Original Research Article

Preliminary phytochemical analysis, In vitro antioxidant and protease inhibitory activity of *Coleus amboinicus*

Running title - Protease inhibitory activity of *Coleus amboinicus*

ABSTRACT:

Introduction: *Coleus amboinicus* may be a semi-succulent perennial plant within the *Lamiaceae* with a pungent oregano-like flavor and odor. Anti-inflammatory activity of most of the drug is due to the inhibition of bradykinin, protease, prostaglandins, and lysosomes. The major plant compounds characterized by antioxidant activity are polyphenols. Neutrophils are a rich source of serine proteinase and are present in lysosomes. leukocytes proteinase play an important role within the event of tissue damage during inflammatory reactions and a significant level of protection was provided by proteinase inhibitors

Aim: This study aims to analyze in vitro antioxidant and protease inhibitory activity of *Coleus amboinicus*

Materials and methods:

Phytochemical screening tests, in vitro antioxidant activity by DPPH radical scavenging activity and in vitro protease inhibitory activity ethanolic extract of Coleus amboinicus were studied by standard protocols. The results obtained were subjected to statistical analysis using one–way analysis of variance (ANOVA) and Duncan's multiple range test to assess the importance of individual variations between the groups using SPSS software. In Duncan's test, significance was considered at the extent of $p<0.05$.

Result: The phytochemical screening analysis showed that the ethanolic extract of *Coleus amboinicus* is rich in protein, amino acid, alkaloid, steroids, saponin, phenols. The DPPH radical scavenging activity showed that the plant extract possessed potent in vitro antioxidant activity although the activity is less when compared to the standard vitamin C. The plant extract also possesses potent protease inhibitory activity in a dose-dependent manner which is a measure of anti-inflammatory activity.

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Comment [S2]: Please clarify the type of phytochemical screening tests performed.

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Conclusion: To conclude the study ethanolic extract of *Coleus amboinicus* possessed potent in vitro antioxidant and protease inhibitory activity.

Keywords:
Coleus amboinicus, antioxidant activity, antimicrobial activity, inflammation, protease.

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INTRODUCTION:

Inflammation comprises systemic/local responses of living tissue towards injury. When cells are damaged by microbes, physical/ chemical agents, the injury is in the form of stress. (1) Despite the dependence on modern medicine and tremendous advances in synthetic drugs, the majority of the world's population can't afford the products of the western pharmaceutical industry and have to use traditional medicine which is derived from plant material. (2) The World health organization has compiled an inventory of medicinal plants with a wide range of pharmacological, biological activities, and interestingly phytochemical constituents. The main role of the anti-inflammatory agents is the inhibition of cyclooxygenase enzymes which are responsible for the conversion of arachidonic acid to prostaglandins. (3) The lysosomal granules of neutrophils carry many serine proteinases (4). Proteinases of leukocytes play a key role in the development of tissue damage during inflammatory processes. According to a previous study it was established that a significant level of protection was provided by proteinase inhibitors (5).

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Coleus amboinicus belongs to the family *Lamiaceae* and *coleus genus*. It is a large succulent aromatic perennial herb with approx 30.9 cm in height and with thick fleshy stem and leaves. The plant is distributed throughout India, it is a folkloric malarial fever, hepatoma the, renal and vesical calculi, cough, chronic asthma, hiccough, bronchitis, helminthiasis, colic, convulsion, the leaves of CA was used in many places around the world to add a punch to their dishes. (6) The strong flavor and aroma of these leaves make them ideal for flavoring certain meats and fishes helping to mask their strong odor. (7,8) The leaf of this plant was traditionally used in the treatment of inflammation, the present study aimed to authenticate that traditional information by in vitro anti-inflammatory screening. The herb *Coleus amboinicus* has got multiple potential and is used for a variety of reasons in different parts of the world. (7)

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it is a folkloric medicinal plant used against malarial fever, hepatopathy, renal and vesical calculi, cough, chronic asthma, hiccough, bronchitis, helminthiasis, colic and convulsion, the leaves of *Coleus amboinicus* was used in many places around the world to add a punch to their dishes [6].

