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Research Article

Phytochemical screening and antibacterial activity of methanolic leaf extract of *Coleus aromaticus* benth

Ashwini S. and Girish K.*

Postgraduate Department of Microbiology, Maharani's Science College for Women, JLB road, Mysore – 570 005, Karnataka, India

ABSTRACT

In the present study, the aqueous extracts of seven plants such as *Cassia auriculata* L., *Coleus aromaticus* Benth, *Lawsonia inermis* L., *Mimosa pudica* L., *Phyllanthus niruri* L., *Tinospora cordifolia* Miers., and *Tribulus terrestris* L., were screened for their antibacterial activity against human pathogenic bacteria like *Escherichia coli, Haemophilus influenzae, Klebsiella pnuemoniae, Proteus mirabilis, Salmonella typhi, Shigella flexneri* and *Staphylococcus aureus.* The antibacterial assay was performed by the agar-well diffusion method. *Coleus aromaticus,* whose aqueous extract recorded antibacterial activity at 10 mg/ml, was subjected to methanol extraction and tested for the presence of phytochemical compounds and also for antibacterial activity at different concentrations *viz*., 0.25 mg/ml, 0.5 mg/ml, 1.0 mg/ml, 2 mg/ml, 4 mg/ml, 6 mg/ml, 8 mg/ml, and 10 mg/ml. Preliminary phytochemical analysis of the leaves extract revealed the presence of reducing sugar, protein, phenolic compounds, alkaloids, flavonoids, tannins, cardiac glycosides, steroids, and terpenoids. Methanolic leaf extract of *C. aromaticus* showed moderate to high activity against all the investigated bacterial pathogens. The results indicated that the methanolic leaf extract of *C. aromaticus* is pharmacologically active and is a good antibacterial agent. Further investigations are required on isolation and characterization of the bioactive principle responsible for antibacterial activity.

Keywords: *Coleus aromaticus;* methanolic extract; phytochemical analysis; bacterial pathogens; antibacterial activity

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 (Bhalodia and Shukla, 2011). However, over the past few decades many of these commonly used antibiotics have become less effective due to emergence of drug resistant bacteria. Multiple drug resistance (MDR) has developed due to the indiscriminate use of antimicrobials (Baskaran et al., 2011). In this context it becomes essential to investigate newer effective drugs. Drugs derived from natural sources play a significant role in the prevention and treatment of human diseases. Plants have been a valuable source of natural products for maintaining human health from ages (Kavishankar et al., 2011). The medicinal use of plants is probably as old as mankind. In many developing countries, traditional medicine is one of the primary health care systems (Houghton, 1995). Herbs are widely exploited in the traditional medicine and their curative potentials are well documented (Dubey et al., 2004). Medicinal plants have curative properties due to the presence of various complex chemical substances called secondary metabolites. Plants are rich in a variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, phenols, steroids, glycosides and volatile oils (Cowan, 1999). The phytomedicines derived from plants have shown great promise in the treatment of various human diseases (Kavishankar et al., 2011). In present scenario, the demand of herbal drugs is growing exponentially throughout the world and major pharmaceutical companies are currently conducting extensive research on plant materials for their potential medicinal values (Pandey et al., 2008).

Coleus aromaticus Benth (Lamiaceae), syn. *Coleus amboinicus* (Lour.) Spreng or *Plectranthus amboinicus* Lour., is commonly known as Indian / country borage (Kumar et al., 2007). It is a commonly available medicinal herb in India. It is a large succulent aromatic perennial herb with hispidly villous or tomentose fleshy stem. Leaves are simple opposite, broadly ovate, crenate, fleshy and very aromatic. The leaves of *C. aromaticus* are often eaten raw with bread and butter and it is used for seasoning meat dishes and in food products (Venkatesh et al., 2010). It grows to a maximum of 1.5 to 2 meters and the plant emanates mild, pleasant odour which increases when cut or crushed (Pritima and Pandian, 2008). The leaves of this plant are tradi-

* Corresponding Author
Email: girishk77@yahoo.com
Contact: +91-9743665772
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tionally used for the treatment of severe bronchitis, asthma, diarrhea, epilepsy, renal and vesicle calculi and fever (Warrier et al., 1995). *C. aromaticus* has been reported to exhibit antilithiotic (Jose et al., 2005), chemopreventive (Prasad et al., 2002) antiepileptic (Buznego and Perez-Saad H, 1999) and antioxidant (Padma et al., 1988) properties. Owing to its biological activities, in the present study, *C. aromaticus* has been screened for its antibacterial activity against seven pathogenic bacteria.

