Research Article

Antimicrobial, Phytochemical and Antioxidant Study of Hydroalcoholic Extracts of *Coleus caninus* (roth) vatke

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ABSTRACT

Coleus caninus (Roth) Vatke (Labiatae) is an important plant in Indian system of medicine. The present study was aimed to determine the secondary plant metabolites present in *Coleus caninus* and investigate its antioxidant and antimicrobial activities. The dried and powdered plant material was extracted with mixture of ethanol and water (7:3) to yield crude extracts. The obtained extract was assayed for Antimicrobial activity by agar-well diffusion method. In addition, a phytochemical screening and in-vitro total antioxidant assay of the hydro-alcoholic extracts was done. The plant extract showed antibacterial activity against *Bacillus subtilis, Escherichia coli, Proteus sp, Serratia marganni* and *Staphylococcus aureus.* The phytochemical analysis carried out revealed the presence of flavonoids, glycosides, phenols, tannins, Saponins and. Alkaloids. Total phenolic and total flavonoid contents in the extract were 10.22±1.05 and 0.93 ± 0.02% w/w of the extracts, respectively. The total antioxidant capacity was found to be 4.356±0.3 Mm Ascorbic acid/g dry mass. This study underscores the importance of application of *C. caninus* in ethno medicine and extracts from *C. caninus* could be used as antioxidant and antimicrobial agents.

Keywords: Antioxidant activity, Antimicrobial activity, Coleus caninus, Phytochemicals.

INTRODUCTION

Coleus caninus (Roth) Vatke (synonym: *Coleus spicatus, Plectranthus caninus*) belongs to Labiatae family and is an important plant in the Indian system of medicine. In India, the genus *Coleus* or *Plectranthus* is found in all the habitats and altitudes, particularly in the Himalaya, the Southern Ghats, and the Nilgiri region. *Coleus amboinicus*, *Coleus barbatus*, *Coleus caninus*, *Coleus mollis* etc are the most common species found in India. In Ayurvedic medicine *Coleus* species have been used to treat heart disease, convulsions, spasmodic pain and painful urination.[1] *Coleus amboinicus* 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.[2] *Coleus barbatus* also known as *Coleus forskohlii* is interesting from a scientific and medicinal standpoint because it produces forskolin, a diterpene used as a vasodilator.[3] The leaves of *Coleus caninus* are chewed in Africa to relieve toothache.[4] The aerial parts of the plant have also been reported to have diuretic, cytotoxic and anti-tumor activities.[5] Phytochemical studies of the genus revealed that Indian *Plectranthus* species are rich in essential oil, and that the most abundant secondary metabolites are diterpenoids, i.e., labdanes, abietanes, and ent-kauranes, as well as triterpenoids.[6]

Medicinal plants represent a rich source of antimicrobial agents. Due to increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, the scientists are forced to search for new antimicrobial substances from various sources like the medicinal plants.[7] The use of indigenous knowledge of traditional medicinal practitioners as leads provides a useful route employed in the search for novel drugs.[8] This is rewarding, and since most indigenous plants used in traditional medicine have not been explored in detail, the potential for discovery of more novel therapeutic compounds through Bioprospecting of the flora in tremendous. Numerous investigations have proved that medicinal plants such as tannins, alkaloids and flavonoids, which exhibit various pharmacological properties.[9] Plant phenolics, in particular phenolic acids, tannins and flavonoids are known to be potent antioxidants and occur in vegetables, fruits, nuts, seeds, roots, leaves and barks. Irrespective of the presence of a large variety of phytoconstituents in the genus *Coleus*, only a few reports regarding the pharmacological investigations on the plants of this genus are available. Hence the present study was aimed to investigate the probable antimicrobial and antioxidant effects of *Coleus caninus* and to analyze various phytochemicals present in the plant.

MATERIALS AND METHODS

Collection of Plant material

Coleus caninus (Roth) Vatke used in the present study was collected from local forest of Hassan, Karnataka, India, during the flowering season of October – November, 2009-2011. The taxonomic identities of plants were confirmed by using Flora of Hassan District and Voucher specimen (RRCBI-8726) deposited in National Ayurveda Dietetics Research Institute, Bangalore (Central Council for Research in Ayurvedic and Siddha, Department of AYUSH, Ministry of Health and F.W, Govt of India, New Delhi).

