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Research Article

ANTIBACTERIAL ACTIVITY OF *COLEUS AROMATICUS* LEAVES

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ABSTRACT

The main aim of this study was to find out the antibacterial activity of Ethanol and hot water leaf extracts of *Coleus aromaticus* (Family: Lamiaceae). For antibacterial test, Disc diffusion technique was used against 3 Gram positive and 2 Gram negative human pathogenic bacteria. Both the extract showed broad spectrum of inhibition by showing antibacterial effect for both Gram positive and Gram negative human pathogen bacterial strains. The zone of inhibition ranged from 9 to 14 mm for Hot water Coleus aromaticus (HWCA) extract and 16 – 27 mm for Ethenolic Coleus aromaticus (ECA) extract for 100 µg concentration. Both extracts showed potent antibacterial activity, of which the ethanol extract demonstrated the strongest antibacterial activity with the MIC value of 25 – 39 µg/ml where as water extract showed around 46 - 62 µg/ml, where *E.coli* showed maximum inhibition against the extract. The results exhibits the scientific evidence for the centuries-old usage of this plant as a medicinal herb.

Keywords: *Coleus aromaticus,* Antibacterial activity, Leaf extract, Medicinal plants.

INTRODUCTION

Antibiotics are one of our most important weapons in fighting bacterial infections and have greatly benefited the health-related quality of human life since their introduction. However, over the past few decades these health benefits are under threat as many commonly used antibiotics have become less and less effective against certain illnesses not only because many of them produce toxic reactions but also due to emergence of drug resistant bacteria. It is essential to investigate newer drugs with lesser resistance. Systematic studies among various pharmacological compounds have revealed that any drug may have the possibility of possessing diverse functions and thus may have useful activity in completely different spheres of medicine.

Drugs derived from natural sources play a significant role in the prevention and treatment of human diseases. In many developing countries, traditional medicine is one of the primary health care systems [1, 2]. Herbs are widely exploited in the traditional medicine and their curative potentials are well documented [3]. About 61% of new drugs developed between 1981 and 2002 were based on natural products and they have been very successful especially in the areas of infectious disease and cancer [4]. Recent trends, however, show that the discovery rate of active novel chemical entities is declining [5]. Therefore, there is a need to bio prospect new sources and if possible from less explored regions and habitats to maximize the discovery of novel bioactive metabolites. Multiple drug resistance (MDR) has developed due to the indiscriminate use of antimicrobials and reemergence of diseases; adverse drug reactions (ADR) and the high costs of antimicrobials have been key contributors to ineffective management of infectious diseases in many developing countries [6, 7]. Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanisms of action [7, 8]. The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world [9]. Much work has been done on ethenomedicinal plants in India [10]. It has been suggested that aqueous and ethanolic extracts from plants used in allopathic medicine are potential sources of antiviral, antitumoral and antimicrobial agents. The selection of crude plant extracts for screening programs has the potential of being more successful in initial steps than the screening of pure compounds isolated from natural products [11].

In an effort to expand the spectrum of antibacterial agents from natural resources, *Coleus aromaticus* belonging to Lamiaceae family (Mint family) has been selected. The leaves of the green type of country borage are often eaten raw with bread and butter. The chopped leaves are also used as substitute for sage (*Salvia officinalis*

L) in stuffing. *Coleus aromaticus* is used for seasoning meat dishes and in food products, while a decoction of its leaves is administered in cases of chronic cough and asthma [12]. It is considered to be an antispasmodic, stimulant and stomachic and is used for the treatment of headache, fever, epilepsy and dyspepsia. It is used to treat conditions such as indigestion, diarrhea, nervous tension, insect bites, toothache, earache, rheumatism, whooping cough, and bronchitis [13]. It is also known to be a very powerful painkiller, stimulates flow of bile aiding digestion. Mast cell stability property of *C. aromaticus* leaves were checked in rat peritoneal mast cells [14]. Freeze-dried aqueous extract of *C. aromaticus* extract clearly established antioxidant potency [15]. *C. aromaticus* has been used historically for menorrhagia in Trinidad [16]. In the present study, antibacterial activity of ethanol and water extract of *Coleus aromaticus* leaves was determined.

MATERIALS AND METHODS

Coleus aromaticus leaves were collected from Herbal garden maintained by Adichunchangiri Maha Samsthana Math, B.G.Nagara, Mandya, Karnataka for experimental purpose. Chloramphenicol, was obtained from Sigma Aldrich Company. (St. Louis, USA). All solvents/chemicals used were of analytical grade and obtained from Merck, (Mumbai, India) and SRL, (Cochin, Kerela). Clinical isolated Bacterial strains were obtained from Dept of Microbiology, AIMS, B.G.Nagara.