MATERIALS AND METHODS

Collection of the plant material and preparation of aqueous extracts

Leaves of seven plants *viz., Cassia auriculata* L., *Coleus aromaticus* Benth, *Lawsonia inermis* L., *Mimosa pudica* L., *Phyllanthus niruri* L., *Tinospora cordifolia* Miers., and *Tribulus terrestris* L., were collected from Mysore, Karnataka, India. The plants were authenticated by Department of Studies in Botany, University of Mysore. Samples (50 g) of the shade-dried powder of leaves of seven plants were macerated with 200 ml distilled water in a warring blender for 10 min. The macerate was first filtered through double-layered muslin cloth and then centrifuged at 5000 g for 30 min. The supernatant was filtered through Whatman No. 1 filter paper and sterilized at 120°C for 30 min. The extracts were preserved aseptically in brown bottle at 4°C until further use.

Collection of the plant material and preparation of methanol extracts

Leaves of *Coleus aromaticus* Benth were collected from Mysore, Karnataka, India. Leaves of *C. aromaticus* were shade dried at room temperature for 2-3 days. These dried leaves were then powdered in a mixer so as to get a coarse powder for extraction in a soxhlet extractor. 100 g each of the powdered material was extracted with 500 ml of methanol separately for 24 hrs. After extraction the solvent was evaporated in rotary flash evaporator. The residues from each extract were dried in the desiccator and the resultant extract was stored in an air tight container at 4°C for further use. The extract was subjected to preliminary phytochemical testing and *in vitro* antioxidant activities.

Phytochemical screening of methanolic leaf extract

The methanolic extract was subjected to phytochemical tests following standard procedures (Khandelwal, 2004) to detect phytoconstituents like proteins, reducing sugars, oils and fats, cardiac glycosides, tannins, saponins, phenols, flavonoids, alkaloids, steroids, anthroquinone, and terpenoids.

Test for reducing sugars (Fehling's test): To 2 ml of the extract, 5ml of a mixture (1:1) of Fehling's solution A and Fehling's solution B was added and the mixture boiled in a water bath for five minutes. A brick-red precipitate indicates the presence of reducing sugars.

Test for protein (Biuret test): To 2 ml of the extract, 2 ml of 5% sodium hydroxide solution and 2 drops of 1% copper sulphate solution were added. Purple colour indicates the presence of proteins.

Test for oils and fats (spot test): A spot was prepared on the filter paper with a drop of extract solution and oil staining on the filter paper indicates the presence of oils and fats.

Test for Cardiac Glycosides (Keller Killani test): 0.2 g of extract was mixed with 2 ml of glacial acetic acid containing 1-2 drops of 2% solution of $FeCl_3$. The mixture was then poured into another test tube containing 2 ml of concentrated H_2SO_4. A brown ring at the inter phase indicates the presence of cardiac glycosides.

Test for anthraquinones: 0.5 g of the extract was shaken with 10 ml of benzene, filtered and 5 ml of 10% ammonia solution added to the filtrate. The mixture was shaken; the presence of a rose-pink colour in the ammoniacal (lower) phase indicates the presence of anthraquinones.

Test for saponins (Foam test): About 0.2 g of extract was mixed with 5 ml of distilled water and shaken vigorously for a stable persistent broth. Formation of foam indicates the presence of saponins. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Test for Tannins (Ferric chloride test): About 0.5 g of the extract was boiled in 10 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or a blue-black colouration.

Test for alkaloids (Mayer's Test, Dragendroff's Test and Wagner's Test): 0.5 g of extract was diluted to 10 ml with acid alcohol, boiled and filtered. To 5 ml of the filtrate was added 2 ml of dilute ammonia. 5 ml of chloroform was added and shaken gently to extract the alkaloidal base. The chloroform layer was extracted with 10 ml of acetic acid. This was divided into three portions. Mayer's reagent was added to one portion, Draggendorff's reagent to the second and Wagner's reagent to the third. The formation of a cream precipitate (with Mayer's reagent) or reddish brown precipitate (with Draggendorff's reagent and with Wagner's reagent) indicates the presence of alkaloids.

Test for Flavonoids (Alkaline reagent test): To 2 ml of extract solution, few drops of sodium hydroxide was added, formation of an intense yellow colour, which turns to colourless on addition of few drops of dilute acetic acid indicates the presence of flavanoids.

Test for Phenol: To 2 ml of extract, added 3-4 drops of ferric chloride solution. Formation of bluish black colour indicates the presence of phenols.