Extraction

Freshly collected plant materials (leaves, roots and stem) were first washed with tap water and then thoroughly with distilled sterile water, so as to remove dust and soil particles. Then it was either air dried or dried in a drying cabinet at 50°C for 5-7 days. Dried plant materials of the plant species were separately crushed and ground into fine powder using food blender. Each powdered plant material (10g) was extracted with mixture of ethanol: water (7:3 ratios) by a Soxhlet apparatus at 45°C for 5h. The obtained extracts were filtered and concentrated in a rotary evaporator at 45 °C under reduced pressure. This crude extract was used for further investigation for potential antimicrobial and antioxidant properties and phytochemical determination.

Anti-microbial Screening

Growth and Maintenance of Test Microorganism

The pathogenic strains used for the screening antibacterial activity, such as *Bacillus subtilis, Escherichia coli*, *Proteus, Pseudomonas aeruginosa, Serratia marganni* and *Staphylococcus aureus* were obtained from Department of Microbiology and Biotechnology, Jnanabharathi Campus, Bangalore University, Karnataka, India. Cultures were maintained as nutrient agar slants in screw-capped bottles and stored at 4°C. All cultures were checked for viability and purity by regular plating and biochemical tests. Test cultures were prepared by transferring a loop full of bacteria from stock culture nutrient broth and incubated at 37 ^{0}C for 24 h.

Antibacterial Activity Test

For Monitoring antibacterial activity by the agar well diffusion method[10], bacterial lawn was prepared by spread plate method (10^6 cells/ml) with 24h old test cultures. Wells were punched for 5 mm deep in 30 min old bacterial lawn. Further, wells were filled with 100 µl aliquots of 50 mg /mL hydro-alcoholic extract of the plant (original stock of the plant extract was diluted by 10% DMSO). Plates were incubated at 37°C for 18-24h.

Antibacterial activities were evaluated by measuring the diameter values of zones of inhibition. Inhibition zones of less than 6 mm were not evaluated. It was confirmed that 10% DMSO itself had no inhibitory effect on any bacterium. A standard antibiotic Chloromycein (50 µg/ml) was used as positive control for comparison. The experiments were performed three-times to minimize errors and mean values are presented.

Preliminary Phytochemical Screening

The crude extract of *Coleus caninus* was tested for the presence of phenols, flavonoids, tannins, Saponin, alkaloids, glycosides and anthraquinones. All the tests were carried out using standard procedures to identify the constituents as described by Trease and Evans[11], Sofowora[12] and Harborne.[13]

Estimation of Total Phenol Content (TPC)

Total phenol content of the extracts was determined calorimetrically using Folin-Ciocalteau method.[14] The aliquots (400 µl) of extract were mixed with 2 ml of Folin-Ciocalteau reagent and 1.6 ml of 4 % sodium carbonate. The mixture was allowed to stand for 2 h with intermittent shaking for reaction. After oxidation the green-blue complex formed was measured at 750 nm). Using phenol, standard curve was prepared and linearity was obtained in the range of 10-50 µg/ml. The amount of total phenolics was expressed in % w/w of the extracts.

Estimation of Total Flavonoid Content (TFC)

Aluminum chloride colorimetric technique[15] was used for total flavonoids estimation. Flavonoids are capable of forming complexes with metal ions and act as antioxidants. A known volume (1.0 ml) of the extract was mixed with 3ml of methanol, 0.2 ml of 10 % aluminum chloride, 0.2 ml of 1 M potassium acetate and 5.6 ml of distilled water. The reaction mixture was allowed to stand at room temperature for 30 min and the absorbance of the reaction mixture was measured at 415 nm. The calibration curve was prepared by using Quercetin at concentrations of 10 to 100 µg/mL in methanol. The total flavonoid content was expressed as Quercetin equivalent in % w/w of the extracts.

