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Research Article

COMPARATIVE EVALUATION OF ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL PLANT OILS ON MULTIDRUG RESISTANT NOSOCOMIAL PATHOGENS

Shilpa M Shenoy[1], A. Veena Shetty[1*] and Vinaya Bhat[2]

[1]Department of Microbiology, K.S. Hegde Medical Academy, Nitte University, Mangalore - 575018, Karnataka, India.

[2]Department of Prosthodontics and Implantology, A.B. Shetty Memorial Institute of Dental Sciences, Nitte University, Mangalore - 575018, Karnataka, India.

ABSTRACT

The problem of microbial resistance is growing and the lookout for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce this problem, and to offer appropriate and efficient antimicrobial drugs to the patient. Down the ages essential oils and other extracts of plants have evoked interest as sources of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases. This study was undertaken to evaluate the antimicrobial activity of commercially available Coconut oil, clove oil, Castor oil, Eucalyptus oil, Neem oil and extracted oreganum oil on the multidrug resistant nosocomial pathogens. The oreganum oil, clove oil eucalyptus oil and neem oil showed antimicrobial activity. With proper studies they can be a beacon of hope in long dark tunnel of dwindling era of new antibiotics.

Keywords: multidrug resistance, essential oil, nosocomial pathogens.

INTRODUCTION

Antimicrobial resistance has been on rise ever since the use of antibiotics. The dream to overcome the resistance of antimicrobials against the microorganisms is still a long way from fulfilment. A report published by the Infectious Diseases Society of America, published in 2009[1], stressed on the increase of the antimicrobial resistance among the gram positive and gram negative pathogens that cause infection in the hospitals and in the community. The report identified "ESKAPE" pathogens *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumanii, Pseudomonas aeruginosa,* and *Enterobacter* species to emphasize that they currently cause the majority of hospital infections and effectively "escape" the effects of antibacterial drugs. Several highly resistant gram-negative pathogens—namely *Acinetobacter* species, multidrug-resistant (MDR) *P.aeruginosa*, and carbapenem- resistant *Klebsiella* species and *Escherichia coli*—are emerging as significant pathogens around the world. The therapeutic options for these pathogens are so extremely less and despite ongoing efforts the number of new antibacterial drugs approved for marketing in the United States and the world continues to decrease[2].

Due to the adverse effects of chemical antibiotics and the resistant microorganisms, the scientists have explored new sources for antimicrobial agents especially among medicinal plants. Numerous plants are known to possess medicinal or food values for humans and are used for treatment of human ailments[3]. Traditional medicine is the main source of medical care for a great proportion of the population of the developing world.

Essential oils and plant extracts based on ethnomedicinal uses are potential sources of new antimicrobial compounds against microbial strains. The combined use of plant extracts or essential oils and antibiotics are useful in decreasing drug resistant problems[4]. Plant products are a great choice and an alternate to synthetic antibiotics due to their ready availability, biodegradability and low cost factor. The antimicrobial property of essential oils from plants was used since old ages, but now there is renewed interest in the search for plants with anti-microbial activity. There are numerous medicinal plant species that are used by Indian population.

The emergence of antimicrobial resistance, coupled with availability of fewer new antimicrobial drugs, led us to conduct this study using the plant oils which are commonly found in the Indian household. We used Coconut oil, Castor oil, Eucalyptus oil, Neem oil, Clove oil and Oreganum oil against the Extended spectrum Beta lactamase(ESBL) and Methicillin resistant *Staphylococcus aureus* (MRSA) producing nosocomial pathogens.

The aim of the study was to determine the antimicrobial susceptibility pattern of organisms producing ESBL in Gram negative bacilli and MRSA obtained from the hospitalised patients.

To determine the antimicrobial activity of the commercially available essential plant oils on the most common ESBL and MRSA organisms isolated from hospitalised patients.

MATERIALS AND METHODS

The most common bacterial nosocomial pathogens *E.coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Acinetobacter baumanii* and *Staphylococcus aureus* were isolated from the various clinical specimens obtained from the hospitalised patients. They were identified by colony morphology, Gram staining and standard biochemical tests. Antimicrobial Susceptibility patterns were determined by Modified Kirby-Bauer method on Muller Hinton agar.

