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ANTIBACTERIAL ACTIVITY OF METHANOL EXTRACT OF *EUCALYPTUS CITRIODORA* IN COMBINATION WITH ANTIBIOTICS

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ABSTRACT: Antibiotics are widely used for treatment of bacterial causing diseases. But indiscriminate use of antibiotics has developed microbial resistance and it is becoming a major issue in health problem all over the world. There are many ways to treat infectious diseases and one of them is use of plant extracts in combination with antibiotics as novel concept. This study was done to evaluate combination effect of methanol extract of *Eucalyptus citriodora* leaves with antibiotics against common pathogenic bacterial species.

Key Words: Antibiotics, Combination effect, Plant extract.

1. INTRODUCTION

Antibiotics have been used for treatment of number of infectious diseases. They are the most important weapons to fight against bacterial infections. Antibiotics have played important role to treat various bacterial infections since their time of introduction, but wide use of antibiotics has led to development of resistant strains, which is becoming a global problem. There are requirement to find some alternatives to tackle the problem of emergence of drug resistant microorganisms [1-2]. There are some alternatives to conventional antibiotics therapy to prevent the spread of infectious diseases such as use of plant extracts or isolated phytochemicals. Other strategy is use of combination of plant extracts or active phytochemicals with antibiotics to fight against various drug resistant microorganisms [3-4]. Synergistic effect from combination of plant extracts or phytochemicals with antibiotics may be new choice for treatment of number of infectious diseases [5]. Plants are rich sources of secondary metabolites such as alkaloids, flavonoids, tannins, and terpenoids with antimicrobial activities and other biological activities. Plant extracts with such chemical diversity may be potential source of antibiotic resistance modifying compounds and therefore plants could be a source of compounds that can increase the sensitivity of bacterial cells to antibiotics. The ability of plant extracts to potentiate antibiotics has not been well explained [6].

Numbers of studies have been reported on combination effect of plant extracts or active phytochemicals with antibiotics against various bacterial and fungal species with significant reduction in the minimum inhibitory concentrations of the antibiotics and enlargement of zone of inhibition. Lee, Young-Seob, et al. investigated synergistic effect of emodin (constituent of *Rheum palmatum*) in combination with ampicillin or oxacillin against methicillin-resistant *Staphylococcus aureus*. Emodin markedly lowered the MICs of ampicillin and oxacillin against the Methicillin-resistant *Staphylococcus aureus* [7]. Coutinho, et al investigated an ethanol extract of *Mentha arvensis* (EEMA) as a resistance-modifying agent in an aminoglycoside-resistant strain of *E. coli* and *methicillin – resistant Staphylococcus aureus.* Although EEMA did not show appreciable antibacterial activity when it was added to the growth medium at 128 µg/ml (1/16MIC), a dramatic reduction in the MIC for gentamicin was observed in the strain *E. coli* 27, demonstrating a potentiating effect of EEMA on aminoglycoside activity [8]. Nweze, Emeka I., et al investigated the interaction of ethanolic extract of the leaves of *Ocimium gratisimum* with different six antibiotics. Zone of ciprofloxacin

was increased in presence of plant extract against *P. aeruginosa, E. coli,* and *P. mirabilis.* Other antibiotics also exhibited synergistic effect with plant extract [9]. Freitas, Eliana, et al investigated the possible in vitro interaction between natural extracts of watercress (*Nasturtium officinale*) and 2-phenylethyl isothiocyanate (PEITC) with a standard antibiotic, against 11 isolates of extended-spectrum *β*-lactamases-*Escherichia coli.* Synergism between both watercress extracts and antibiotic, as well as for PEITC with antibiotic was observed [4].

Eucalyptus citriodora Hook (family: Myrtaceae.) is a tall, evergreen and graceful tree which is normally cultivated to produce essential oil, for fuel, timbers and medicinal purpose [1, 2]. The leaves of *E. citriodora* contain a fragrant volatile oil that is known to have a various activities such as antibacterial, anti-inflammatory, antiseptic, analgesic, deodorant, diuretic, expectorant and other activities. Leaves contain number of phytochemicals including phenolic compounds, flavonoids, sesquiterpenes, aldehydes, ketones and tannins. Citronellol is found as main constituent in essential oil and it is best known for its aromatic properties. Essential oils are also widely used in modern cosmetics, food, and pharmaceutical industries [10-13].

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

Leaves of *Eucalyptus citriodora* Hook was collected from local area and identified by experts. After proper collection, leaves were washed with tape water to remove dust particles and then dried at room temperature for few days. Dried leaves were grinded to form powder, which was stored it in airtight bottle till use properly.

