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Original Research Article

Quantitative analysis of chemical constituents in medicinal plant *coleus aromaticus* extracts

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ABSTRACT

Background: Indian medicinal plants have great potential towards curing many diseases. Medicinal plant *Coleus aromaticus* is known for its wide medical applications. The main objectives of the study undertaken were to analyze the phytochemicals and compare the concentration present in the dialyzed *Coleus aromaticus* protein extract with various solvents.
Methods: Various extracts of the medicinal plant *Coleus aromaticus* leaves such as dialyzed Coleus aromaticus protein, hydro alcoholic extract, ethanol extract and chloroform extract were prepared and analyzed for various phytochemical concentrations such as Total phenols (Folin-Ciocalteu method), Flavonoids (Aluminum chloride method), total sugars (Dubois method) and Proteins (Bradford's method). Standard graph for each component was plotted.
Results: The protein concentration in dialyzed *Coleus aromaticus* protein extract is considerably higher (5.8µg/10µl) than hydro alcoholic extract (1.6µg/10µl), ethanol extract (5.2µg/10µl) and chloroform extract (2.8µg/10µl). The other phytochemicals like total phenol, flavonoids and total sugars were low in concentration in the dialyzed *Coleus aromaticus* protein extract compared to hydro alcoholic extract, ethanol extract and chloroform extract.
Conclusions: In this study, the protein concentration in dialyzed *Coleus aromaticus* protein extract is considerably higher than the same in other solvent extracts. This is the basis for further studies to unfold the antioxidant activity of *Coleus aromaticus* protein in vitro.

Keywords: Dialyzed *Coleus aromaticus* protein (DCAPE), Hydro alcoholic extract (HAE), Ethanol extract (EE) and Chloroform extract (CE)

INTRODUCTION

Medicinal plants are very good sources of drugs for traditional systems of medicine. Indian medicinal plants have lot of potential towards curing many diseases. Medicinal plant extracts contain various types of bioactive compounds known as phytochemicals. These phytochemicals can be used in treatment as anticancer, antimicrobial, antioxidant, anti-inflammatory agents etc,.[1] Recent studies show that these phytochemicals are safe, broadly effective and have less adverse effects. However in vivo studies of these phytochemicals are necessary to demonstrate their efficacy, safety and to verify their

bioavailability.[2] Coleus aromaticus plant is well known for its medical applications. Various researches on Coleus aromaticus have highlighted this.[3-5] It is necessary to explore, isolate and identify the medicinally important phytochemicals which could be used to treat various diseases. This study is one among many towards such efforts.[6,7] Crude extract of Coleus aromaticus plant leaves used for phytochemical screening tests provides insight, regarding the type of phytochemicals existing in this extract. Phytochemicals screening tests is a simple and quick procedure which gives information to various types of phytochemicals and also important tool in bioactive compound analysis. But still phytochemicals screening with different polarities and their separation remains big challenge for the process of identification and characterization of them.[8,9]

In the present study, various extracts (Dialyzed Coleus aromaticus protein, Hydro alcoholic, Ethanol and Chloroform) of the medicinal plant Coleus aromaticus leaves were analyzed for proteins, phytochemical components such as total phenols, flavonoids and total sugars and compared with dialyzed Coleus aromaticus protein extract.

METHODS

Plant materials

The coleus aromaticus plant leaves were freshly collected from in and around Tumkur, Karnataka, India. The plant material was identified and authenticated by botanist.

Preparation of crude extracts

100g of cleaned coleus aromaticus leaves collected from authentic source, cleaned with 0.1% $KMnO_4$ solution, followed by washing with double distilled water, crushed, shade dried and powdered (British Pharmacopoeia 100 mesh) and stored in glass bottle. The 4gm of coleus aromaticus leaves powder was mixed with 200 ml of double distilled water and vortexed for 4 hours at 20^{o}C using magnetic stirrer. The vortexed mixture is then centrifuged at 8000 rpm for 20 minutes and the supernatant was separated. The supernatant was subjected to 65% ammonium sulphate precipitation and vortexed overnight. The mixture was centrifuged at 8000 rpm for 20min at -4^{O}C. The precipitated protein (residue) was collected and subjected to dialysis using 2.5kDa molecular cutoff bio-membrane against double distilled water for 76 hours with an interval of 6 hours. The dialyzed precipitated (Dialyzed Coleus aromaticus protein) was separated and stored at -10^{O}C for further analysis.

