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RESEARCH ARTICLE

ENHANCED ANTIBACTERIAL ACTIVITY OF SALT WATER EXTRACTS OF *COLEUS AROMATICUS*

#Ananda Vardhan Hebbani, #Roopasri Ranganatha and *John Barnabas

*Centre for R&D in Life Sciences,
*Microbiology Research Laboratory,
#Post Graduate-Department of Biochemistry,
*Post Graduate-Department of Microbiology,
Dayananda Sagar Institutions, Dr.C.D. Sagar Centre for Life Sciences, Shavige Malleshwara Hills, Kumaraswamy Layout, Bangalore-560078, India

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ABSTRACT

The undertaken exercise was to analyze the exacerbating effect of common salt on the antibacterial activity of *Coleus aromaticus,* a commonly found medicinal herb in India. Water, ethanol, acetone and salt water (1.2% NaCl) extracts of *Coleus aromaticus* were examined for their antibacterial efficacy by disc diffusion method on both Gram positive (viz. *Bacillus subtilis*, *Staphylococcus aureus* and *Streptococcus pyogenes*) and Gram negative (viz. *Escherichia coli, Klebsiella pneumonia and Pseudomonas aeruginosa)* bacteria. Results of the study disclosed that the salt water extract of the leaf exhibited a profound antibacterial activity on both Gram type of bacteria, in comparison with the other extracts. The results thus support the age old practice of consuming the leaf in combination with salt, as a better smoothening (anti tussessive) agent against throat infections and a natural cure for tonsils.

INTRODUCTION

Medicinal plants have always played a crucial role from time immemorial. The use of medicinal plants to alleviate and cure illnesses has been prevalent since the early times. Herbs and plant materials like stem, bark, resin, leaf, pollen, fruit, seed and roots find an important place in all forms of native and medicinal therapy and this is cosmopolitan[1,5]. The first attempt of recording medicinal plants was done by Dioscorides in 1st Century AD – "*De Materia Medica*" which became the prototype for modern pharmacopoeias[1]. Plants produce a large number of substances called phytochemicals that exhibitantimicrobial activity. These can be categorized into various groups such as phenolics, polyphenols, quinones, flavones, flavonoids, flavonols, tannins, coumarins, terpenoids, alkaloids, lectins, polypeptides and polyacetylenes[1]. It has been reported that terpenes are amongst those chemicals responsible for the medicinal, culinary and fragrant uses of medicinal and aromatic plants[2]. Many reports are available on antiviral, antibacterial, antifungal, antihelminthic, antimolluscal and anti-inflammatory properties of plants[3]. With the discovery of antibiotics, the treatment of illnesses by these traditional methods had taken a backseat for a long period. However, commonly used antibiotics are recently reported of becoming less chosen, not only because of the toxic side effects, but also because of the emergence of drug resistant microorganisms[4].

***Corresponding author:** johnbarnabas@gmail.com

With the increase in population, the comman people are becoming aware of the problems with the abuse of antibiotics, and also for scientific reasons; fact that the effective lifespan of any antibiotic is limited with intense side effects upon administration hence medicinal plants have taken the limelight again. Drug discovery from natural sources have become the trend of late. Drug discoverists are thus investigating plants anew with an eye on their antimicrobial usefulness[1]. The essential oils of many medicinal plants are being considered as alternative remedies for many infectious diseases due to their number of their desirable characteristics such as low toxicity, high efficiency and broad spectrum activity[6]. *Coleus aromaticus* is a commonly found medicinal plant which belongs to *Labiatae* family (mint family). It's approximately 30-90cm in height with fleshy stem with thick fleshy leaves. The leaves are simple, ovate, succulent having a distinct, strong but yet a pleasant aroma. The plant is commonly referred to as Indian Borage and the leaves are mostly used in flavoring various meat dishes and also in curing common ailments. Decoction of the leaves is administered in cases of chronic cough, asthma and tonsils[1] and is also reported to possess antilithotic, chemopreventive and anti-oxidant potential[4, 5, 6, 7].

It is an established fact that, NaCl (common salt or table salt) is used in food industries as a preservative, due to its inhibitory effect on bacterial growth. At appropriate concentrations, NaCl has been shown to work as a synergist with the essential oils extracted from plants.

In previous observations, antimicrobial activity of clove essential oil got enhanced by the addition of 1.2% w/v of NaCl,[8] this encouraged us to explore and assess the effects of addition of sodium chloride to the leaf extracts of *Coleus aromaticus* and evaluate the changes in its antibacterial potential against both Gram-positive and Gram-negative bacteria.

MATERIALS AND METHODS

Samples: Mature, healthy *Coleus aromaticus* leaves were collected during the winter season (December – February 2010). The leaves were surface sterilized by thoroughly washing with distilled water, followed with 0.1% mercuric chloride solution and again with distilled water.

