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Comparing the Efficiency of Various Extracts of *Coleus aromaticus* against Human Respiratory Pathogens

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ABSTRACT

Coleus aromaticus, an Indian medicinal plant used to treat various respiratory ailments was screened for their antimicrobial activity against a few respiratory pathogens. Acetone, ethanol and water extracts were prepared from the leaves of the chosen plant. The antimicrobial activity was determined against pathogens associated with respiratory conditions *i.e. E.coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Citrobacter freundii, Staphylococcus aureus,Methicillin Resistant Staphylococcus aureus, Proteus mirabilis, Burkholderia cepacia* and *Candida albicans.* The leaf extracts demonstrated significant activity in the disc diffusion assay and the zones of inhibition ranged from 5mm to 14mm, while the MIC values ranged from 0.312mg/ml to 20.0mg/ml. Hence, the antimicrobial activity recorded for the plant extracts validates their traditional uses to treat various respiratory infections.

Keywords: *Coleus aromaticus*, MIC, zones of inhibition, respiratory pathogens

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INTRODUCTION

Since olden days, various medicinal plants have been used to treat human diseases. It has been estimated that between 60-90% of the populations of developing countries use traditional and botanical medicines almost exclusively and consider them to be a normal part of primary healthcare[1]. Plants are rich in a great diversity of phytochemicals such as phenolic acids, flavonoids, tannins, lignin,and other small compounds[2]. The expanding bacterial resistance to antibiotics has become a growing concern worldwide[3]. Intensive care physicians consider antibiotic-resistant bacteria a significant or major problem in the treatment of patients[4]. Increasing bacterial resistance limits therapeutic options and hence attention has turned towards plants as alternative therapy against resistant strains[5,6]. A vast number of medicinal plants have been recognized as valuable resources of natural antimicrobial compounds[7]. Medicinal plants are promising and offer considerable potential for the development of new agents effective against infections currently difficult to treat[8]. The most frequent bacteria includes *Escherichia coli, Pseudomonas aeruginosa, Pseudomonas cepacea ,Staphylococcus aureus, Streptococcus pneumoniae, Haemophilus influenza* and *Klebsiella pneumonia* which colonize the respiratory tract of young children and the elderly as commensals, and quite often results in diseases whenever the condition warrants[9]. The primary benefits of using plant derived medicines are that they are relatively cheaper than synthetic alternatives, offering profound therapeutic benefits and more affordable treatments. Many of the plant materials used in traditional medicine are readily available in rural areas and this has made traditional system of medicine relatively cheaper than modern medicine. *Coleus aromaticus* Benth., (Fam. Lamiaceae), syn. *Coleus amboinicus* Lour. Spreng or *Plectranthus ambonicus* Lour, is commonly known as Indian/ country borage. It is used against various disorders in indigenous system of medicine, such as severe bronchitis, asthma, diarrhoea, epilepsy, renal and vesicle calculi, fever, common cold, cough, headache, indigestion, urinary diseases, vaginal discharges, colic, dyspepsia, convulsions[10], stimulates the functions of liver, indicated in kidney and bladder stones, dysentery, cholera and several other infections.

MATERIALS AND METHODS

Preparation of plant materials

Fresh leaves of *Coleus aromaticus* were collected from local gardens of Tiruchirappalli during the month of December, 2012 and the specimen was identified by a botanist of our college and the voucher specimen was deposited at the department. The freshly collected leaves were spread

to dry under shade at normal room temperature for seven days in the shade. Upon drying, the leaves were pounded using mortar and pestle into smaller particles and then blended to powder and the powder was stored in airtight containers and kept under normal room temperature (28 ± 2°C) until required.

Collection of test organisms

The test organisms were clinical isolates obtained from patients previously diagnosed with respiratory tract infection from a local hospital, Tiruchirappalli, Tamil Nadu, India. Sputum, throat and mouth specimens were collected with the aid of the hospital staff following standard procedures described by Cheesborough[11]. Sputum and the swab samples were cultured on blood agar, Deoxycholate Citrate agar and Mac Conkey agar plates at 37°C for 24 h. Discrete colonies were isolated and cultured on Mueller Hinton agar slants and were immediately transported to the Microbiology laboratory and identified using standard biochemical tests. All the test bacteria were maintained in a refrigerator at 4°C on Mueller Hinton agar slants until required[12].

Extraction procedure

10 g of powdered sample was soaked in 100 ml solvent contained in a 500 ml sterile conical flask. The flask was covered with cotton plug and then wrapped with aluminium foil and shaken vigorously at 3 h intervals for 48 h at room temperature. The crude extract was then filtered using muslin cloth and then Whatman no.1 filter paper. The filtrate was evaporated to dryness using rotary evaporator (Model 349/2, Corning Limited) maintained at 40°C and the dried substance was stored in airtight bottles until required.

Antimicrobial Susceptibility Testing

Disc Diffusion test

All isolates were tested for susceptibility to the extracts and antimicrobial agents on Mueller Hinton agar (Hi-Media India) by the standard disc diffusion method recommended by the National Committee for Clinical Laboratory Standards. The diameter of the zone of inhibition of growth was recorded, and interpreted by the criteria of NCCLS.