The stable radical DPPH has been used widely for the determination of primary antioxidant activity, that is, the radical scavenging activities of pure antioxidant compounds, plant and fruit extracts, and food materials. The assay is predicated on the reduction of DPPH radicals in methanol which causes an absorbance drop at 517 nm. Anti-inflammatory activity is due to the inhibition of bradykinin, protease, prostaglandins, and lysosomes. The major plant compounds characterized by antioxidant activity are polyphenols. These are present in most plants and are considered to prevent free radicals associated damages in numerous ways including direct scavenging of radicals and inhibition of enzymes involved in free radical production (9). Our team has extensive knowledge and research experience that has translate into high quality publications (10),(11),(12),(13),(14),(15),(16),(17),(18),(19),(20),(21),(22),(23),(24),(25),(26),(27),(28),(29)In vitro protease inhibitory activity of *Coleus amboinicus* was not studied so far hence the aim of the present study is to study in vitro antioxidant and protease inhibitory activity of *Coleus amboinicus.*

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MATERIALS AND METHODS:

Comment [S11]: Please mention the method for *Coleus amboinicus* extraction to get the extract used for the subsequent tests.

1. Phytochemical Screening test

- Test for phlobatannin

1ml of the extract was treated with 1ml of 1% HCl and boiled for 10 mins. The formation of red color precipitate indicates the presence of phlobatannin.

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- Test for Carbohydrates

Three to five drops of Molisch reagent was added with 1 mL of the extract and then 1 mL 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 5 mL of distilled water. The development of a red or dull violet ring at the junction of the liquids showed the presence of carbohydrates.

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- Test for Flavonoids

Few drops of 1% liquid ammonia were taken in a test tube and along with it 1ml of the extract was added resulting in the formation of yellow color 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. H2SO4 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-1-picrylhydrazyl (DPPH) radical was assessed by the method of Barros et al, (30). 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 scavenged (%) = Control OD-Sample OD X100

Control OD

3. In vitro protease inhibitory activity of *Coleus amboinicus*

The test was performed according to the modified method of Dharmalingam et al., (31). The reaction mixture (2 ml) was made with 0.06 ml trypsin, 1ml of 20mM Tris HCl buffer (pH 7.4)

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and 1ml test sample of different concentrations. The reaction mixture was incubated for 10 minutes at 37ºC. Then, 1ml of 0.65% (W/V) casein was added. The mixture was re-incubated for 20 min. After incubation, 2 ml of 2M HClO4 was added to terminate the reaction. The cloudy suspension was centrifuged at 7830 rpm for 15 minutes. The absorbance of the supernatant was measured at 280 nm against. The Tris-HCl buffer was used as blank. The experiment was performed in triplicate. Anti-inflammatory activity was measured by calculating % inhibition against a range of concentrations.

% inhibition= (1-Ac/At) 100; where Ac is absorbance of control; At is absorbance of the test.

4. 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 $p<0.05$.

RESULT:

The qualitative phytochemical analysis of *Coleus amboinicus* was done and it was shown that the extract is rich in protein, amino acid, alkaloid, steroids , saponin and absent in carbohydrates terpenoids and flavonoids (Table 1). The in vitro antioxidant activity of the extract is evaluated using DPPH radical scavenging activity. The activity of the extract was analysed in various concentrations ranging from 100 to 500 µg/ml. It was shown that the extract possessed potent antioxidant activity in a dose dependent manner, although its activity was less compared to that of the standard vitamin C (Figure1). The results of protease inhibitory activity also showed that the activity increases with increase in concentration. To compare the activity of the extract we have used the standard antiinflammatory drug Diclofenac. Here also the extract's activity is less compared to diclofenac in all the tested concentrations (FIgure 2).