2.2 Extraction of Plant Material

Dried powder was extracted with Methanol using magnetic stirrer for 24 hours at room temperature. Then, extract was filtered through Whatmann filter paper No1. Solvent was evaporated to obtain the dry extracts and it was stored properly.

2.3 Antibiotics and Strains used in experiment

Antibiotics (Amoxicillin, Ciprofloxacin, Ceftazidime, and Erythromycin) were purchased from *Hi Media* Laboratories. The microbial strains are identified strains and were obtained from National Chemical Laboratory (NCL), Pune, India.

2.4 Antibacterial assay

Antibacterial activity was followed by previously described standard method [14-15]. Mueller Hinton Agar was used for antibacterial susceptible testing. The test organism was activated by inoculating a loopful of the strain in 25 ml of Mueller Hinton Broth and kept overnight for incubation at 30-35°C. The assay was performed by agar well diffusion method. Compare the above inoculums with previously made 0.5 MacFarland solutions and introduced into molten Mueller Hinton Agar, then poured into petri dishes when temperature reached 40-42° C. The media was solidified and wells were prepared in the seeded agar plates with the help of a cup borer (8.0 mm). 100 µL of plant extract and antibiotic was filled in wells and in case of combination, equal volume (50 µL) of each was added in the wells. The plates were incubated and then zone of inhibition was measured with the help of zone reader.

3. RESULTS AND DISCUSSION

Table 1: - Antibacterial activity of methanol extract of *Eucalyptus citriodora* and its combination with antibiotics.

Plant Extract/Antibiotics	Microorganisms (Zone of Inhibition*)			
	B. subtilis NCIM2063	*E. coli* NCIM2065	*P. aeruginosa* NCIM2200	*S. aureus* NCIM5345
Plant Extract	11.0	10.3	10.7	10.8
Amoxicillin	26.1	21.8	25.6	22.9
Plant Extract + Amoxicillin	23.0	19.9	24.8	19.9
Ciprofloxacin	38.7	32.1	34.4	26.3
Plant Extract + Ciprofloxacin	38.9	32.9	36.4	23.9
Ceftazidime	Nz	Nz	Nz	Nz
Plant Extract + Ceftazidime	10.1	11.1	10.7	9.9
Erythromycin	31.0	25.2	27.4	23.0
Plant Extract + Erythromycin	28.6	24.0	29.3	24.7

(*B. subtilis- Bacillus subtilis, E. coli- Escherichia coli, P. aeruginosa- Pseudomonas aeruginosa, S. aureus- Staphylococcus aureus)*

(Concentration of plant extract -1 mg/mL, Concentration of antibiotic – 25 µg/mL, Nz- No zone of inhibition, * - Zone of inhibition in mm)

Combination of plant extracts or active constituents of plant extract with antibiotics may be novel approach to treat various infectious diseases. Interaction of plant extracts with antibiotics may be resulted in synergistic, additive or antagonistic effect. Various studies have been reported on synergistic interaction of plant extracts with antibiotics [16].

This study was carried out to evaluate combined antibacterial activity of methanol extract of leaves of *Eucalyptus citriodora* and different antibiotics. Methanol extract showed effective inhibition against test bacteria (Zone of inhibition in range of 10.0-11.0 mm). This result proved the potential of plant extract to fight against various diseases causing microorganisms.

Antibiotics tested in study exhibited very effective inhibition against test bacteria. Interaction of plant extract with different antibiotics resulted in synergistic, additive or antagonistic effect. Combination of plant extract and amoxicillin showed decreased activity than amoxicillin alone, thus this combination showed antagonistic effect against all test bacteria. Ciprofloxacin with plant extract showed indifferent activity against *B. subtilis* and *E. coli*. This combination resulted in positive interaction against *P. aeruginosa* and decreased inhibition against *S. aureus*. Ceftazidime did not show inhibition against all test bacteria and in presence of plant extract mostly showed indifferent or antagonistic effect against test bacteria. Erythromycin in presence of plant extract showed positive interaction against *P. aeruginosa* and *S. aureus*.

In this study combination of plant extract and antibiotics mostly showed indifferent or antagonistic effect. Synergistic, antagonistic or indifferent effect may be due to formation of certain complex against microorganisms. It is required to carry out more combination of various plant extracts with antibiotics against various microorganisms, so right combination may be administrated to treat infectious diseases. This is only *in vitro* experiment and such combination must be followed by toxicity

test and *in vivo* study to ensure its real efficacy for therapeutic applications.

4. CONCLUSION

This study proved the importance of plant extracts to inhibit the growth of microorganisms and it indicate that plant extract may be new source of alternatives to conventional antibiotics. Combination of plant extract with antibiotics leads to new choice for treatment of infectious diseases and plant extracts may be act as activity modifying agent for antibiotics.

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