Test for terpenoids (Salkowski test): To 0.2 g of the extract 2 ml of chloroform was added. Concentrated

Table 1: Antibacterial activity of aqueous extracts of different plants against some human pathogenic bacteria at 10 mg/ml concentration

S. No.	Human pathogenic bacteria	Zone of inhibition (mm)						
		Cassia auriculata	***Coleus aromaticus***	***Lawsonia inermis***	***Mimosa pudica***	***Phyllanthus niruri***	***Tinospora cordifolia***	***Tribulus terrestris***
1	*Escherichia coli*	0.0	13.7 ± 0.3	0.0	0.0	0.0	0.0	0.0
2	*Haemophilus influenzae*	0.0	12.6 ± 0.2	0.0	0.0	0.0	0.0	0.0
3	*Klebsiella pnuemoniae*	0.0	15.2 ± 0.4	0.0	0.0	0.0	0.0	0.0
4	*Proteus mirabilis*	0.0	12.4 ± 0.3	0.0	0.0	0.0	0.0	0.0
5	*Salmonella typhi*	0.0	13.9 ± 0.4	0.0	0.0	0.0	0.0	0.0
6	*Shigella flexneri*	0.0	14.4 ± 0.3	0.0	0.0	0.0	0.0	0.0
7	*Staphylococcus aureus*	0.0	15.7 ± 0.4	0.0	0.0	0.0	0.0	0.0

Values are means of two experiments and each with three replications ± SE

Table 2: Phytochemicals of *Coleus aromaticus* methanolic leaf extract

S. No.	Phytochemicals	
1	Reducing Sugar	+
2	Protein	+
3	Phenol	+
4	Alkaloids	+
5	Flavonoids	+
6	Tannins	+
7	Cardiac glycosides	+
8	Steroids	+
9	Terpenoids	+
10	Saponins	--
11	Anthroquinone	--
12	Oils and fats	--

+ Present; --- Absent

H_2SO_4 (3 ml) was carefully added to form a layer. A reddish brown colouration of the interface indicates the presence of terpenoids.

Test for steroids: 0.2g of extract was mixed with 2 ml of chloroform and concentrated H_2SO_4 was added sidewise. A red colour produced in the lower chloro---form layer indicated the presence of steroids. Another test was performed by mixing 0.2g of extract with 2 ml of chloroform. Then 2 ml of each of concentrated H_2SO_4 and acetic acid were poured into the mixture. The development of a greenish coloration indicates the presence of steroids.

Antibacterial activity assay

The pure cultures of human pathogenic bacteria such as *Escherichia coli, Haemophilus influenzae, Klebsiella pnuemoniae, Proteus mirabils, Salmonella typhi, Shigella flexneri* and *Staphylococcus aureus* were obtained from the culture collection centre, Department of Microbiology, J.S.S. Medical College, Mysore, India. The cup diffusion method was used to screen the antibac---terial activity using Mueller Hinton Agar (MHA) ob---tained from Himedia (Mumbai). The MHA plates were inoculated with 1 x 10^5 cfu cultures of test bacteria. Agar cup of 5 mm diameter were made in the plates. 0.25 mg/ml, 0.5 mg/ml, 1.0 mg/ml, 2 mg/ml, 4 mg/ml, 6 mg/ml, 8 mg/m1, and l0 mg/ml solutions of each aqueous/ methanol extract were prepared using dis---tilled water. 50 µ1 volume of each aqueous/ methanol extract was introduced into wells in MHA plates al---ready seeded with the standardized inoculums (1x10^5 cfu/ml) of the test bacterial cells. 50 µ1 of distilled wa---ter/ methanol was introduced to one of the well as control. The compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 37°C for 24 h. Chloramphenicol discs (30 mcg) was used as positive control for comparison. After incuba---tion, inhibition zones formed around the wells and discs were measured with transparent ruler. Triplicates were maintained and the experiments were conducted twice.

Table 3: Antibacterial activity of methanolic leaf extract of *Coleus aromaticus* against some human pathogenic bacteria at different concentrations

Human pathogenic bacteria	Zone of inhibition (mm)							
	0.5mg/ml	1.0mg/ml	2.0mg/ml	4.0mg/ml	6.0mg/ml	8.0mg/ml	10.0mg/ml	CHL (30mcg)
Escherichia coli	0.0	5.8±0.33	11.0±0.35	14.5±0.33	20.7±0.36	25.0±0.33	29.1±0.36	24.6 ± 0.31
Haemophilus influenzae	0.0	0.0	6.0±0.26	11.6±0.33	14.0±0.28	18.5±0.29	21.8±0.34	18.4 ± 0.35
Klebsiella pnuemoniae	0.0	6.5±0.31	12.5±0.35	18.6±0.34	23.0±0.25	28.5±0.33	34.3±0.29	24.0±0.32
Proteus mirabilis	0.0	5.5±0.25	10.8±0.24	15.4±0.28	18.5±0.43	24.4±0.26	28.6±0.35	20.6±0.43
Salmonella typhi	0.0	5.5±0.31	9.5±0.25	13.8±0.25	17.0±0.24	21.7±0.29	26.5±0.29	25.4±0.28
Shigella flexneri	0.0	6.8±0.24	12.6±0.32	17.5±0.25	21.5±0.32	26.5±0.32	31.8±0.34	24.8±0.34
Staphylococcus aureus	0.0	7.0±0.43	13.0±0.43	18.5±0.29	22.7±0.34	27.0±0.26	34.2±0.24	20.1±0.29