Preparation of Hot water extract of *Coleus aromaticus* (HWCA)

Leaves of *Coleus aromaticus* was thoroughly washed with double distilled water 50 gm of leaves was homogenized with 100 ml of boiling hot water using pestle and mortar, 400 ml of hot/cold water was mixed with the residue and kept for stirring for 30 minutes. The pooled extract was centrifuged at 10,000 rpm for 15 minutes at 4^0C. Collected supernatant was concentrated by freeze drying using lyophilizer. The Extract obtained was called as HWCA (Hot water extract). Extract was filtered through 0.22 micron filter and stored at -20 ^{0}C for further use.

Preparation of ethanol extract of *Coleus aromaticus* (ECA)

50g of dried leaves of *Coleus aromaticus* were homogenized with 500ml of distilled ethanol using mortar and pestle. This was centrifuged at 7000rpm for 10 minutes. Clear supernatant was concentrated using rotary evaporator at 38^0 to 40^o C. The extracts were dissolved in ethanol and kept in -20^0C for further use.

Proximate analysis of the extracts:

The protein content at extracts was determined by Bradford method [17]. The total sugar was estimated by the phenol–sulphuric acid method (18), Total phenolic content was determined by the Folin–Ciocalteau reagent (19), chlorophyll and β-carotinoids content was estimated according to the method described by Sadasivam and Manickam (20) and Ascorbic acid (21), content was also determined.

Antibacterial activity of HWCA/ECA

Antibacterial activity was evaluated by the well diffusion method on nutrient agar medium (22). This was confirmed by the inhibitory effect on bacterial growth as reflected by the inhibition zone compared to known antibiotics. The sterile nutrient agar medium (20 ml) in Petri dishes was uniformly smeared using sterile cotton swabs with test pure cultures of human pathogenic bacteria *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Escherichia coli,* and *Salmonella enteritidis* The nutrient agar media was prepared by dissolving 0.3% beef extract, 0.3% yeast extract, 0.5% peptone, 0.5% NaCl and 1.5% agar in 1liter of distilled water. The wells of 5mm diameter were made using sterile cork borer in each petri plates and the buffer extract, Different concentration of Samples were added, a blank well loaded without test compound was regarded as control. For each treatment triplicates were maintained. The plates were incubated at 37 ^{0}C for 24 hr and the resulting zone of inhibition was measured by comparing control and the standard antibiotic.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC)

The minimum inhibitory concentration of HWCA/ECA was determined by serial dilution method in the nutrient broth (23), the inoculum was prepared from fresh overnight broth culture in nutrient broth. Plates were incubated for 24 hours at 37 ^{0}C. MIC was recorded as lowest extract concentration demonstrating no visible growth in the broth. Minimum bactericidal concentrations (MBC) were recorded as the lowest concentrations that showed no growth with the CA extracts

RESULTS AND DISCUSSION

Coleus aromaticus is a common plant with medicinal properties. In our study, when the hot aqueous and ethenolic extract of *C.aromaticus* was tested for antibacterial activity against human pathogenic Gram positive and Gram negative bacteria's like *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Escherichia coli,* and *Salmonella enteritidis*.

Proximate analysis

Proximate analysis of hot water and ethanol extract showed that the extracts was rich in Polyphenolics, α-tocopherol, Proteins, Sugars and Vitamin –C and Chlorophyll. Ethanol extract had β-carotene in extra. Table.1 shows the results of proximate analysis of HWCA and ECA.

Table 1: Proximate analysis of active components in extracts of *Coleus aromaticus*

Different extracts	Protein mg/g	Total sugar mg/g	Vitamin-C mg/g	Total polyphenols mg/g	Chlorophyll mg/g	β-carotene mg/g	α-tocopherol mg/g
HWCA extract	2.1	1.7	0.14	1.6	0.028	Nil	0.61
ECA extract	1.6	1.3	0.08	2.9	0.031	0.418	1.09

Antibacterial activity of HWCA and ECA

Both HWCA and ECA were tested for antibacterial effect. Table.2 shows the effect of HWCA (100 µg), ECA (100 µg) and Chloramphenicol (15 µg) on bacterial strains. Ethnaolic extract (ECA) shows good antibacterial activity when compared to hot water extract (HWCA). The inhibition zone of ECA (100 µg) against bacterial strains ranged from 16 to 27 mm where as HWCA showed 9 to 14 mm.

Table.2: Antibacterial activity of the extracts of *Coleus aromaticus* using disc diffusion assay. *Coleus aromaticus* extracts / mean length of inhibition zones (mm) ± S.D.

Bacterial cultures	HWCA extract (100µg)	ECA extract (100µg)	Chloramphenicol (15 µg)
Zone of inhibition(mm)			
Gram positive bacteria	10±2	19±1	18±1
Staphylococcus aureus			
Bacillus subtilis	11±0.5	16±1	16±1
Bacillus cereus	9±0.8	17±1.5	19±1
Gram negative bacteria	14±1	27±1	30±2
Escherichia coli			
Salmonella enteritidis	11± 0.8	21±1	20±1

The sterile nutrient agar medium (20 ml) in petri dishes was uniformly smeared with test pure cultures. HWCA and ECA(100µg) was added to the well. Chloramphenicol (15 µg) used as positive control. The plates incubated at 37 ^{0}C, for 24 hrs and zone of inhibition measured in mm. Analysis was carried out in triplicates and the values are expressed as mean ± SD

Dose dependent antibacterial activity of HWCA and ECA

Fig. 1 and Fig. 2 shows the dose dependent antibacterial effect of HWCA and ECA on the bacterial strains. When compared to all other strains, *E.coli* showed more Inhibition zone. ECA extract showed more potency in it by showing more of inhibition zone than HWCA for all the strains.

