

Screening of phytochemical constituents and assessment of anti-cholesterol and antioxidant potentials of *Coleus amboinicus*

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Abstract

Background: Coleus amboinicus is also called "karpoora valli" in Tamil. It has a lot of medicinal values, it is also used as an ornamental plant and spice. It is also commonly called Indian mint. Hypercholesterolemia is the highest risk factor and cause of cardiovascular diseases like atherosclerosis, myocardial infarction. High LDL is the cause of high cholesterol levels.

Aim-: Screening of phytochemical constituents and assessment of anti-cholesterol and antioxidant potentials of coleus amboinicus.

Materials and Methods-: Phytochemicals screening test and DPPH radical scavenging activity and anticholesterol activity were done using the ethanolic leaf extract of the plant and the results obtained were analyzed statistically by a one-way analysis of variance (ANOVA) followed by Duncan's multiple range test was used to see the statistical significance among the groups. The results with the p<0.05 level were considered to be statistically significant.

Results: The phytochemical screening showed the rich presence of secondary metabolites in the extract. The results showed that the extracts possessed in vitro antioxidant activity in a concentration-dependent manner. The anticholesterol activity of the extract is also evident from the results. The p value is less than 0.05 and is statistically significant.

Conclusion-:It was concluded that ethanolic extract of Coleus amboinicus possessed potent anti-cholesterol and antioxidant activity.

Keywords: Hypercholesterolemia, Anti-cholesterol, Antioxidant, Coleus amboinicus, DPPH radical scavenging, Phytochemical screening, innovative technology; novel method

INTRODUCTION

Hypercholesterolemia is the 6th major risk factor for death in the world ¹. Cholesterol can increase the risks of stroke, heart diseases and other vascular diseases, one third of the ischemic heart diseases is globally attributable due to high blood cholesterol. The root cause for atherosclerosis and other cardiac complications is due to hypercholesterolemia. People/individuals with elevated low density lipoprotein (LDL) cholesterol are highly prone to start developing coronary heart disease through various multiple stages of the process. LDL cholesterol lowering is the primary target of the therapy. Statins (ST's) are drugs of first choice in hypercholesterolemic patients, some of them are very intolerant to ST's specially and in those at high cardiovascular risk².

The border line devices between nutrients and drugs are nutraceuticals; they provide supplementation of particular nutrients and drugs with beneficial effects on health. The nutraceuticals which are derived from plants have been suggested to improve plasma lipid profile ³. Coleus amboinicus is also called "karpoora valli" in Tamil. It has a lot of medicinal values, it is also used as an ornamental plant and spice. It is also commonly called as Indian mint and it is a semi- succulent perennial plant belonging to the family Lamiaceae⁴. The leaves are mainly used for the treatment of asthma, headache, stomach pain, fever, renal diseases and epilepsy. It is used to treat conditions such as diarrhoea, toothache, insect bites and whooping cough ⁵. Various pharmacological properties of C.amboinicus have been reported including antiepileptic, antimutagenic and antifungal properties ⁶. This plant is used for infertility in both males and females and also for reproductive issues such as child birth ⁷. The leaves are consumed as breast milk stimulants ⁸. A few studies have shown the absence of toxicity of plant extracts in vitro and in vivo ⁹ which makes the use of this species very safe in the treatment of multi-resistant infections ¹⁰.

Antimicrobial activities of extracts, oils and nanoparticles of C. amboinicus have also been reported. C.amboinicus is reported to contain several classes of phytochemicals including alkaloids, terpenoids, proteins, phlobatannins, flavonoids and others ¹¹. The details of these bioactive constituents have been reported. Our team has extensive knowledge and research experience that has translate into high quality publications ^{12–21}. We wanted to find out about the anticholesterol activity coleus of amboinicus and this study is being done as anticholesterol activity on coleus amboinicus has never been done before.

MATERIALS AND METHODS

1. Phytochemical screening test Test for Phlobatannins

1ml of the extract was treated with 1ml of 1% Hcl and boiled for 10min. The formation of red colour precipitate indicates the presence of phlobatannin.