Values are means of two experiments and each with three replications ± SE; CHL: Chloramphenicol

RESULTS

Antibacterial activity of aqueous extracts

Antibacterial activity of aqueous extracts of seven plants against different human pathogenic bacteria is presented in Table 1. Out of the aqueous leaf extracts of seven plants studied, leaf extract of only one plant, *Coleus aromaticus* Benth, showed significant antibacterial activity. At the concentration of 10 mg/ml, aqueous extract of no other plant tested showed any antibacterial activity. Antibacterial activity of different concentration of aqueous extract of *C. aromaticus* showed a variable effect against different human pathogenic bacteria studied. Among the pathogenic bacteria tested *S. aureus* was highly susceptible followed by *K. pnuemoniae* and *S. flexneri.*

Phytochemical screening of methanolic leaf extract

Phytochemical screening of methanolic leaf extract revealed the presence of reducing sugars, proteins, phenolic compounds, alkaloids, flavonoids, tannins, cardiac glycosides, steroids and terpenoids (Table 2).

Antibacterial activity of methanolic leaf extract of *Coleus aromaticus*

Methanolic leaf extracts of *Coleus aromaticus* Benth, showed significant antibacterial activity. The antibacterial activity of different concentration of methanolic leaf extract of *C. aromaticus* is presented in Table 3. A variable antibacterial activity of different concentrations of *C. aromaticus* methanolic leaf extract against different human pathogenic bacteria was observed. Among the pathogenic bacteria tested *K. pnuemoniae* and *S. aureus* were highly susceptible followed by *S. flexneri*, *E. coli* and *P. mirabilis*, whereas *H. influenzae* was least susceptible. With increasing concentration there was increased activity against all the bacteria tested. Comparatively better inhibition was observed with methanolic extracts than aqueous extracts. In methanol extract, the lowest MIC value observed was 1.0 mg/ml except *H. influenzae* (2.0 mg/ml).

DISCUSSION

Presently, the principle focus of pharmaceutical research is on the ethnobotanical approach for the discovery of new drugs. Plants have always been the sources of biologically active compounds used for the treatment of various infectious diseases (Dev, 1997; Perumalsamy and Ignacimuthu, 2000). The side effects associated with the use of allopathic drugs have resulted in an increased demand for the phytopharmaceutical products (Samy, 2008). In the present study, the antibacterial activity of the aqueous and methanol extracts of *Coleus aromaticus* was determined against seven different human pathogenic bacteria. 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 (Kusumoto, 1995). The methanolic crude extract of *C. aromaticus* leaves exhibited significant antibacterial activity against all the tested human pathogenic bacteria. The activity observed was better than the standard antibiotic chloramphenicol. It is obvious from the results that *C. aromaticus* is a fairly good antibacterial herb and has broad spectrum of activity. Antimicrobial activities including antibacterial activity of different solvent extracts as well as essential oils of *C. aromaticus* have been reported (Koba et al., 2007; Shiney Ramya et al., 2012; Thenmozhi et al., 2011). Antibacterial action of the methanolic leaf extract of *C. aromaticus* against bacterial pathogens explains the fact that this herb may be utilized for the treatment of several difficult-to-treat infections in humans.

The therapeutic value of medicinal plants lies in the various chemical constituents in them. The phytochemical analysis of the methanolic leaf extract revealed the presence of reducing sugar, protein, phenolic compounds, alkaloids, flavonoids, tannins, cardiac glycosides, steroids, and terpenoids. The antibacterial activity of the leaf extracts of *C. aromaticus* as recorded in present study may therefore be attributed to the presence of above phytochemicals. Alkaloids, flavonoids, tannins and terpenoids are plants metabolites well known for their antimicrobial activity (Cowan, 1999; Singh and Bhat, 2003). Broad spectrum activity of methanol extract in this study shows that the active ingredients of the leaves were better extracted with methanol. The results of present investigations indicate that *C. aromaticus* can be used as a potential

source of useful drugs for the treatments of the various bacterial pathogens. However, further studies are needed to isolate and characterize the bioactive prin---ciples to develop new antimicrobial drugs.

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