Micro broth dilution method to determine MIC

The antibacterial activity of the extracts was determined by the micro broth dilution method using a microtitre plate. The lowest concentration of antibiotic at which there was no visible growth was recorded as MIC.

Determination of cellular toxicity using sheep erythrocytes

The method described by Xian-guo and Ursula[13] was employed to study cellular toxicity. Briefly, 10-fold serial dilutions of the extract were made in phosphate buffered saline. A total

volume of 0.8 ml for each dilution was placed in an eppendorf tube. A negative control tube (containing saline only) and a positive control tube (containing plant extract, 5 mg/ml) were also included in the analysis. Fresh sheep erythrocytes were added to each tube, to give a final volume of 1 ml. Solutions were incubated at 37°C for 30 min and all tubes were centrifuged for 5 min and then observed for haemolysis. Complete hemolysis was seen by a clear red solution without any deposit of erythrocytes. Hemolysis was also checked microscopically for presence or absence of intact RBCs.

RESULTS & DISCUSSION

Screening for antibacterial activity by disc diffusion method

Activity of a given extract was determined by measuring the zones of inhibition (ZOI). Because zones of inhibition were often asymmetrical, experiments were repeated three times and the average was recorded.

Antibacterial activity of *Coleus aromaticus*

Table 1 displays the results of the antibacterial testing for the various extracts of *Coleus aromaticus* against the drug-resistant bacterial isolates and standard strains respectively. Measured inhibitory zones displayed in the table shows that among the four extracts used for the study, acetone extract demonstrated inhibitory activity against all the bacterial isolates under study.

Table 1: Inhibitory effects of the different extracts of *Coleus aromaticus* on respiratory pathogens by disc diffusion method (1000µg/10µl/disc)

Bacteria	Dia of Zones (mm)		
	CAA	CAE	CAAq
Escherichia coli	13	7	-
Pseudomonas aeruginosa	14	8	-
Citrobacter freundii	7	6	6
Staphylococcus aureus	10	-	6
Methicillin Resistant S. aureus	7	-	-
Proteus mirabilis	6	6	11
Klebsiella pneumoniae	10	5	6
Burkholderia cepacia	7	6	7
Candida albicans	10	-	-

CAA – Coleus aromaticus acetone extract, CAE - Coleus aromaticus ethanol extract, CAAq - Coleus aromaticus aqueous extract

It is also seen that the extracts had no varied response to Gram positive and negative organisms. No zones were produced by the dimethyl sulfoxide (DMSO), the suspending solvent and the

solvent only discs, indicating their non-involvement in the inhibitory role. Among the extracts of *Coleus aromaticus*, the acetone extract clearly demonstrated a far more superior effect than its counterparts and were also comparable with a few antibiotics and hence it was chosen for further analysis.

B. Minimal Inhibitory Concentration of the acetone extract by broth dilution method

The MIC values of the acetone extract of *Coleus aromaticus* against the drug-resistant bacterial isolates and standard strains respectively are given in table 2. As shown, the MIC values were lower for the drug resistant *S.aureus, E.coli* and *K.pneumoniae* than the other isolates.

Table 2: Minimal inhibitory concentrations of *Coleus aromaticus* acetone extract (CAA) on a few respiratory pathogens (mg/ml)

Bacteria	CAA
Escherichia coli	1.25
Pseudomonas aeruginosa	2.5
Citrobacter freundii	10.0
Staphylococcus aureus	10.0
Methicillin Resistant S. aureus	0.312
Proteus mirabilis	20.0
Klebsiella pneumoniae	10.0
Burkholderia cepacia	10.0
Candida albicans	20.0

Toxicity testing

Absence of haemolysis of the erythrocytes of sheep and human erythrocytes by the plant extracts at recommended doses further confirmed its non-toxicity.

Already, several antibiotic resistant strains of this once manageable disease have emerged, with doctors and scientists scrambling to find new kinds of antibiotics. Bacteria and microbes evolve to build up these resistances because of the overuse of antibiotics. Studies have revealed that they have been systematically been overprescribed over the past decades, giving the most mundane strains of bacteria plenty of opportunities to build up defenses against them. This limits therapeutic options. Hence alternative drug sources were sought after. Results of the extraction with various solvents show that acetone was the more efficient solvent, followed by ethanol and water. The acetone extract produced the highest yield amongst all the three extracts. This is a clear indication that the solvent system plays a significant role in the solubility of the bioactive components and influences the antibacterial activity. The above results illustrate that *Coleus aromaticus* showed a wide spectrum of antibacterial activity against all human pathogenic bacteria tested. The high potency of acetone extract showed more potency of antibacterial effect

than the other two extracts. This may be because the alcohol extract is rich in polyphenol and other bioactive components, which are responsible for its antioxidant activities. Similar observation has been reported that grape seed extracts rich in polyphenols exhibit antibacterial and antioxidant activities and it is reported that active compound responsible for the inhibition of *E.coli* and *Salmonella enteritidis* have been identified as gallic acid. The acetone extract showed a broad spectrum of antibacterial activity by inhibiting both Gram positive and Gram negative bacteria.

CONCLUSION

Among the extracts tested, the acetone extract demonstrated potent anti-bactericidal activity against all the isolates tested, the extract can be used to treat respiratory infections. The results provide a scientific basis for the centuries-old usage of this plant as a medicinal herb.

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