In-vitro antioxidant assay

Determination of total antioxidant capacity

The total antioxidant capacity was evaluated by the Phosphomolybdenum method.[16] The assay is based on the reduction of Mo (VI) to (V) by the antioxidant extract and subsequent formation of a green phosphate Mo (V) complex at acidic pH. An aliquot of 0.3 ml of the extract containing a reducing species in DMSO was combined in an eppendorf tube with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate). The tubes containing the reaction solution were incubated in water bath at 95°C for 90 min. Then the absorbance of the solution was measured at 695 nm using spectrophotometer against blank after cooling to room temperature. Methanol (0.3 ml) in the place of extract was used as the blank. The total antioxidant capacity was expressed as mM equivalent to ascorbic acid per gram dry mass.

RESULTS

Results obtained in the present study relieved that the tested medicinal plant extract posse's potential antibacterial activity against *B. subtilis, E. coli*, *Proteus sp, S. marganni*, and *Staphylococcus aureus* (Table 1). The highest antibacterial activity of 13 mm in *E. coli* and least activity recorded in *B. subtilis* measured 9 mm. The extracts of *C. caninus* showed no zone of inhibition against *Pseudomonas aeruginosa* and *Klebsiella sp.*

Phytochemical screening by simple chemical tests showed presence of phenols, flavonoids, tannins, Saponin and

alkaloids (Table 1). Table 2 showed the total flavonoid and phenol contents, and total antioxidant capacities of the plant extract. The total phenol content and total flavonoid content showed strong correlation with total antioxidant activity. This indicates that the antioxidant activity of the extract from *Coleus caninus* is due to its phenolic constituents. These results are in accordance with other reports in the literature, which showed positive strong correlation between antioxidant activities and total phenolics.[17-19]

Table 1: Result of antibacterial screening of hydroalcoholic extracts of *Coleus caninus* by the agar cup method.

Test bacteria	Zone of inhibition (mm)	
	Hydro-alcoholic extract (50 mg/ml)	DMSO (10%)
Bacillus subtilis	9	-
Escherichia coli	13	-
Proteus sp	12	-
Pseudomonas aeruginosa	-	-
Serratia marganni	12	-
Staphylococcus aureus	10	-
Klebsiella sp	-	-

Values are mean inhibition zone (mm) of three replicates

Table 2: Phytochemical screening of hydro alcoholic extracts of *Coleus caninus*

Constituents	Tests	Hydro alcoholic extract
Phenols	Ferric chloride test	++
Alkaloids	Dragendroff's test	++
Saponin	Frothing test	+
Tannins	Gelatin test	+
Flavonoids	Sodium hydroxide test	++
Glycosides	Borntrager's test	+
anthraquinones	Borntrager's Test	-

+, positive; ++, strongly positive; -, negative

DISCUSSION

Various phytochemicals have been known to possess medicinal properties and hence widely used in Indian systems of traditional medicine. With three thousand species, the Labiatae are very much a family of medicinal plants. Plants belonging to the Labiatae family are very rich in polyphenol compounds. In India, the genus *Coleus* or *Plectranthus* is a popular traditional plant and found in all the habitats. Therefore our aim was to study the antimicrobial and antioxidant capacity of the Labiatae member, *Coleus caninus*. In this study, various phytochemicals like phenols, flavonoids, Saponin, alkaloids, tannins, etc were present in *Coleus caninus* indicating their potential medicinal uses. Therefore, these secondary metabolites present in this plant may contribute directly to their antimicrobial and antioxidant action.

Table 3: Total flavonoid and phenol contents, and total antioxidant capacities of the plant extract

Extract	Total phenol (%)	Total flavonoid (%)	Total antioxidant capacity (Mm Ascorbic acid/g dry mass)
Coleus caninus	10.22±1.05	0.93 ± 0.02	4.356±0.3

Values are mean ± SD of three individual experiments

CONCLUSION

The present study confirmed the in-vitro antimicrobial and antioxidant potential of *Coleus caninus*. The phytochemical screening revealed chemical constituents that form the foundation of their pharmacological activity. These data further support the view that the plants is promising sources of natural antioxidants, and could be seen as potential sources of useful drugs. Nonetheless, further in-vivo studies and purification of the compounds responsible for antioxidant activity are needed.