Table 1: Phytochemical Analysis of Coleus amboinicus

Phytochemical	Presence	Absence
Protein	+	

Comment [S23]: Please change it to $HClO_4$

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Amino acid	+	
Carbohydrates		-
Terpenoids		-
Flavonoids		-
Saponin	+	
Steroids	+	
Alkaloid	+	

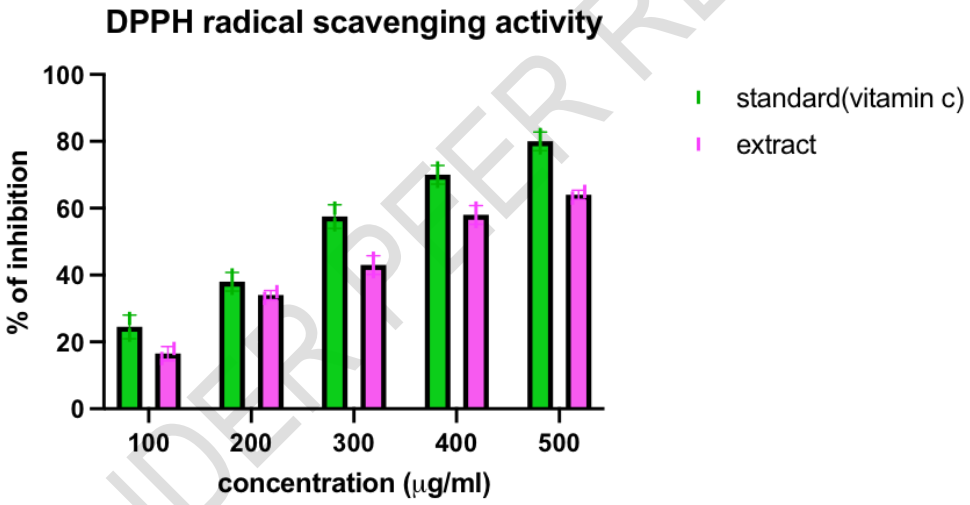

Figure1: In vitro antioxidant activity of Coleus amboinicus. The X-axis represents concentration and Y-axis represents the percentage of inhibition. Yellow colour represents Standard (Vitamin c) and blue represents *coleus amboinicus* extract. Each bar represents the mean SEM of 3 independent observations. The p value <0.05 level was considered to be statistically significant.

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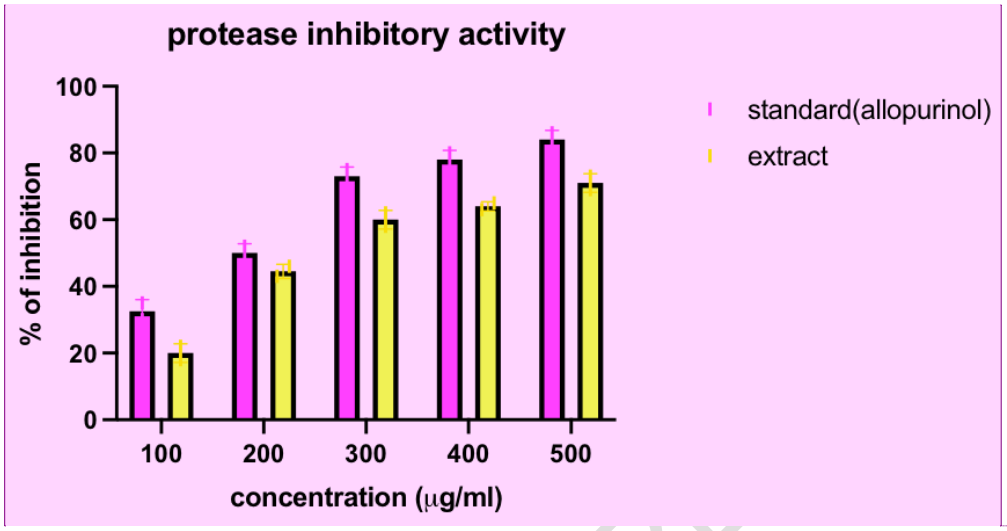

Figure 2: Protease inhibitory activity of Coleus amboinicus. The X-axis represents concentration and Y-axis represents the percentage of inhibition. Purple colour represents standard (Diclofenac) and green represents *Coleus amboinicus* extract. Each bar represents the mean ± SEM of 3 independent observations. The p value <0.05 level was considered to be statistically significant.