Detection of MRSA and ESBL

Screening for Methicillin resistant *Staphylococcus aureus*(MRSA) was done by disc diffusion method using Cefoxitin disc(30 µg). Detection of Extended Spectrum Beta Lactamse (ESBL) enzyme was done by double disc diffusion method for screening and confirmatory tests set by CLSI guidelines. Screening for ESBL production was done by placing Cefotaxime (30µg), Ceftazidime(30µg) and Ceftriaxon(30µg) on inoculated Mueller-Hinton agar plates and incubated for 24 hours at 37°C. When the inhibition zones of the drugs for gram negative bacteria were ≤27mm for Cefotaxime(30µg), ≤22mm for Ceftazidime(30µg), ≤25mm for Ceftriaxon(30µg) respectively ,the strain was suspected as a potential ESBL producer. The potential ESBL isolate in *E.coli* and *Klebsiella pneumoniae* was then confirmed using Cefotaxime (30µg), ceftazidime (30µg), alone and in combination with clavulanic acid on Muller hinton agar. The test organism was identified as a ESBL producer when the zone of inhibition of ceftazidime (30µg) or Cefotaxime (30µg) combined with inhibitor showed difference of ≥5mm compared with cephalosporins alone [5].

ESBL production in *P.aeruginosa* and *A.baumanii* was detected by double disc synergy test [6]. Synergy was determined between a disc of amoxyclav (20 µg amoxycillin and 10 µg clavulanic acid) and a 30-µg disc of each 3GC test antibiotic placed 15 mm apart on a lawn culture of the isolate. The test organism was considered to produce ESBL if the zone size around the antibiotic disc increased towards the amoxyclav disc. This increase occurs because the clavulanic acid present in the amoxyclav disc inactivates the ESBL produced by the test organism

Antimicrobial Activity of the plant oils

Five commercially available plant oils were procured: Eucalyptus oil, Neem oil, Castor oil, Coconut oil and Clove oil; Ooms laboratory Shimoga. Oreganum oil was extracted through standard procedures. All the oils were checked for authenticity by a pharmacognosist. The purchased oils were not diluted and not altered chemically by any solvent or processing.

Antibacterial activity of the oils were tested using the agar well diffusion method[7]. The test inoculum (0.5 McFarland's turbidity) was spread onto Muller-Hinton agar by using a sterile cotton swab. Then the wells were punched by a sterile well puncture. Twenty µl of oils were added to each well and incubated at 37°C for 24 h. The presence of zones of inhibition was regarded as the presence of antimicrobial action. The diameter of zone of inhibition was measured in mm. Antimicrobial activity was expressed in terms of average diameter of the zones of inhibition measured.

MIC and MBC

The broth microdilution method was used to determine Minimum inhibition concentration (MIC) and Minimum bactericidal (MBC). All tests were performed in Mueller-Hinton broth. Briefly, serial doubling dilutions of the extract were prepared in a 96-well microtiter plate ranging from 100% to 0.098%. Finally, 10 µl of bacterial suspension (106 CFU/ml) was added to each well. The plates were wrapped loosely with cling film to ensure that the bacteria did not get dehydrated. The plates were prepared in triplicates, and then they were placed in an incubator at 37°C for 18-24 hours[8]. The results were expressed as mean and or ranked in order of importance as percent.

RESULTS

The organisms selected for the study were multidrug resistant organisms. Organisms which were found to be resistant to three or more different classes of antibiotics were defined as multidrug resistant organisms. All the gram negative bacterial isolates included in the study were resistant to third generation cephalosporin. But were susceptible to carbapenems, and Staphylococcus aureus was resistant to cefoxitin but susceptible to vancomycin.

The essential plant oils exhibited very good antimicrobial activity on the nosocomial bacterial pathogens as compared to the control antibiotics, i.e Imipenem for gram negative bacteria and Vancomycin for *S.aureus*. The oreganum oil exhibited the most antibacterial activity against the tested bacteria. It showed better antibacterial activity as compared to the control antibiotics. Eucalyptus oil and clove oil also exhibited better antibacterial activity as compared to the neem oil, coconut oil and castor oil. The oils exhibited the highest antibacterail activity against *E.coli and S.aureus* as compared to other tested organisms (Table 1).

