested the use of medicinal plants as a source of a variety of drugs. Antimicrobial effect of various plant oils have mostly been studied against antibiotic sensitive bacteria and fungi. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plants. These products are known by their active substances like essential oils containing phenolic ingredients. Essential oils of various medicinal plants including pine oil (*Pinus sylvestris*), tulsi oil (*Ocimum gratissimum*), eucalyptus oil (*Eucalyptus citriodora*) and black pepper oil (*Piper regnellii*) have been used by several workers; however, their effect against MDR Gram-negative pathogens has remained a less explored field.

ESSENTIAL OILS AS ANTIMICROBIAL AGENTS:

Aromatic plants have been traditionally used in folk medicine as a cure for many diseases as well as for extending the shelf life of food materials. The medicinal and preservative properties of most of the plants are due to essential oils produced as a result of secondary metabolism. In spite of production of a number of new

antibiotics by pharmaceutical industries in last three decades, resistance development by microorganisms limited the use of these drugs for the treatment of diseases. The growing problem of antibiotic resistance has made it compulsory to look for the suitable alternatives.

Essential oils of many medicinal plants have been used for evaluation of their antibacterial and antifungal activities by many workers¹⁻⁴. The oils as well as their major components *i.e.* thymol and eugenol are responsible for antimicrobial activity. Resistance of *Salmonella typhi* towards clove oil was also demonstrated earlier⁵. The activity of pine oil was demonstrated against fungi and gram-positive bacteria by many workers^{6, 7}. Essential oils of citronella (*Citronella sp.*), lemongrass (*Cymbopogon citratus*), peppermint (*Mentha piperita*), palmarosa (*Cymbopogon martini*) and geranium (*Pelargonium graveolens*) had shown moderate activity against most of the MDR pathogens giving moderate zone of inhibition. These oils were also tested against gram positive, gram-negative bacteria and some fungi by some workers^{8, 9}. Effect of eucalyptus oil against *Salmonella typhi* has also been previously demonstrated with similar results¹⁰. Maximum antimicrobial activity was shown by clove oil with largest zone diameter as also demonstrated earlier⁵, followed by pine oil and thyme oil.

TULSI OIL (*OCIMUM GRATISSIMUM*)

Ocimum gratissimum (Tulsi) is widely distributed in tropical and warm temperate regions. The plant is commonly used in folk medicine to treat different diseases such as upper respiratory tract infection, diarrhoea, headache, skin disease, pneumonia *etc.* Moreover, lot of work has been done to show the antimicrobial properties of this plant to some selected pathogens. It has been reported to be active against several species of bacteria and fungi. The volatile oil of

Ocimum contains mostly phenols, particularly thymol, those are probably responsible for its reported antimicrobial action^{11,12}.

The antimicrobial activity of four species of *Ocimum* *canum*, *O. gratissimum*, *O. trichodon* and *O. urticifolium* has been demonstrated^{1,13}. The antibacterial activity of essential oil of *Ocimum gratissimum* was also reported¹¹. The essential oil of *tulsi* inhibited *Staphylococcus aureus* at a concentration of 0.75 µg ml⁻¹. The minimal inhibitory concentrations for *Shigella flexneri*, *Salmonella enteritidis*, *Escherichia coli*, *Klebsiella sp.* and *Proteus mirabilis* were ranging from 3 to 12 µg ml⁻¹. *Pseudomonas aeruginosa* was considered resistant because it gave no zone of inhibition. The compound that showed antibacterial activity in essential oil of *O. gratissimum* was identified as eugenol and structural findings were supported by gas chromatography / mass spectra retention data. The antibacterial activity of different extracts from the leaves of *Ocimum gratissimum* against *Staphylococcus aureus*, *E. coli*, *Salmonella typhi* and *Salmonella typhimurium* was studied¹². Multidrug resistant bacteria and fungi of clinical origin were found to be inhibited by five herbal extracts¹⁴. Use of essential oils and plant extracts to control multidrug resistant bacteria infecting upper respiratory system in human population was studied¹⁵.

