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Preliminary Phytochemical Analysis, *In vitro* Antioxidant and Protease Inhibitory Activity of *Coleus amboinicus*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Coleus amboinicus is a Lamiaceae-related semi-succulent perennial plant with a spicy oregano-like flavour and odour. The majority of the drug's anti-inflammatory effect is attributable to inhibition of bradykinin, protease, prostaglandins, and lysosomes. Polyphenols are the most common plant chemicals with antioxidant action. Neutrophils and lysosomes are both rich sources of serine proteinase. Proteinase inhibitors provided a considerable amount of protection in the case of tissue injury during inflammatory reactions, and proteinase inhibitors were found to be effective.
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 $p<0.05$.

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Results: The ethanolic extract of Coleus amboinicus is high in protein, amino acid, alkaloid, steroids, saponin, and phenols, according to the phytochemical screening study. The plant extract has significant in vitro antioxidant activity, however it was less than that of normal vitamin C, as measured by DPPH radical scavenging activity. The plant extract also has significant protease inhibitory activity, which is a measure of anti-inflammatory action, in a dose-dependent way **Conclusion:** It is concluded that ethanolic extract of *Coleus amboinicus* possessed potent in vitro antioxidant and protease inhibitory activity.

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

1. 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 their reliance on modern medicine and enormous breakthroughs in synthetic medications, the bulk of the world's population cannot afford the products of the western pharmaceutical industry and must rely on traditional plant-based treatment [2]. The World Health Organization has prepared a list of therapeutic plants that have a diverse variety of pharmacological, biological, and phytochemical characteristics. The inhibition of cyclooxygenase enzymes, which are responsible for the conversion of arachidonic acid to prostaglandins, is the main function of anti-inflammatory medicines [3]. Neutrophils have a lot of serine proteinases in their lysosomal granules [4]. Leukocyte proteinases are important in the development of tissue damage during inflammatory events. Proteinase inhibitors are known to provide a significant level of protection, according to a recent study [5].

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. Coleus amboinicus is a folkloric medicinal plant that is used to treat malarial fever, hepatopathy, renal and vesical calculi, cough, chronic asthma, hiccough, bronchitis, helminthiasis, colic, and convulsion. The leaves of Coleus amboinicus were used in many parts of the world to add a punch to their dishes. [6]. The leaves have a strong flavour and perfume, making them suitable for seasoning various meats and fishes and masking their strong stink [6,7] The leaf of this plant has long been used to treat inflammation, and the current study attempted to validate that knowledge through in vitro anti-inflammatory testing. Coleus amboinicus is a versatile herb that is used for a variety of purposes in different parts of the world [8].

The stable radical DPPH has been frequently used to assess primary antioxidant activity, or the ability of pure antioxidant chemicals, plant and fruit extracts, and food components to scavenge radicals. The assay is based on the reduction of DPPH radicals in methanol, which results in a 517 nm absorbance decrease. 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 [10-29]. 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.*

2. MATERIALS AND METHODS

2.1 Phytochemical Screening Test

- Test for phlobatannin

One milliliter 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.

- Test for Carbohydrates
- Molisch reagent (three to five drops) was added to the mixture. One millilitre of the extract and one millilitre of concentrated sulphuric acid were carefully introduced via the test tube's side. After two minutes, the mixture was allowed to sit for two minutes before being diluted with 5 mL of distilled water. Carbohydrates were detected by the

formation of a red or dim violet ring at the liquid-liquid interface. 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
- Two millilitres of material were combined with two millilitres of HCl. Then 6 drops of HCN and 2 drops of picric acid were added, yielding a creamish pale yellow ppt that indicated 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 millilitre of chloroform was combined with one millilitre of extract, followed by ten drops of acetic anhydride and five drops of strong sulphuric acid. The presence of steroids is indicated by the creation of a dark red or dark pink colour.

2.2 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 (%) =

$$\frac{\text{Control OD-Sample OD}}{\text{Control OD}} \times 100$$

2.3 *In vitro* Protease Inhibitory Activity of *Coleus amboinicus*

The test was carried out using Dharmalingam et almodified .'s approach [31] 0.06 ml trypsin, 1 ml 20mM Tris HCl buffer (pH 7.4), and 1 ml test sample of various concentrations were used to make the reaction mixture (2 ml). At 37 degrees Celsius, the reaction mixture was incubated for 10 minutes. After that, 1 mL of 0.65% (W/V) casein was added. After 20 minutes, the mixture was re-incubated. After incubation, the reaction was stopped with 2 ml of 2M HClO4. The hazy suspension was centrifuged for 15 minutes at 7830 rpm. 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.

2.4 Statistical Analysis

To determine the significance of individual differences between groups, the data were subjected to statistical analysis using one-way analysis of variance (ANOVA) and Duncan's multiple range test. Duncan's test considered significant at the level of p0.05.

3. RESULTS

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 (Fig. 1). 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 (Fig. 2).

