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To Study Antimicrobial Activities of Essential Oils Against MDR Clinical Isolates of *Pseudomonas aeruginosa*

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***Abstract*— Pseudomonas aeruginosa is commonly resistant to antibiotics, and because of this it is a dangerous and dreaded pathogen. Case-fatality rates are due to drug resistance profile of Pseudomonas. Overuse of drug has enhanced resistance causing havoc of rapid spread of this organism. Examining present scenario it is mandatory to look for medicine readily available from natural resources. Essential oils are sources of novel antimicrobial compounds. Traditional medicines are trusted as a source of potential antimicrobial agents thus essential oil from sources like medicinal plants, herbs and spices are used. The true purpose of this study was to reveal the antibacterial properties of essential oil such as Cinnnamomum zylanicum (Dalchini oil), Eucalyptus globulus (Nilgri oil), Eugenia caryophyllata (Clove oil), Ocimum sanctum (Tulsi oil), Allium sativum (Garlic oil) against MDR strains of P. aeruginosa.These MDR strains possessed resistance for antibiotics Gentamicin (GEN) Meropenem (MRP), Cefazolin (CZ), co-trimoxazole (COT). These oils expressed antimicrobial activity against clinical isolates of Pseudomonas aeruginosa.The Cinnnamomum zylanicum oil had strong inhibitory activity against multidrug resistant strains as compared with other oils. Oils potentially inhibit biofilm producing MDR strains.**

***Index Terms*— Antibiotics,Biofilm,Essential oil, MDR, Pseudomonas aeruginosa.**

I. INTRODUCTION

Essential oils (also called volatile oils) are aromatic oily liquids obtained from materials (buds, flowers, barks, seeds, leave,twigs,wood,herbs,fruits and roots) [5].Essential oils and their components are widely used in medicine as constituents of different medical products, in the food industry as flavouring additives and also in cosmetics as fragrances [1].Many essential oils are associated with antimicrobial activity, and are found to be effective. Infectious diseases accounts for high proportion of health problems in the developing countries including India. Microorganisms have developed resistance to many antibiotics and as a result, immense clinical problem in the treatment of infectious diseases has been created [2]. The resistance of the organisms increased due to indiscriminate use of commercial antimicrobial drugs commonly used for the treatment of infectious disease. This situation forced the researchers to search for new antimicrobial substance from various sources including medicinal plants [3]. As the essential oils and other extracts of plants have evoked interest as a source of natural products. They have been screened for their potential uses as alternative remedies for the treatment of many infectious diseases [4]. Essential oils (also called volatile oils) are aromatic oily liquids obtained from plant materials (flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots) [24]. Essential oils have been shown to possess antibacterial, antifungal, antiviral insecticidal and antioxidant properties [6].

Antibiotics are also known to kill “good/beneficial” indigenous bacteria, which may have protective role against pathogenic bacteria [7],[8]. Another important point to consider is that antibiotics have been found to be less effective in biofilm-growing bacteria [9]. Besides this, the remarkable ability of *P. aeruginosa* to form biofilms in many environments renders antibiotic treatments inefficient and therefore promotes chronic infectious diseases [9]. Several different epidemiological studies track its emergence as multi-drug-resistant *Pseudomonas aeruginosa* (MDRPA) strains in clinical isolates. Genome based resistance mechanism is evolving due to nonspecific antibiotic use [16]. Beyond its natural resistance to many drugs, its ability to form biofilm, a complex biological system, renders ineffective the clearance by immune defense systems and antibiotherapy [17].

The remarkable ability of *P. aeruginosa* to form biofilms in many environments renders antibiotic treatments inefficient and therefore promotes chronic infectious diseases [18]. *Pseudomonas aeruginosa* is a notoriously difficult organism to control with antibiotics or disinfectants and has become increasingly recognized as an emerging opportunistic pathogen of clinical relevance [10]. The wrong and excessive dose of antibiotics is a serious problem in antimicrobial chemotherapy which causes resistance and ineffective antimicrobial treatment [20]. Thus, antibiofilm compounds could be interesting antibiotic adjuvants to prevent or treat chronic infections [18].The aim of this study was to evaluate the antibacterial activity of essential oil on MDR clinical isolates of *P.aeruginosa*.

II. MATERIALS AND METHODS

A. Medicinal plants

Cinnnamomum zylanicum (Dalchini), *Eucalyptus globulus* (Nilgri), *Eugenia caryophyllata* (Clove), *Ocimum sanctum* (Tulsi), *Allium sativum* (Garlic).