Discussion:

Phytochemical screening test refers to the screening and identification of the medicinally active substances found in plants. Some of the bioactive substances that can be derived from plants are flavonoids, alkaloids, carotenoids, tannin, antioxidants, and phenolic compounds. (7,8,32) The phytochemical screening test of *Coleus amboinicus* showed the presence of phlorotannin, Carbohydrates, Flavonoids, Alkaloids, Terpenoids, proteins, saponins, steroids. Previous studies showing a protective effect of diets rich in fruits and vegetables against cancer have focused attention on the likelihood that biologically active plant secondary metabolites exert anti-carcinogenic activity. (7,8,32) This huge group of compounds, now collectively termed 'phytochemicals', provides much of the flavor and color of edible plants and therefore the

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beverages derived from them. Many of those compounds also exert anti-carcinogenic effects in animal models of cancer, and much progress has been made in defining their many biological activities at the molecular level.

Many secondary metabolites found in plants have a task in defense against herbivores, pests, and pathogens. and a few of the issues in determining the precise role(s) of such metabolites were highlighted. The role of secondary metabolites in defense may involve deterrence/antifeedant activity, toxicity, or acting as precursors to physical defense systems(8,10). (33,34)Oxidative stress (OS) can be prevented by using antioxidants. Plants with phenolic contents have antioxidant properties. The present study was designed to investigate the antioxidant properties and phenolic contents (total phenols, flavonoids, flavonoids, and proanthocyanidins) of methanolic extracts from coleus amboinicus. (7,32) In this study, free radical scavenging activity was observed in a dose-dependent manner for the extract which might be due to the presence of the phytochemicals.

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Proteinases have an important role in arthritic reactions. Neutrophils are a rich source of serine proteinase which is localized to carry in their lysosomal granules. leukocytes proteinase played an important role in the development of tissue damage during inflammatory reactions and was protected by proteinase inhibitors.(35) Inflammation refers to the body's process of fighting against harmful things, such as infections, injuries, and toxins, in an attempt to heal itself. When something damages your cells, your body releases chemicals that trigger a response from your immune system. There are many causes for inflammation, but the mechanisms are common to all.(36) The inflammatory agent acts in the cell membranes inducing the activation of phospholipase A2 and consequently, liberates arachidonic acid and metabolites. According to the inflammatory mediators like cytokine, histamine, serotonin, leukotrienes, and prostaglandin increase the vascular permeability to all or any migration leukocytes cells to act on the site of inflamed tissue. Any interruption of this sequence of events results in the reduction of the liberation of the mediators causing the microcirculation to come back to a normal hemodynamic state.(36,37) From this study, we can see the dose-dependent protease inhibitory potential of *Coleus amboinicus* extract.

The results obtained from our studies on coleus amboinicus have shown a potential anti-inflammatory activity. The extracts inhibited the protease activity. This indicates that plants are more useful in studies of inflammation and various related physiological studies, aging, and diseases such as cancer, neurological disorder, etc. The limitations of the present study is that only in vitro analysis was conducted, in that itself only a limited number of analysis was done. Detailed in vitro and in vivo analysis can be done to study the exact mechanism of action of the plant in future. The isolation of active principle in future can also help to develop an anti inflammatory drug from the plant.

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CONCLUSION:

In the present study, results indicate that the methanol extracts of *Coleus amboinicus* possess anti-inflammatory properties. These activities could also be per the strong occurrence of polyphenolic compounds like alkaloids, flavonoids, tannins, steroids, and phenols. The extract also functions as radical inhibitors or scavengers, which can also contribute to the therapeutic property of the plant. In short, ethanolic extract of coleus amboinicus possessed potent in vitro antioxidant and protease inhibitory activity.

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