The MIC was defined as the lowest concentration of the extract at which the microorganism does not demonstrate the visible growth. The MBC was defined as the lowest concentration of the extract at which the incubated microorganism was completely killed. The average of 3 values was calculated providing the MIC and MBC values for the tested oils. Among the oils studied, oregano oil exhibited the lowest inhibitory and bactericidal concentration against all the microorganisms tested. Eucalyptus oil, clove oil and neem oil followed not far behind (Table 2).

DISCUSSION

The emergence of resistance to conventional antimicrobials is a serious problem that physicians face. This necessitates constant development of newer agents, which can inhibit the growth of or kill resistant organisms. The oils of medicinal plants have been used for treatment of various ailments since times immemorial. Numerous studies have been conducted to screen the antimicrobial activity of different plant extracts and essential oils against human pathogens, but this study was conducted to find the antimicrobial activity of commonly found plant oils in every Indian household against the multidrug resistant nosocomial pathogens. These oils are commonly used as food flavouring agents, in small doses in clinical setting for numerous medical conditions such as liver and gallbladder disturbances, abscesses, headaches, appendicitis, epilepsy, hemorrhoids, constipation, diarrhoea, intestinal obstructions, skin diseases, hyper activity in children and to avert threatened abortion in pregnant women, antiparasitic [9,10,11].

Our investigation on commonly used plant oils showed that the oreganum oil had the highest activity against the Multi Drug Resistant (MDR) organisms tested. It showed better activity than the control antibiotics used in the study. Our results are also supported by previous authors oreganum oil has shown great antimicrobial activity against numerous microorganisms, it has been proved to be antibacterial-not only against the human pathogens but also against animal, plant pathogens and food spoiling bacteria, antifungal. Clove oil exhibited good antimicrobial activity against the MDR organisms in our study.

The studies conducted by L Mayaudi[12], H.J.D Doroman[13], D Stojkovic[14] found that Oregano and clove oil had had highest inhibitory activity on the tested pathogens. Clove oil exhibited great antibacterial activity against Multidrug resistant *Pseudomonas*[15] and *E.coli*[16]. A large scale study conducted by Mendell Friedman[17] on 96 essential oils and 23 compounds against the bacteria isolated from food and clinical cases found that oregano oil and clove oil were effective against *E.coli, L.monocytogens, S.enterica, C.jejuni*. Similarly, another study[18] found that among the 56 essential oils tested exhibited highest antimicrobial activity against *A.baumanii, E.coli, K.pneumoniae, P. aeruginosa, and S.aureus.*

Eucalyptus oil also exhibited great antimicrobial activity against the multidrug resistant organisms tested in our study. In support to our study, studies conducted by T Takahashi[19] and Fatemeh

Mohsen Nezhad[20] found that antimicrobial activity was found greatly among the Gram positive organisms including MRSA. Biljana's[21] study found strong antimicrobial activity of Eucalyptus essential oil on *S.pyogens, E.coli, Candida , Acinetobacter* species and *Klebsiella* species.

Neem an evergreen tree has been a source of numerous medicinal properties. Our study showed a moderate antimicrobial activity against the MDR organisms. Our work is in support with the study done by M M Nijire[22],they found that neem oil had comparatively much lesser activity against the MDR organisms. Whereas studies conducted by other authors found that the ethanolic and dichloromethane leaf extracts of Neem were more active towards the bacterial species used in their study [23]. Aditi Grover.et al[24] found that the methanol extract of Neem leaves were effective as 100µg/ml Gentamicin against *Bacillus subtilis* and 20µg/ml Tetracycline against both *Staphylococcus aureus* and *Proteus vulgaris* and 10µg/ml Gentamycin against *Pseudomonas aeruginosa*. R K Upadhyaya[25] and his group found that among all essential oils, they tested almond and neem oils were found to be highly bactericidal, as they exhibited lowest MIC and MBC values and high growth inhibition zone diameter in comparison to antibiotics. Present study reveals significantly higher broad-spectrum antibacterial activity in essential oils than antibiotics i.e., tetracycline, ampicillin and ciprofloxacin.