Table 1. Phytochemical Analysis of Coleus amboinicus

Phytochemical	Presence	Absence
Protein	+	
Amino acid	+	
Carbohydrates		-
Terpenoids		-
Flavonoids		-
Saponin	+	
Steroids	+	
Alkaloid	+	

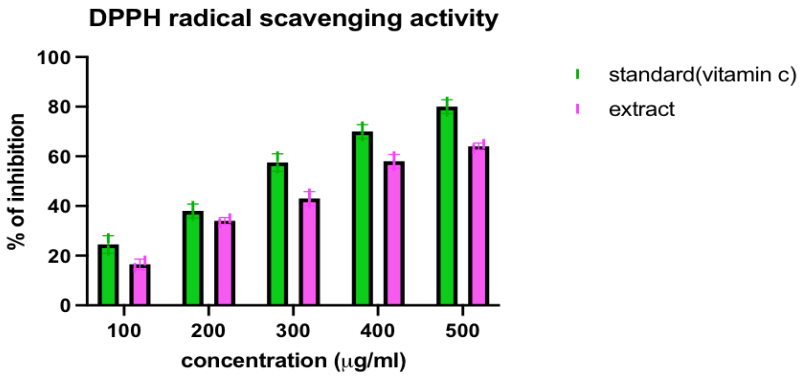

Fig. 1. *In vitro* antioxidant activity of Coleus amboinicus. The X-axis represents concentration and Y-axis represents the percentage of inhibition. Yellow colour represents Standard (Vitamin c) and blue represents *coleus amboinicus* extract. Each bar represents the mean SEM of 3 independent observations. The p value <0.05 level was considered to be statistically significant

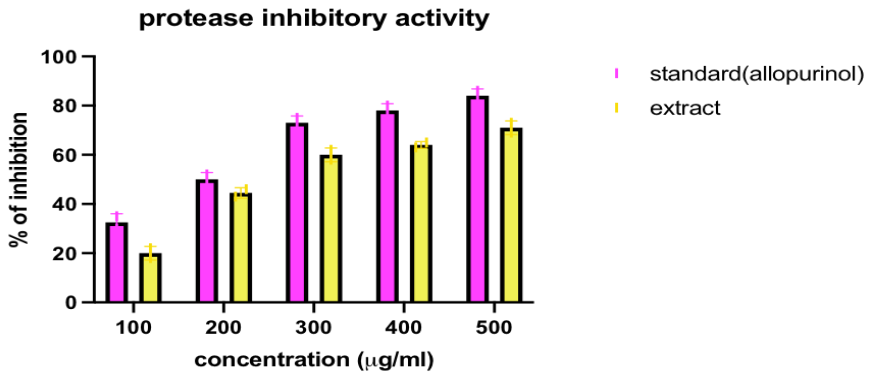

Fig. 2. Protease inhibitory activity of Coleus amboinicus. The X-axis represents concentration and Y-axis represents the percentage of inhibition. Purple colour represents standard (Diclofenac) and green represents *Coleus amboinicus* extract. Each bar represents the mean ± SEM of 3 independent observations.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

4. DISCUSSION

The screening and identification of medicinally active chemicals present in plants is referred to as a phytochemical screening test. Flavonoids, alkaloids, carotenoids, tannin, antioxidants, and phenolic compounds are some of the bioactive molecules derived from plants [7,8,32]. The

phytochemical screening test of *Coleus amboinicus* showed the presence of phlorotannin, Carbohydrates, Flavonoids, Alkaloids, Terpenoids, proteins, saponins, steroids. Previous research has focused attention on the possibility that biologically active plant secondary metabolites have anti-carcinogenic action, as evidenced by studies showing a protective effect of a diet rich in fruits and vegetables against cancer [7,8,32]. This large category of substances, now known as 'phytochemicals,' is responsible for most of the flavour and colour of edible plants, as well as the beverages made from them. Numerous of those chemicals have also been shown to have anti-carcinogenic properties in animal cancer models, and significant work has been made in characterising their many biological actions at the molecular level.

Many secondary metabolites in plants play a role in protecting plants from herbivores, pests, and pathogens. and a few of the challenges in understanding the precise function(s) of such metabolites were discussed. Secondary metabolites may play a role in defence by functioning as deterrents/antifeedants, toxins, or as precursors to physical defensive 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.

Proteinases play a crucial part in the development of arthritic symptoms. Serine proteinase is abundant in neutrophils, and it is stored in their lysosomal granules. Proteinase inhibitors protected leukocyte proteinase, which played a significant part in the development of tissue damage during inflammatory reactions [35]. Inflammation is the body's attempt to cure itself by fighting against harmful substances such as infections, injuries, and toxins. When your cells are damaged, your body releases substances that cause your immune system to respond. Inflammation has numerous causes, but the mechanics are the same in all of them [36]. The inflammatory agent acts in the cell membranes inducing the activation of phospholipase A2 and consequently, liberates arachidonic acid and metabolites. Inflammatory mediators such as cytokines, histamine, serotonin, leukotrienes, and prostaglandins increase vascular permeability, allowing all or any migrating leukocytes to operate on the inflamed tissue location. When this series of events is disrupted, the mediators' liberation is reduced, forcing the microcirculation to return to a normal hemodynamic condition [37]. We can see that Coleus amboinicus extract has dose-dependent protease inhibitory activity from this investigation.

The results of our research on coleus amboinicus revealed that it may have anti-inflammatory properties. The protease activity was suppressed by the extracts. This suggests that plants are more effective in research on inflammation and related physiological studies, ageing, and diseases like cancer, neurological disorders, and so on. 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.

5. CONCLUSION

The results of this investigation show that Coleus amboinicus methanol extracts have anti-inflammatory activities. Polyphenolic substances such as alkaloids, flavonoids, tannins, steroids, and phenols may also have a role in these actions. The extract also acts as a radical scavenger or inhibitor, which may add to the plant's medicinal properties. In brief, coleus amboinicus ethanolic extract had significant antioxidant and protease inhibitory action in vitro.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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