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B. Extraction procedure

The different parts of above mentioned plants were dried at room temperature. After cleaning and removal of the sand and foreign material, all the dried material was ground to fine powder using a grinder. The oil was extracted with n-hexane (1:4 w/v) by continuous extraction in a soxhlet apparatus for 12 hours.

C. Microbial strains

A total of 10 clinical isolates were isolated from patients and were procured from department of microbiology (Pravara Rural Hospiatal),Pravara Instituite of Medical Science-DU, Loni. Sample Processing and identification of organism was done in microbiology department: Two sterile swab sticks were used to collect the pus samples. 1st swab stick was used for gram staining and IInd swab stick was used for culture. Direct smear with gram stain were screened for the presence of inflammatory cells and type of microbial flora. IInd swab was inoculated on MacConkey agar (MA) then it was incubated at 37°C for 24 - 48 hrs. After observing the growth on MA, it was then subcultured on MA &. The colonial morphology and identification was done as per standard microbiology procedures [11].

D. Antibiogram testing

Selective colonies from the culture plate were inoculated into 2ml of peptone water. Incubated at 37°C for 2 hr. Turbidity was compared to that of 0.5 McFarland standards. A cotton swab was immersed and rotated in this inoculums, the swab was then pressed to the inner surface of the tube so as to remove excess inoculums. It was then used for carpet streaking on Muller Hinton agar plate. The required antibiotic discs were then placed aseptically on this medium with sterile forcep. The plate was then incubated 24 hr at 37°C. Next day the zone size was recorded and reported as sensitive or resistant by comparing the zone size to the Kirby-bauer chart. Antimicrobial susceptibility testing of isolates was performed by standard Kirby Bauer disc diffusion methods according to CLSI protocol [12].Depending on the isolate that is *Pseudomonas aeruginosa*, antibiotic discs were selected from among the following to determine antibiotic sensitivity pattern of *Pseudomonas aeruginosa* isolates:

Gentamicin (GEN) (10mcg/disc), Meropenem (MRP) (10 mcg/disc), Cefazolin (CZ) (30 mcg/disc), co-trimoxazole (COT) (25 mcg/disc) was tested (HIMEDIA, MUMBAI, INDIA).

Similarly antimicrobial activities of the essential oils were studied with the well diffusion method. Wells 5 mm in diameter were punched into the agar and filled with 20–30 µl of the oils. The plate was then incubated 24 hr at 370C and at the end of the period, the diameter of inhibition zones were measured in (mm) using a vernier scale.

III. RESULT

A total of 10 Multi drug resistance (MDR) isolates were selected for this study. MDR strain was detected for ≥ 3 antibiotics group.According to the antibiogram analysis in the following data (Table I) all the isolates were resistance and followed unique pattern of resistance.

Table I: Antibiogram analysis of *Pseudomonas aeruginosa*

Sr.No.	Antibiotic Resistant Pattern	Total number of isolates	Isolates
1	CZ^r,MRP^r,COT^r, GEN^r	10	P209,P406,P449,P461 , P782, P966, P85 ,P656,P770, P940

Out of 10 clinical MDR isolates 7 (70%) were biofilm producing and 3(30%) were non- biofilm producing.

Table II: Effect of essential oils on *Pseudomonas aeruginosa*

	Essential oils	MDR (Biofilm producing)							MDR (Non- biofilm producing)		
Sr.No.	Plants	Essential oils inhibitory effect on clinical isolates (mm)									
		P85	P209	P406	P656	P770	P782	P940	P461	P449	P966
1	*C. zylanicum* (Dalchini oil)	24 (++++)	21 (++++)	23 (++++)	25 (++++)	26 (++++)	28 (++++)	24 (++++)	27 (++++)	28 (++++)	25 (++++)
2	*E. globulus* (Nilgri oil)	22 (++++)	18 (+++)	25 (++++)	23 (++++)	27 (++++)	18 (+++)	25 (++++)	17 (+++)	30 (++++)	26 (++++)
3	*E.caryophyllata* (Clove oil)	16 (+++)	21 (++++)	19 (+++)	15 (+++)	18 (+++)	21 (++++)	24 (++++)	19 (+++)	17 (+++)	22 (++++)
4	*O.sanctum* (Tulsi oil)	18 (+++)	19 (+++)	13 (+++)	11 (++)	10 (+++)	14 (+++)	13 (+++)	10 (++)	9 (++)	8 (++)
5	*A. sativum* (Garlic oil)	3 (+)	2 (+)	7 (+)	5 (+)	0 (-)	2 (+)	3 (+)	0 (-)	2 (+)	4 (+)

* Diameter of inhibition zone: (++++) - 20mm and more; (+++) - 12-20 mm; (++) - 6-12 mm; (+) - 2-6 mm; (-) no antibacterial activity [13].