REFERENCES

1. Dubey MP, Srimal RC, Nityanand S, et al., Pharmacological studies on coleonol, a hypotensive diterpene from *Coleus forskohlii*, J Ethnopharmacol, 3, 1981, 1–13.
2. Warrier PK, Nambiar VPK, Ramankutty C, Indian Medicinal Plants, Orient Longman Ltd., Madras, 1995, 1-5.
3. Alasbahi RH, Melzig MF, Plectranthus barbatus: A Review of phytochemistry, ethnobotanical uses and pharmacology - Part 1, Planta Med, 76(7), 2010, 653-661.
4. Kokwaro JO, Medicinal Plants of East Africa ed. 1, East African Literature Bureau, Nairobi, 1976.
5. Jaslin Edward J, Padmaja V, Evaluation of Antioxidant Activity of Aerial Part Extract of *Coleus spicatus*. Benth On Chromium (VI) Induced Oxidative Stress In Albino Rats, International Journal of Pharm Tech Research, 4(4), 2012, 1745-1749.
6. Waldia S, Joshi BC, Pathak U, Joshi MC, The genus *Plectranthus* in India and its chemistry, Chem. Biodiver, 8, 2011, 244-252.
7. Debasmita D, Mahesh CS, Shakti R, Bimoch PP, Nagen KD, Rabindra NP, Antimicrobial activity of medicinal plants used by aborigines of Kalahandi, Orissa, India against multidrug resistant bacteria, Asian Pacific Journal of Tropical Biomedicine, 2012, 846-854.
8. Amusan OOG, Sukati NA, Dlamini PS, Sibandze FG, Some Swazi phytomedicines and their constituents, African Journal of Biotechnology, 6, 2007, 267-272.
9. Emam SS, Comparative study of the alkaloid and tannin contents of some *Reseda* species, Journal of Applied Sciences Research, 6, 2010, 888-896.

10. Saranraj P, Sivasakthivelan P, Screening of Antibacterial Activity of the Medicinal Plant *Phyllanthus amarus* Against Urinary Tract Infection Causing Bacterial Pathogens, Applied Journal of Hygiene, 1(3), 2012, 19-24.
11. Trease GE, Evans WC, Pharmacognosy, 11th edition. Brailliar Tiridel and Macmillan Publishers, London, 1989.
12. Harborne JB, Phytochemical Methods, Chapman and Hall Ltd. London, 3, 1973, 135-203.
13. Sofowora A, Medicinal plants and Traditional Medicine in Africa. Spectrum Books Ltd, Ibadan, 1993.
14. Mahnaz K, Mannan H, Mina CN, Zahra K, Yousef A, Abbas H, Mohammad RO, et al., Comparison of antioxidant activity and total phenolic contents in some *Stachys* species, African Journal of Biotechnology, 8 (6), 2009, 1143-1147.
15. Alanassova, Georgieva MS and Ivancheva K, Total phenolic and total flavonoid contents, antioxidant activity and biological contaminants in medicinal herbs, J. of chemical technology and Metallurgy, 46, 2011, 81-88.
16. Jaishree V, Shabna V, A Comparative study of In-vitro antioxidant and DNA damage protection of soxhlet vs microwave assisted extracts of *Michelia champaca* Linn. Flowers, Indian Journal of Natural Products and Resources, 2(3), 2011, 330-334.
17. Piluzza G, Bullitta S, Correlations between phenolic content and antioxidant properties in twenty four plant species of traditional Ethnoveterinary use in the Mediterranean area, Pharm Biol, 49(3), 2011, 240-47.
18. Naima S, Muhammad RK, Maria S, Antioxidant activity, total phenolic and total flavonoid contents of whole plant extracts *Torilis leptophylla* L, BMC Complementary and Alternative Medicine, 12(1), 2012, 221.
19. Anand SP, Mohan G, Antioxidant activity, Phenol and Flavonoid contents of *Acacia sinuata* (Lourr) Merr, Bioscience Discovery, 5(1), 2014, 24-27.

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