The diameter of the clear zone were measured and plotted after subtracting the diameter of the well (5 mm). Results are mean ± S.D for three independent assays each performed in triplicate.

Minimum Inhibitory Concentration (MIC) and Minimum bactericidal concentrations (MBC) of HWCA and ECA

MIC and MBC was done Serial dilution method. MIC value of HWCA extract ranged from 46 – 62 µg/ml, ECA extract's MIC values ranged from 25 – 39 µg/ml. *E.coli* showed the MIC value of about 42 and 25 µg/ml concentration against HWCA and ECA respectively. MBC values of HWCA and ECA ranged from 96 – 130 µg/ml and 50 – 75 µg/ml respectively. Table.2 shows the MIC and MBC values of HWCA and ECA extracts.

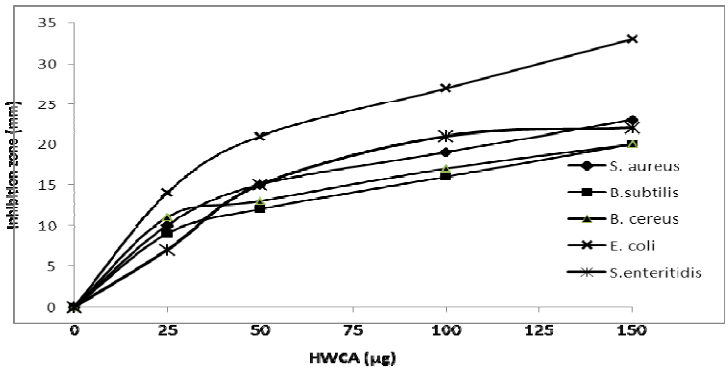

Fig. 1: Dose dependent antibacterial activity of HWCA against different human pathogenic strains in agar diffusion assays. The diameter of the clear zone were measured and plotted after subtracting the diameter of the well (5 mm). Results are mean ± S.D for three independent assays each performed in triplicate.

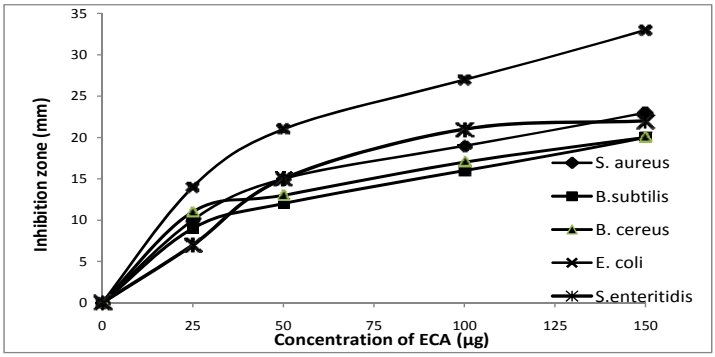

Fig 2: Dose dependent antibacterial activity of ECA against different human pathogenic strains in agar diffusion assays.

Table 3: Minimum Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) of HWCA and ECA extracts in serial dilution method

Bacterial cultures	HWCA extract (µg/ml)		ECA extract (µg/ml)		Chloramphenicol (µg/ml)
	MIC	MBC	MIC	MBC	MIC
Staphylococcus aureus	58 ± 2	110 ± 2	37 ± 1.5	70 ± 2	14 ± 1
Bacillus subtilis	56 ± 1.5	100 ± 3	39 ± 1	75 ± 3	14 ± 0.5
Bacillus cereus	52 ± 2	120 ± 2	33 ± 1.5	65 ± 2.5	12 ± 1
Escherichia coli	46 ± 1.5	96 ± 1.5	25 ± 3	50 ± 1.5	15 ± 1
Salmonella enteritidis	62 ± 1.5	130 ± 3	34 ± 1.5	68 ± 2	13 ± 1.5

The above results illustrate that *Coleus aromaticus* showed a wide spectrum of antibacterial activity against all human pathogenic bacteria tested. ECA extract showed more potency of antibacterial effect than HWCA extract. 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.

Both HWCA and ECA showed a broad spectrum of antibacterial activity by inhibiting both Gram positive and Gram negative bacteria. As the extract showed potent antibactericidal activity against *E.coli*, the extract can be used to treat cases of diarrhea caused by these organisms in infected individuals. . The results provide a scientific basis for the centuries-old usage of this plant as a medicinal herb.

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