PEPPER OIL (*PIPER REGNELLII*)

The essential oils of *Piper cermum* and *Piper regnellii* (black pepper) showed growth inhibitory activities against *Staphylococcus aureus* and *Candida albicans*¹⁶. Oil of *Piper regnellii* suppressed the growth of *Staphylococcus aureus* and *Bacillus subtilis*, a moderate activity against *Pseudomonas aeruginosa* and weak activity against *E. coli*¹⁷. Similarly, an aqueous extract of the leaves of *Piper regnellii* displayed a weak activity, whereas ethyl acetate extract gave good activity against *Staphylococcus aureus* and *B. subtilis*¹⁸. Gram negative bacteria were not inhibited by the extracts below a concentration of 1000 µg ml⁻¹. The antibacterial properties of *P. regnellii* (black pepper) justify its use in traditional medicine for treatment of wounds contaminated through bacterial infections.

THYME (*THYMUS VULGARIS*)

The antimicrobial and fungicidal activities of *Thymus vulgaris* (thyme), *Rosamarinus officinalis* (rosemary) and *Calamintha nepeta* were analyzed¹⁹ and found that all oils showed biotoxic effect, the most active being those from *Calamintha* and *Thymus*. Thyme essential oils for their inhibitory effects was tested against nine strains of gram negative bacteria and six strains of gram positive bacteria and observed that the bacteriostatic activity was more marked against gram positive bacteria, whereas antibacterial activity was more marked against gram negative bacteria²⁰. Carvacrol was identified to be a predominant compound in thyme oil²¹. The essential oil of *Thymus revolutus* was tested against eleven bacteria and four fungi at different concentrations and was found to exhibit significant antibacterial and antifungal activity. The composition of essential oils of some *Thymus* species was analyzed and *in vitro* antimicrobial activities of its components were reported and antibacterial activity of

methanolic extract of *Thymus pubescens* was detected^{22,23}.

Similarly, antimicrobial activity of twenty specimens of essential oils of 11 *Thymus sp.* on *Staphylococcus aureus*, *S. pyogens* and *S. pneumoniae* was tested and recorded that all samples of oil possessed strong antibacterial activity²⁴. Bactericidal activity of essential oils extracted from two varieties of *Thyme* was examined against *E. coli*, *S. aureus*, *B. subtilis*, *K. pneumoniae* and *P. aeruginosa* and concluded that high phenol rich aromatic content of oils was responsible for strong antibacterial activity²⁵.

Antimicrobial activity of hydro distilled essential oils of *Thyme* was tested against *Candida albicans*, *E. coli*, *Listeria monocytogenes*, *Proteus mirabilis*, *Salmonella sp.* and *S. aureus* and observed that these organisms show significantly different sensitivities to thyme oil as tested by disk diffusion method²⁶. It was concluded that the antimicrobial activity of essential oils may be related to more than one component in the oil. The antimicrobial activity of essential oils of *Thymus vulgaris*, *Ocimum gratissimum*, *Eugenia caryophyllata* and *Melaleuca viridiflora* was reported by determination of their respective MIC on enteropathogenic and bacterial strains responsible for food spoilage and observed that the oils from *Thymus*, *Ocimum* and *Eugenia* exhibited wide inhibition spectrum¹.

EUCALYPTUS OIL (*EUCALYPTUS CITRIODORA*)

Essential oil of eucalyptus was used to study its antimicrobial activity against multidrug resistant *E. coli*, *Proteus*, *Klebsiella*, *Pseudomonas* and *S. aureus*. The results of the study revealed that the oil of eucalyptus has antibacterial activity against gram positive as well as gram negative bacteria²⁷. The antibacterial activity of leaf extracts of *Eucalyptus camaldulensis* and the antimicrobial activity of combination of eucalyptus oil and 1, 8-cineole with chlorhexidine digluconate against microorganisms was studied²⁸⁻³⁰.