Chemicals

Standard reagents and chemicals of analytical grade were used. Quercetin and bovine serum albumin were procured from Hi Media (Mumbai, India). Ethanol, chloroform, gallic acid, aluminum chloride, folin-Ciocalteu reagent were purchased from Merck procured from Chetana chemicals, Mysore, India.

Phytochemical screening

Various extracts of the medicinal plant Coleus aromaticus such as hydro alcoholic, ethanol and chloroform were prepared. These extracts along with Dialyzed Coleus aromaticus protein (DCAPE) obtained by the method described above were analyzed for phytochemical components such as total phenols (Folin-Ciocalteu method), flavonoids (Aluminum chloride method), total sugars (Dubois method) and proteins (Bradford's method).

Estimation of total phenolic content

The total phenolic content was estimated by "Folin - Ciocalteu method". The estimation is based on the principle that, phenolic compounds react with phosphomolybdic /phosphotungstic acid, in the alkaline medium. This can be measured spectrophotometrically at 730nm. Total phenolic content was calculated using a standard gallic acid.[10]

Estimation of flavonoids

The total flavonoids content was determined by "Aluminum chloride method". Flavonoids react with AlCl3 to form acid stable complexes, which can be measured spectrophotometrically at 415nm. Quercetin was used as the standard. The concentrations of flavonoids in the test samples were calculated from the calibration plot.[11]

Estimation of total sugars

The sugar conc. of the extract is estimated by "Dubois method". The sugar complexes present in the extract, react with phenol in acidic medium to form brown color, read immediately at 520nm. The total sugar concentration was calculated according to the standard glucose calibration curve.[12]

Estimation of proteins

Bradford assay relies on the binding of the dye Coomassie Blue G250 to protein. The dye binds most readily to arginyl and lysyl residues of proteins. Blue form of the dye which binds to protein, can be estimated by determining the amount of dye at 595nm. The concentrations of proteins in the test samples were calculated from the calibration plot. Varying concentrations of bovine serum albumin (10-100µg/µl) used for standard calibration curve.[13]

RESULTS

Phytochemicals like total phenols (1.55µg/10µl), flavano-

ids (1.3µg/10µl) and total sugars (21µg/10µl) are less in dialyzed Coleus aromaticus protein extract (DCAPE) compared to ethanol extract (5.2µg/10µl, 2.1µg/10µl and 32µg/10µl) and chloroform extract (4.9µg/10µl, 1.9µg/10µl and 31µg/10µl) respectively (Table 1 and Figure 1).

Table 1: Concentration of the phytochemicals in the leaf extracts of coleus aromaticus.

	DCAPE	EE	CE	HAE
Total phenols (µg/10µl)	1.5	5.2	4.9	0.6
Flavonoids (µg/10µl)	1.3	2.1	1.9	0.3
Total sugars (µg/10µl)	21	32	31	29
Proteins (µg/10µl)	5.8	5.2	2.8	1.6

All data are expressed as average of three replicate. DCAPE; Dialyzed Coleus aromaticus protein extract, HAE: Hydro alcoholic extract, EE: Ethanol extract & CE: Chloroform extract.

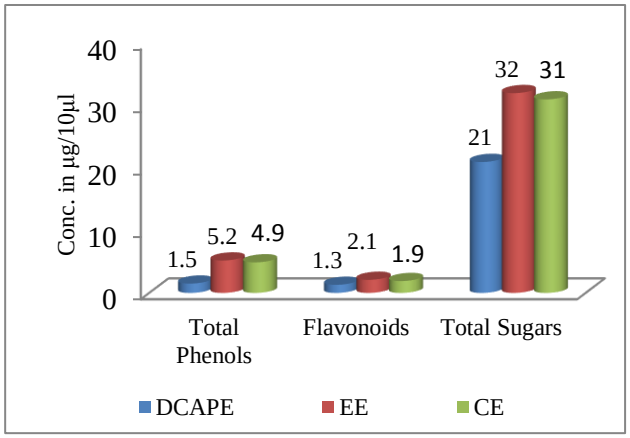

Figure 1: Total Phenols, Flavonoids and Total Sugars concentrations in DCAPE, EE and CE of Coleus aromaticus.

