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DIFFERENTIAL ANTIBACTERIAL ACTIVITY OF EXTRACTS FROM COLEUS AROMATICUS LEAF

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ABSTRACT

The aim of the present study was to look at the differential 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 reveal that the salt water extract of the leaf showed a profound antibacterial activity on both the categories 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.

Key words: *Coleus aromaticus,* Leaf extract, Sodium Chloride, Antibacterial

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1. INTRODUCTION

Medicinal plants have always played a crucial role in the existence of the human civilization. The use of medicinal plants to alleviate and cure illnesses has been prevalent since the origin of mankind. 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 all over the world[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 (phytochemicals) having antimicrobial 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 quite 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]. With the population increasing and the public becoming more aware of the problems with the misuse of antibiotics, medicinal plants have come back into the limelight. Scientists have also realized the fact that the effective lifespan of any antibiotic is limited and 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 because of possessing a number of 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 and thick fleshy leaves. The leaves are simple, ovate, succulent having a distinct, strong yet pleasant aroma. The plant is commonly referred to as Indian Borage and the leaves are 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's already known that, NaCl (common salt or table salt) is used in the food industries as a preservative since it inhibits the growth of bacteria and at appropriate concentrations NaCl has been shown to work as a synergist with the essential oils extracted from plants. The previous observations that the antimicrobial activity of clove essential oil got enhanced by the addition of 1.2% w/v of NaCl[8] encouraged us to explore and assess the effects of addition of sodium chloride to the leaf extracts of *Coleus aromaticus* and evaluating the changes in its antibacterial potential against both Gram-positive and Gram-negative bacteria.

2. MATERIALS AND METHODS

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.

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 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 water extract.
   b. **Ethanol and Acetone extracts:** Thoroughly washed mature leaves were shade dried and then powdered with the help of 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 (*B.subtilis*, *S.aureus* and *S.pyogenes*) and gram negative bacteria (*E.coli, K.pneumoniae and P.aeuroginosa*) were procured from Department of Microbiology, Sagar Hospitals, Bangalore, 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. The nutrient agar plates were prepared by pouring 15 ml of molten media into sterile petriplates. The plates were allowed to solidify for 5 minutes and 0.1 % inoculum suspension was swabbed uniformly and the inoculum was allowed to dry for 5 minutes. Sterile discs (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. 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. `

3. RESULTS AND DISCUSSION

The antibacterial activity assessment by disc diffusion method for gram +ve and gram –ve bacteria are summarized in Table 1 and Table 2 respectively.

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©	Antibiotic
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

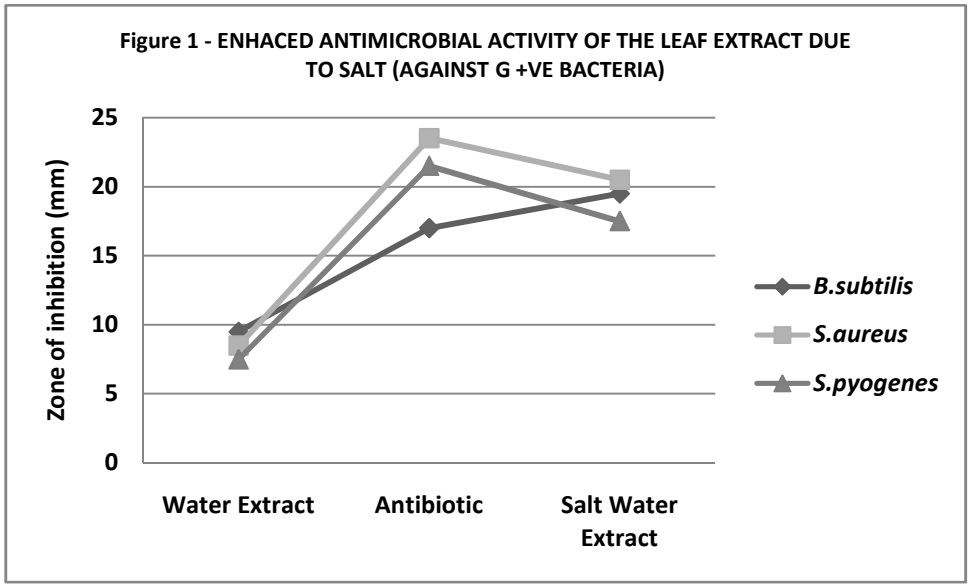
Table 2 Antibacterial activity of *Coleus aromaticus* leaf extracts against gram negative bacteria using disc diffusion method.