Chemicals: Ethanol: 99.9%., Changshu Yangyuan, GB 678-90., Lot No. 100103, Acetone: 99% SDFCL D09A/0509/3003/13, NaCl: 99.5% RFCL.

Antibiotic Discs (for control): Chloramphenicol(Himedia) 30mcg/disc, 6mm, Filter paper: Whatmann No.1 filter paper.

Equipment : Blender: Mixer Maharaja Whiteline, MX-107, 500 Watts Soxhlet Extractor: 240 Volts, LABSTAR(Heating Mantle), Borosil(Soxhlet),Mumbai.

1. Preparation of leaf extracts of *Coleus aromaticus*

a. **Water extract:** 100 grams of dry, sterilized leaves were macerated with 100ml sterile distilled water in a mortar and pestle for 10 minutes. The macerate was filtered through double layered muslin cloth and centrifuged at 4000 *g* for 30 minutes. The supernatant was then filtered through Whatmann No.1 filter paper, sterilized at 120^0 C for 30 minutes. This is maintained as a stock of water extract.

b. **Ethanol and Acetone extracts:** Thoroughly washed mature leaves were shade dried and then powdered with a blender. 25 grams of the powder was filled in the thimble and extracted successively with appropriate volumes of ethanol and acetone using a Soxhlet extractor for 48 h. The extracts were cooled and concentrated using an evaporator and preserved at 4^0C in airtight brown bottle until further use[3].

c. **Salt water extract:** 100 grams of dry, sterilized leaves were macerated with 100ml of 1.2% NaCl solution in a mortar and pestle for 10 minutes. The macerate was filtered through double layered muslin cloth and then centrifuged at 4000 *g* for 30 minutes. The supernatant was then filtered through Whatmann No.1 filter paper, sterilized at 120^0 C for 30 minutes. This is maintained as a stock of salt water extract.

2. Antibacterial activity:

a. Preparation of inoculums: The cultures of Gram positive bacteria; *Bacillus subtilis*, *Staphylococcus aureus* and *Streptococcus pyogenes* and Gram negative bacteria;(*Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeuroginosa* were procured from the Post Graduate- Department of Microbiology, Dayananda Sagar Institutions, Bangalore-78, India. Biochemical tests were performed to confirm the bacterial strains. Stock cultures were maintained at 4°C on slopes of nutrient agar. Broth cultures for the experiments were prepared by transferring a loopful of cells from the stock cultures to test tubes of nutrient broth**.**

b. Antibacterial test: The disc diffusion method[9] was used to screen the antibacterial activity. Nutrient agar plates were prepared by pouring 25 ml of molten media into sterile petriplates. The plates were allowed to solidify for 5 minutes and 0.1 ml of bacterial inoculum suspension was inoculated by spread plate method. Sterile discs of 5mm were soaked in the different leaf extracts (water extract, ethanol extract, acetone extract and salt water extract) and were placed on the surface of medium, allowed to diffuse for 5 minutes and the plates were kept for incubation at 37°C for 24 hrs in an inverted position. At the end of incubation, inhibition zones formed around the disc were measured with transparent ruler in millimeter. The same procedure was followed for discs dipped in salt water to figure out the inhibitory effect of salt alone on the different bacterial strains. Standard antibiotic discs were used as a standard reference. These studies were performed in duplicates.

RESULTS AND DISCUSSION

The antibacterial activity assessment by disc diffusion method for Gram positive and Gram negative bacteria are summarized in Table 1 and Table 2 respectively. It is evident from Table 1 and 2 that there is a profound rise in antibacterial activity of the salt water extract in comparison with that of other extracts. There is steady rise in antibacterial activity of the leaf based on the type of extract used. Although the antibacterial activity got enhanced with non polar solvents like ethanol and acetone, the activity of salt water extracts appeared to be the highest and almost equivalent to that of standard antibiotics, which is quite an interesting observation. As salt itself is an antibacterial agent, we tried to look at the antibacterial efficacy of the same concentration (1.2%) of salt alone, and interestingly it had very less efficacy, suggesting an intricate mechanism of the salt's interaction with the phenolic components of the leaf extracts. It is evident from Figure 1 and Figure 2 that salt has a kind of stimulating effect on enhancing the antibacterial activity of *Coleus aromaticus.* Salt water extract of leaf seems to be almost comparable to the standard antibiotics in its antibacterial activity than just the leaf extract. The most important observable feature of the present work is the broad spectrum antibacterial activity of *Coleus aromaticus*, supporting the previous observations made about the medicinal value of the leaf [4,5,6,10,11, 12]. It is observed that the leaf extract proves to be a little more effective against Gram-positive bacteria than Gram-negative. Although the exact reason for this difference is not known, it is proposed that it might be due to the variability in the structure of their cell envelopes. It is known that the cell envelopes of gram-negative bacteria are more complex than that of gram-positive. Gram-negative possess two layers that protect the cell and provide rigidity, whereas the Gram-positive bacteria lack the outer membrane, making them more susceptible to the action of phenolic components of the leaf extract. As these