Coconut and its numerous products find its uses in our daily life, coconut oil is consumed on daily basis in coastal Karnataka region. Hence it was used in our study, according to our results commercially available coconut oil did not show any antimicrobial activity against the multi drug resistance organisms. Our results support a study conducted by Chiaw Mei Sia et.al[26], they also found that the commercial available coconut oil did not exhibit any antimicrobial properties. But there are studies conducted on the fermented coconut oil [27] which exhibited antibacterial activity against *Bacillus subtilis, Escherichia coli, Pseudomonas fluorescence, Bacillus cereus* and *Salmonella.* Studies conducted on mesocarp extracts, extracted from different solvents also exhibited antimicrobial activity against *E.coli* and *Salmonella* spp[28]. Castor oil was selected in the study due to its easy availability in every household. The commercially available Castor oil used in this study did not exhibit any antimicrobial activity against the multidrug resistant nosocomial bacteria. In contrast to our study, the studies conducted by other authors to evaluate the antimicrobial property of castor seeds have exhibited antimicrobial activity[29]. But the tests were conducted on freshly prepared ethanolic, methanolic aqueous extracts and freshly extracted oils from the seeds. These studies show that the aqueous extract was comparitvely less active against the tested organisms. They found that *P.aeruginosa* and *E.feacalis* were resistant to the extracts as compared to other organism[30]. Further studies are required to confirm the activity of the commercially available oils against the multidrug resistant pathogens. Through our study we observed that the freshly prepared oils exhibited better antibacterial activity as compared to the commercially available oils. This may be one of the reason as our study did not show any antibacterial activity in coconut and Castor oils.

Natural drugs have been a part of the evolution of human, healthcare for thousands of years. Nowadays nearly 88% of the global populations turn to plant derived medicines as their first line of defence for maintaining health and compacting diseases. One hundred and nineteen secondary plant metabolites derived from plants are used globally as drugs, 15% of all angiosperms have been investigated chemically and of that 74% of pharmacologically active plant derived components were discovered [31]. Plants are rich in a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, etc. which have been found In-vitro to have medicinal properties. Pharmacological studies have accepted the value of medicinal plants as potential source of bioactive compounds[32]. Phyto-chemicals from medicinal plants serve as lead compounds in antimicrobial discovery[33,34]. This may be due to the known botanical facts that trees from different location grown on different geographical, climatic and zoological conditions may demonstrate different properties. The disparity sometimes may also be attributed to the superior equipments and chemicals, extraction methods. Hence the need of the hour is to standardise the different extraction procedures and identify the antimicrobial component by researched method. This is very much required to combat the headlong evolution of multidrug resistance in the pathogens.

Table 1: Antibacterial activity of the plant oils by well diffusion method

Test material	Mean Zone of Inhibition				
	E.coli	***K.pneumoniae***	***P.aeruginosa***	***A. baumanii***	***S. aureus***
1. Oregano oil	22.17±0.75	20.11±0.66	18.03±0.87	17.9±0.55	20.11±0.0
2. Eucalyptus oil	16.50±0.83	18±0.65	16.65±0.06	15.75±0.65	18.17±0.76
3. Clove oil	15±0.63	16.50±0.45	12.08±0.78	13.05±0.98	18.16±0.85
4. Neem oil	12±0.65	13.25±0.05	12.06±0.80	10.06±0.09	15.5±0.67
5. Coconut oil	6	6	6	6	6
6. Castor oil	6	6	6	6	6

Table 2: MIC and MBC expressed by the oils by microtitre plate method

Test material	Organism									
	E.coli		***K.pneumoniae***		***P.aeruginosa***		***A. baumanii***		***S. aureus***	
	MIC	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*	*MIC*	*MBC*
1. Oregano oil	0.048	0.097	0.097	0.781	0.195	0.781	0.195	0.781	0.048	0.097
2. Eucalyptus oil	0.097	0.390	0.390	0.781	0.390	1.562	0.390	1.562	0.097	0.781
3. Clove oil	0.097	0.390	0.781	1.562	0.781	3.125	0.781	3.125	0.390	0.781
4. Neem oil	0.781	3.125	1.562	3.125	1.562	3.125	3.125	6.250	0.781	1.562

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