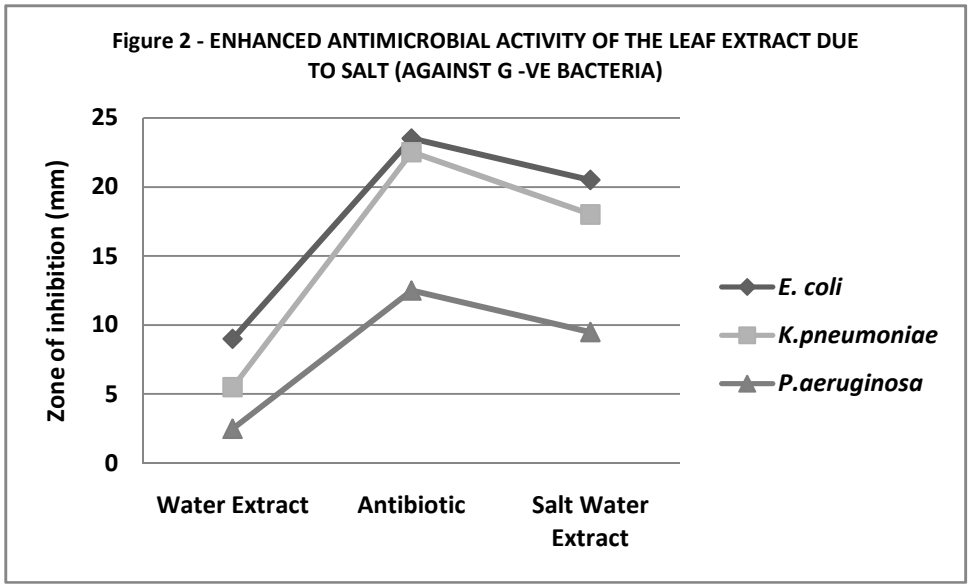
Organism	Water extract	Ethanol extract	Acetone extract	Salt water extract	Saltwater©	Antibiotic
Zone of Inhibition (mm)						
E.Coli	**9±1.4**	**11.5±0.7**	**12.3±0.4**	**20.5±0.7**	**1.5±0.7**	**23.5±2.1**
K.pneumoniae	**5.5±0.7**	**8.5±0.7**	**9.3±0.4**	**18±1.4**	**Negligible**	**22.5±2.1**
P.aeruginosa	**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

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. More over the antibacterial activity of salt water extracts appears to be almost equivalent to that of standard antibiotics, which is quite an interesting observation.

It is evident from Figure 1 and Figure 2 that the salt water extracts of *Coleus aromaticus* is much better and almost comparable to the standard antibiotics in its antibacterial activity than just the leaf extract. Thus the present results scientifically prove the correctness of a general house-hold medicational practice of consuming this leaf with salt.





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 bacteria. 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 bacteria. Gram-negative bacteria 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 components are hydrophobic in nature, they get partitioned in the lipids of the bacterial cell membrane and mitochondria, thus distorting the structure and rendering them more susceptible to the antimicrobial action, leading to leakage of the cell contents and the extensive loss of cell contents would eventually lead to 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's an encouraging observation that the combination of leaf extract and salt at appropriate concentrations seems to be acting as a broad spectrum antibacterial agent, which might not be possible with salt alone. 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. Thus the results of our study serve as a testimonial to the age old practice of using medicinal plant extracts to cure various ailments. Also, it reinforces the correctness of ancient therapies which were always thought to be a safer alternative mediation to allopathic medication.

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