Table 1: Antibacterial activity of *Coleus aromaticus* leaf extracts against gram positive bacteria using disc diffusion method

Organism	Water extract	Ethanol extract	Acetone extract	Salt water extract	Saltwater©	Chloramphenicol
			Zone of Inhibition (mm)			
B.subtilis	9.5±0.7	12.5±0.7	15±1.4	19.5±0.7	2.5±0.7	17±1.4
S.aureus	8.5±0.7	14±1.4	12.5±2.1	20.5±0.7	1.5±1	23.5±0.7
S.pyogenes	7.5±0.7	10±1.4	11±1.4	17.5±0.7	2.5±0.7	21.5±0.7

© - Control, Values are mean ± S.D of two replicates

Table 2: Antibacterial activity of *Coleus aromaticus* leaf extracts against gram negative bacteria using disc diffusion method

Organism	Water extract	Ethanol extract	Acetone extract	Salt water extract	Saltwater©	Chloramphenicol
			Zone of Inhibition (mm)			
E.coli	9±1.4	11.5±0.7	12.3±0.4	20.5±0.7	1.5±0.7	23.5±2.1
K.pneumoniae	5.5±0.7	8.5±0.7	9.3±0.4	18±1.4	Negligible	22.5±2.1
P.aeruginosa	2.5±0.7	7.5±2.1	6.5±0.7	9.5±0.7	0.5±0.7	12.5±0.7

© - Control, Values are mean ± S.D of two replicates

components are hydrophobic in nature, they get partitioned in the lipids of the bacterial cell membrane , thus distorting the

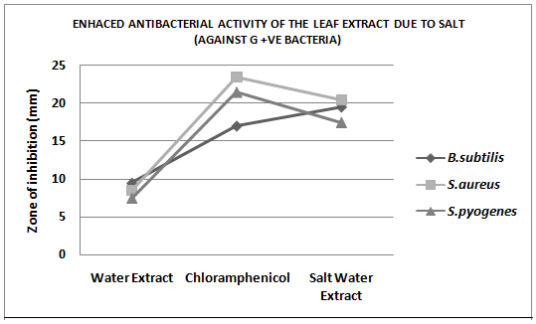

Figure 1. Influence of salt on the antibacterial (against G +ve bacteria) activity of *Coleus aromaticus* leaf extract

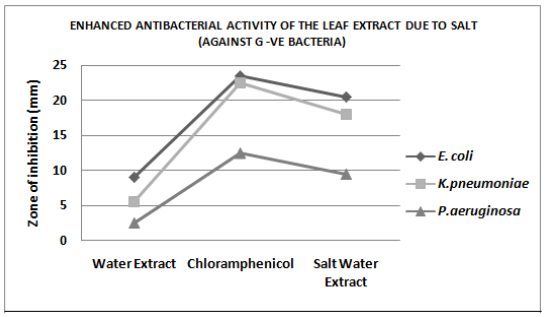

Figure 2. Influence of salt on the antibacterial (against G -ve bacteria) activity of *Coleus aromaticus* leaf extract

structure and rendering them more susceptible to the antimicrobial action, leading to leakage of the cell contents and the extensive loss would eventually lead to cell death[13]. It has been reported that the most important component of the *Coleus aromaticus* leaf extract is cavarcol which is capable of disrupting the outer membranes of bacteria[14, 15]. It is interesting to note that the combination of leaf extract and salt at appropriate concentrations seemed to be acting as a broad spectrum antibacterial agent, which might not be possible with salt alone. It's already known that appropriate concentrations of salt with essential oil prolong the lag time of few bacteria or decrease the growth of bacteria[8]. Although the exact underlying mechanism for the synergistic role of NaCl with the phenolic components of the leaf extract still remains to be understood, the present results undoubtedly reveal the fact that NaCl exacerbate the antibacterial efficacy of the leaf extract. The variations in the antibacterial efficiencies of the leaf extracts with different concentrations of NaCl and other salts still remain to be explored.

Conclusions

Coleus aromaticus is undoubtedly an excellent herb with lots of medicinal applications. Our results scientifically prove the correctness of a general house-hold medicational practice of consuming this leaf with salt. Thus the results of our study serve as a testimonial to the age old practice of using this medicinal plant extracts to cure various ailments. Keeping in view the problems of multi drug resistance patterns posed by the evolving microorganisms and the side effects of chemical compounds on the physiology of the human system, our work reinforces the uses and advantages of ancient therapies which were always thought to be a safer alternative to allopathic medication.

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