IV. DISCUSSION

A traditional use of all medicinal plant is carried out world - wide. Medicinal plants are considered as treasure of various curative therapies. Thus it is important to investigate scientifically those plants which have been used in traditional medicines as potential sources of novel antimicrobial compounds [14].The present study conducted on 10 clinical isolates of rural tertiary care hospital of Pravara Institute of Medical Sciences,Loni. This isolates are classified as MDR as they are totally resistant to antibiotics and follow a unique resistance pattern CZ^r,MRP^r,COT^r,GEN^r. These clinical isolates were then further tested for essential oils Cinnnamomum zylanicum (Dalchini oil), Eucalyptus globulus (Nilgri oil), Eugenia caryophyllata (Clove oil), Ocimum sanctum (Tulsi oil), Allium sativum (Garlic oil).Maximum activity was revealed for Cinnnamomum zylanicum (Dalchini oil), followed by Eucalyptus globulus (Nilgri oil), Eugenia caryophyllata (Clove oil), Ocimum sanctum (Tulsi oil) and Allium sativum (Garlic oil).

These oils were tested on MDR strains of *P.aeruginosa* comprising biofilm producers and nonbiofilm producers. As per the data *C. zylanicum* (Dalchini oil) showed maximum activity for MDR as well as biofilm producing organism followed by *E. globulus* (Nilgri oil) and activity was further reduced for other oils sequentially (Table-II). Similarly, in vitro antimicrobial activity of *Cinnamon zelyanicum* (bark) against human pathogenic fungi and commensally bacteria was studied by Chaumont *et al* [25].A report indicated cinnamon oil showed significant inhibitory effect against *P. aeruginosa* followed by clove oil, eucalyptus oil had no significant inhibition and is different from existing report [24]. *Eucalyptus globulus* (Nilgri oil), *Eugenia caryophyllata* (Clove oil) and *Cinnnamomum zylenicum*(Kalmi-Dalchini oil) showed antifungal activity whereas *Allium sativum* (Garlic oil) had failed to develop zone of inhibition [26].

Another report by Tarek *et al* stated that Cinnamon was the only essential oil which showed antibacterial activity against *P. aeruginosa* at the lowest concentration. Whereas other oils like Peppermint, clove did not show any activity even at the highest concentration [19]. This data states in accordance to present study on effective *Cinnamon zelyanicum* oil. Efficient antimicrobial activity was observed for *E. globulus* for organism like *E.coli* ,*S.aureus* by Bachir Raho G *et al* [15] similarly high activity was revealed for *P.aeruginosa* in the present study. These essential oils were tested against strong biofilm forming *P.aeruginosa* and were found effective in developing zone of inhibition. It reveals that essential oil pose certain mechanism to control biofilm forming ability of *P.aeruginosa*. A previous data of Husain *et al* illuminate property of clove essential oil reduces biofilm formation by *P.aeruginosa* [21].

An important characteristic of essential oils and their components is their hydrophobicity, which enable them to partition the lipids of the bacterial cell membrane and mitochondria, disturbing the cell structures and rendering them more permeable [22],[23].These data is supporting the notion of Bansod and Rai that plant essential oils have a role as pharmaceuticals and preservatives [26].

V.CONCLUSION

The essential oil effect are well demonstrated in this and earlier research work .Thus from this study it is evident that essential oil inhibit MDR and biofilm producing organism. As less sequential data is available on effect of essential oils on biofilm as well as MDR strains, henceforth surveillance study is vital for analysing the circulation of frequently changing resistance profile of this dreaded pathogen. It will prove beneficial in controlling further emergence of MDR strains. As essential oils can reduce biofilm formation, this data can prove a boon for treating fatal diseases where biofilm prevent disease curing. Present study confirms essential oils possess in-vitro antimicrobial activity against pathogen. It needs further investigative study to detect the changing circulation pattern of notorious *P. aeruginosa* with respect to drug resistance and efficacy of essential oils towards this emerging MDR strains in hospital settings. This valuable data proves helpful for clinicians to decide proper treatment strategy and avoid judicial use of antibiotics. Instantaneously looking ahead for essential oils is best combined therapy to enhance patient's immunity and to achieve effective drug concentration. This will also cover-up strategy used by microorganism to combat immune system as well as reduce emerging resistance profile of *P.aeruginosa*.

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