Test for Carbohydrates

Three to five drops of molisch reagent was added with 1ml of the extract and then 1ml of concentrated sulphuric acid was added carefully through the side of the test tube. The mixture was then allowed to stand for two minutes and diluted with 5ml of distilled water. The development of a red or dull violet ring at the junction of the liquid showed the presence of carbohydrates.

Test for Flavonoids

Few drops of 1% of liquid ammonia were taken in a test tube and

along with it 1ml of the extract was added resulting in the formation of yellow colour thereby indicating the presence of Flavonoids.

Test for Alkaloids

2ml of sample was mixed with 2ml of HCl. Then 6 drops of HCN was added and further 2 drops of picric acid was added that resulted in a creamish pale yellow ppt indicating the presence of alkaloids.

Test for Terpenoids

2 ml of sample along with 2ml of chloroform and 3ml of con. H_2SO_4 was added. Red color ppt obtained indicates the presence of terpenoids.

Test for proteins

One milliliter of ninhydrin was dissolved in 1 mL of acetone and then a small amount of extract was added with ninhydrin. The formation of purple colour revealed the presence of protein.

Detection of saponins

Foam test: A fraction of the extract was vigorously shaken with water and observed for persistent foam.

Test for steroids

One milliliter of chloroform was mixed with 1 mL of extract and then ten drops of acetic anhydride and five drops of concentrated sulphuric acid were added and mixed. The formation of dark red colour or dark pink colour indicates the presence of steroids.

2. DPPH free radical scavenging activity of Coleus amboinicus

Scavenging of 2, 2-Diphenyl-1picrylhydrazyl (DPPH) radicals was assessed by the method of Hatano et al, (1989). DPPH solution (1.0 ml) was added to 1.0 ml of extract at different concentrations (0.1 to 0. 5mg/ml). The mixture was kept at room temperature for 50 minutes and the activity was measured at 517 nm. Ascorbic acid at the same concentrations was used as standard. The capability to scavenge the DPPH radical was calculated and expressed in percentage (%) using following formula:

DPPH radical scavenging (%) = ControlOF-Sample OD X100

Control OD

3. In vitro anti-cholesterol activity of Coleus amboinicus

The anti-cholesterol assay was carried out as described as per the kit method (Spinreact, S.A.U-Ctra Santa Coloma, Girona, Spain). Cholesterol was dissolved in chloroform at a concentration of 2.5 mg mL/ml. Ten microliter of the extract was pipetted into a microtiter plate followed by the addition of 2000 µL of R1 reagent and 10 µL of cholesterol as sample. Twenty microliter of distilled water and 2000 µL of R1 reagent were used as blank. Negative control consisted of 20 µL cholesterol and 2ml R1; standard consisted of 20 µL simvastatin and 2000 mL R1 reagent. The contents were incubated between 0-30 min at room temperature and the absorbance was read at 500 nm in a UV-Vis spectrophotometer against reagent blank. Anti-cholesterol assay of the extract was calculated using the following equation:

Inhibition (%) = Negative control-Sample \times 100

Negative control

STATISTICAL ANALYSIS

The data were subjected to statistical analysis using one – way analysis of variance (ANOVA) and Duncan's multiple range test to assess the significance of individual variations between the groups. In Duncan's test, significance was considered at the level of p<0.05.

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RESULTS

The qualitative analysis for phytochemicals showed that the extract of Coleus amboinicus is rich in protein, amino acids, alkaloids, steroids and saponins. There was absence of carbohydrates, terpenoids, flavonoids in the extract (Table 1). The in vitro antioxidant activity of the extract was studied using DPPH radical scavenging activity. Vitamin C was used as the control. The activity of both the extract and standard was done at varying concentrations ranging from 100 to 500 μ g/ml. The results showed that extract possessed the potent antioxidant activity in a concentration dependent manner. But the activity of VItamin C was higher compared to the extract in all the tested concentrations (Figure 1). The in vitro anticholesterol activity of the extract was evaluated in varying concentrations, in which simvastatin was used as the standard, which

is a synthetic anti cholesterol drug. The plant extract's anticholesterol activity increases with increase in the concentration, although the activity was less compared to the synthetic drug (Figure 2).