METHODS OF TESTING

The alcoholic extracts of 82 traditional Indian medicinal plants were tested against several opportunistic pathogens and observed that the extracts of five plants showed strong and broad-spectrum antimicrobial activity as compared to the rest³¹. Among various extracts, alcoholic extracts of *Embllica officinalis* (amla) showed highest activity against test bacteria. Five plants were selected including *Embllica officinalis*, *Terminalia chebula*, *T. bellirica*, *Plumbago zeylanica*, *H. antidysenterica*, to determine the antimicrobial potency of their alcoholic extracts and their minimum inhibitory concentration (MIC) against several pathogenic or potentially pathogenic organisms³². Maximum potency (lowest MIC) was shown by *Embllica officinalis* (amla) against *Staphylococcus aureus*, *S. epidermidis* and *Salmonella typhimurium*. MIC for amla ranged from 950-1200 µg ml⁻¹. Synergistic antimicrobial action of these alcoholic extracts was also recorded against *S. aureus*, *E. coli* and *Candida albicans*. Phytochemical analysis of these extract revealed the presence of biologically active substances like tannins, glycosides, flavonoids and saponins.

Experiments were carried out to determine the potential of powder and essential oil of dried ground leaves of *Cymbopogon citratus* (lemon grass) to control aflatoxin contamination of melon seeds³³. Essential oil of lemon grass was mixed with the seeds inoculated with aflatoxin producing *Aspergillus flavus* strain. The essential oil at a concentration of 0.1 and 0.25 % (v/w) significantly reduced the concentration of aflatoxin in melon seeds. At higher doses (0.5 and 1.0 % v/w), the essential oil completely prevented aflatoxin production and growth of *A. flavus*.

Fifty two plant oils and plant extracts were tested against Gram positive and Gram negative organisms. Lemon grass oil and oregano oil inhibited all organisms. MIC of thyme oil was 0.03% (v/v) against *Candida albicans* and *E. coli*³⁴. The antimicrobial activity of volatile oil of black pepper, clove, geranium, nutmeg, oregano and thyme against 25 different genera of bacteria was studied³⁵. These included animal and plant pathogens, food poisoning and spoilage bacteria. The volatile oils exhibited considerable inhibitory effects against all the organisms tested.

Disk diffusion method originally developed by Bauer³⁶ and has been commonly used to test the antimicrobial activity of essential oils. The essential oils of aegle, ageratum, citronella, eucalyptus, geranium, lemongrass, orange, palmarosa, patchouli and peppermint were tested for antibacterial activity against 22 bacteria, including

gram positive cocci and rods, gram negative rods and 12 fungi by the disk diffusion method³⁷. The oils of lemongrass, eucalyptus, peppermint and orange were found to be effective against all 22 bacterial strains. The MIC of eucalyptus, lemongrass, palmarosa and peppermint oils ranged from 0.16 to >20 $\mu\text{l ml}^{-1}$ for 18 bacteria and from 0.25 to 10 $\mu\text{l ml}^{-1}$ for 12 fungi. Essential oils of cinnamon, clove, eucalyptus, oregano, rosewood, sage, tea tree and red thyme were also tested using disk diffusion method³⁸.

The antimicrobial activity of aqueous extracts of dry fruits and seeds of *Caesalpinia digyna* at various concentrations were tested on some human pathogens using disk diffusion method³⁹. The fruit extract showed broad spectrum of antimicrobial property and was more effective than the seed extract. *Staphylococcus aureus* was inhibited the most and *Pseudomonas aeruginosa* the least by fruit extract. *Salmonella typhi* was inhibited the most and *Yersinia enterocolitica* the least by the seed extract. Similarly, the disk diffusion method was used to evaluate the zone of microbial growth inhibition at various concentrations of thyme oil. High aromatic compound content of phenol rich oils accounted for strong antibacterial activity²⁵.

Disk diffusion method has been commonly used to test the antimicrobial activity of essential oils. Use of essential oils is suggested to overcome the problem of antibiotic resistance.

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