However hydro alcoholic extract (HAE) showed less total phenol (0.6µg/10µl), flavonoids (0.3µg/10µl) and high total sugars (29µg/10µl) when compared to Dialyzed Coleus aromaticus protein extract (Figure 2).

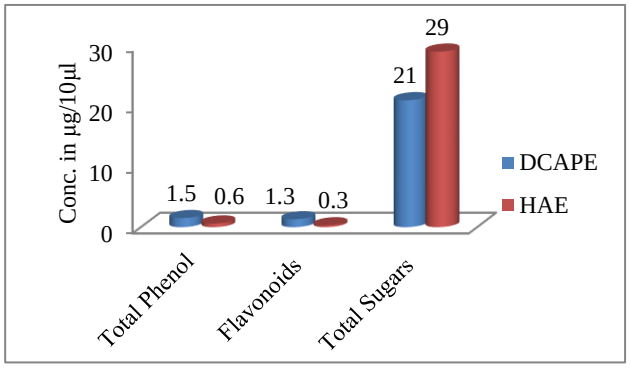

Figure 2: Total Phenols, flavonoids and total sugars concentrations in DCAPE and HAE of Coleus aromaticus.

The protein concentration in dialyzed Coleus aromaticus protein extract is considerably higher (5.8µg/10µl) than ethanol extract (5.2µg/10µl), chloroform extract (2.8µg/10µl) and hydro alcoholic extract (1.6µg/10µl) (Figure 3).

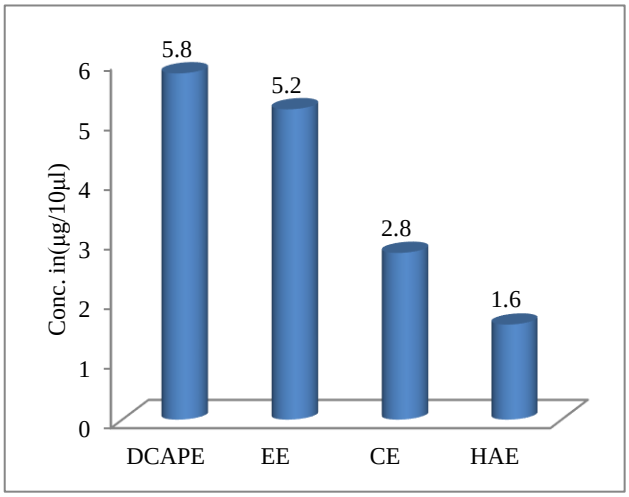

Figure 3: Protein concentration in various extracts of Coleus aromaticus.

DISCUSSION

This preliminary quantitative phytochemical profiling has revealed that the phytochemical composition varies with the solvent used. Hence, based on the phytochemical of interest, it is necessary to use the appropriate solvent for extraction and isolation.[9] In the present study, the total phenols, flavonoids and total sugars showed higher conc. in organic solvent extraction such as ethanol and chloroform. Hydro alcoholic extract showed high total sugars less total phenols, flavonoids and proteins when compared to dialyzed Coleus aromaticus protein extract.

The secondary metabolites such as flavonoids and phenols are well known for their antioxidant activity and we know that antioxidants are specific compounds that protect human and animal cells against the damaging effects of free radicals. The flavonoids and phenols of coleus aromaticus extracts exhibit the greatest antioxidant activity through the scavenging of free radicals, which participate in various pathophysiologies of diseases.[14] The protein content in dialyzed coleus aromaticus protein extract is considerably higher than the same in other solvent extracts. Plant proteins have been investigated in the search for novel antioxidants in the past few years but generally there is still a demand to find more information concerning the antioxidant potential of plant species as they are safe and also bioactive.[15]

CONCLUSION

The Coleus aromaticus have showed significant antioxidant activity due to bioactive components like total phenols, flavonoids and are subjected to

pharmaceutical drug formulations. However, role of Coleus aromaticus protein as an antioxidant has not been studied till date. In this study the protein concentration in dialyzed Coleus aromaticus protein extract is considerably higher than the same in other solvent extracts. This is the basis for further invitro studies to unfold the antioxidant activity of Coleus aromaticus protein.

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