Table	1:	Phytochemical	screening	of
Coleus	am	boinicus		

Phytochemical	Presen ce	Absen ce
Protein	(+)	
Amino acids	(+)	
Carbohydrates		(-)
Terpenoids		(-)
Flavonoids		(-)
Alkaloids	(+)	
Steroids	(+)	
Saponin	(+)	



- I Standard (Vitamin C)
- Extract

Figure 1: The figure represents the in vitro antioxidant activity of *Coleus amboinicus*. X axis represents the concentration and the Y axis represents the percentage of inhibition. Blue color represents standard (Vitamin C) and green color represents the extract. Each bar represents Mean ± SEM of 3 independent observations. P value \leq 0.05 was considered to be statistically significant.



Standard (Simvastatin)

Figure 2: The figure represents the in vitro anticholesterol activity of *Coleus amboinicus*. X axis represents the concentration and the Y axis represents the percentage of inhibition. Red color

DISCUSSION

Phytochemical screening of coleus amboinicus is rich in protein, amino acid, alkanoids, steroids, saponin, and is absent in carbohydrates, terpenoids, flavonoids. The natural bioactive compounds found in plants are phytoconstituents. They form an integrated part of defence systems and its world against various diseases and work with various nutrients and fibres ²². Usage of plant derived medicines are safer than synthetic alternatives, more affordable treatments and more therapeutic benefits ²³. represents standard (Simvastatin) and the pink color represents the extract. Each bar represents Mean \pm SEM of 3 independent observations. P value ≤ 0.05 was considered to be statistically significant.

The beneficial activity of *Coleus amboinicus* might be attributed to the presence of these phytoconstituents.

DPPH radical scavenging activity revealed the antioxidant activity of ethanolic extract of Coleus amboinicus. The activity was observed in a concentration dependent manner. The antioxidant activity was compared with the standard vitamin C. However the plant extract showed less activity compared to the standard in all the tested concentrations. The important role as a health protecting factor in food is played by antioxidant compounds, the main characteristic of an antioxidant is its ability to trap free radicals. Antioxidant compounds like polyphenols ,phenolic acids and flavonoids are commonly found in plants and they have been reported to have multiple biological effects, including antioxidant activity.²⁴.

The plant also possesses potent anticholesterol in vitro. Medicinal plants are indispensable components of earth as they are used to cure different infectious diseases all over the world and are a source of many effective and powerful drugs¹¹. The study results showed the potent anticholesterol activity of the extract in a concentration dependent manner. The activity was compared with the standard drug. Compared to simvastatin, the anticholesterol activity of the extract is less in all the doses. The crude nature of the extract might be the reason for reduced activity of the plant extract compared to the standard drug. Further purification and characterization can increase the activity of the extract. The standard simvastatin is well known to regulate the synthesis of cholesterol. Simvastatin is a HMG-CoA reductase inhibitor which acts by blocking the HMG-CoA reductase enzyme, which catalyzes the rate-limiting step in de novo cholesterol synthesis.¹. Statins are a widely used class of drug and they have adverse side effects like cognitive loss, neuropathy, pancreatic and hepatic dysfunction, and sexual dysfunction, Myositis and Myalgia ²⁵. The use of medicinal plants can reduce the side effects caused by synthetic drugs. Since our plant showed anticholesterol activity, if detailed studies were done on the plant, it can be used for formulating drugs hypercholesterolemia. against The limitation is that only in vitro analysis was done. Detailed in vitro and in vivo studies

in future might provide a way to develop a safe anti cholesterol drug from the plant.

Conclusion

The present study established the in vitro antioxidant and anticholesterol activity of *Coleus amboinicus*. The presence of phytochemicals might have contributed to the beneficial property of the plant.

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STATEMENT OF CONFLICT OF INTEREST

The author declares that there is no conflict of interest in the present study.

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