

In-vitro Antioxidant and Anti-Inflammatory Studies of *Coleus malabaricus* Benth. and its Morphotype on a Comparative Account with *Coleus zeylanicus* and *Coleus amboinicus* of Family Lamiaceae

Beesha S Kamal ^{1,*}, Kumar M ²

¹ College of Pharmaceutical Sciences, Government T.D Medical College, Alappuzha, Kerala, India; beeshanireesh123@gmail.com;

² Department of Pharmaceutical Chemistry, Vinayaka Mission's College of Pharmacy, Vinayaka Mission's Research Foundation, Salem, Tamil Nadu, India; kumarvmcp@yahoo.co.in;

* Correspondence: beeshanireesh123@gmail.com;

Scopus Author ID 55850610800

Received: 26.07.2024; Accepted: 6.10.2024; Published: 14.02.2025

Abstract: The family Lamiaceae includes aromatic medicinal plants of ethnomedicinal value and many of them are widely used in traditional medicine to treat inflammatory and painful conditions. The current article intends to put forward a comparative analysis of antioxidant and anti-inflammatory activities of alcoholic extracts of whole plants of *Coleus malabaricus* and its morphotype with the most studied and well-documented plants of the genus *Coleus* (Family: Lamiaceae), *Coleus zeylanicus* and *Coleus amboinicus* by various *in-vitro* methods. The preliminary phytochemical screening of the plant extracts showed phytoconstituents such as steroids, alkaloids, flavonoids, phenolic compounds, and tannins. The total phenolic and flavonoid content of alcoholic extracts was determined by Folin-Ciocalteu and aluminum chloride colorimetric assays, respectively. The antioxidant potential was screened using hydroxy radical scavenging assay, nitric oxide radical scavenging assay, and anti-inflammatory activity by *in-vitro* protein denaturation and lipoxigenase inhibition method. All the extracts exhibited anti-inflammatory activity in a dose-dependent manner. Comparable results have been obtained at higher concentrations with the standard drug diclofenac. The antioxidant and anti-inflammatory activities are correlated well with the phenolic and flavonoid content. The ethanolic extracts of these *Coleus* plants thus exhibited good anti-inflammatory and antioxidant potential and may be an important source of novel antioxidant and anti-inflammatory agents.

Keywords: antioxidant; anti-inflammatory; *Coleus malabaricus*; *Coleus zeylanicus*; ethnomedicine.

Abbreviations: CA - *Coleus amboinicus*, CM1- *Coleus malabaricus* Benth., CM2- *Coleus malabaricus* Benth. (morphotype), CZ - *Coleus zeylanicus*, GAE -Gallic acid equivalent, QE-Quercetin equivalent, IC₅₀ – Half maximal Inhibitory concentration.

© 2025 by the authors. This article is an open-access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

The human immune system is always on alert to fight against various infectious pathogens and poisons invading the body. As part of this, it triggers inflammatory reactions to heal the body tissues. These proinflammatory activities of the human innate immune mechanism sometimes progress uncontrolled, leading to oxidative stress conditions and the generation of excessive reactive oxygen species. Oxidative stress and inflammation are considered the central mechanisms involved in various disease pathologies such as cancer,

diabetes, Parkinson's disease, asthma, arthritis, and Alzheimer's [1,2]. Antioxidants can prevent these oxidative reactions by scavenging free radicals, chelating metal ions, and acting as electron donors. Plants rich in antioxidants have been suggested as a potential solution for managing some of these problems. Polyphenols, flavonoids, alkaloids, and terpenoids are the most characterized phytochemicals for producing antioxidant and anti-inflammatory activities. Plants possessing these phytochemicals are always a great choice as antioxidant and anti-inflammatory agents instead of their synthetic counterparts owing to their lack of side effects, easy availability, and cheaper. Though several studies have focused on the traditional knowledge of medicinal plants, many floras have been left unexplored. In this regard, the scientific validation of this traditional medical knowledge is necessary [3-6].

The genus *Coleus* belongs to the family Lamiaceae (the mint family). It includes 236 genera and about 6900 -7000 species, which makes it the largest family of the order Lamiales and the sixth largest family of flowering plants. It is found to be widespread throughout tropical and warm regions like Africa, Australia, and India [7]. Among the different species, *Coleus amboinicus* (*Plectranthus amboinicus*) and *Coleus barbatus* (*Plectranthus barbatus*, *Coleus forskalaei*) account for about 68% of all traditional uses of the genus [8]. *Coleus* species constitute Flavonoids, glycosides, phenolic compounds, and volatile components. The aromatic nature of the plants belonging to the genus is attributed to essential oil production. Isolated diterpenoids and abietane diterpenoids, triterpenoids of the essential oil are mainly responsible for different pharmacological activities such as antibacterial, antifungal, and antitumor, and these compounds make *Coleus* an important genera for drug development [9]. Ethnobotanical uses of the genera include treating digestive disorders, skin conditions, respiratory conditions, infections and fever, genitourinary conditions, and musculoskeletal conditions. Owing to these reasons, more than 500 varieties of *Coleus* species are cultivated throughout the world nowadays. In spite of the worldwide distribution of the species, the chemistry and biological activities of many species have to be studied more deeply [7-9].

The present study is intended to compare preliminary phytochemical, antioxidant, and anti-inflammatory studies of the plant *Coleus malabaricus* and its morphotype with the traditionally valuable and most documented species of the genus *Coleus zeylanicus* and *Coleus amboinicus*. *Coleus malabaricus* is used by the tribal communities of south India, especially of Kerala and Tamil Nadu, to cure a wide range of ailments, including cough, cold asthma, inflammations, and cardiac problems [7,10,11]. However, reports on the scientific validation of their application in ethnomedicine are very limited in the literature. The species also exhibit extreme polymorphism, and almost eight polymorphic species were reported from Kerala [12]. The morphotype for the particular study was collected from the same area, and the present study's findings would also be valuable from a chemotaxonomic viewpoint.

2. Materials and Methods

2.1. Procurement & authentication of plants.

Fresh plant samples (5 kg) of *Coleus malabaricus* (CM 1) and morphotypes of *Coleus malabaricus* (CM 2) were gathered from Thollayiram, Wayanad, Kerala. Both the plant specimens were identified as *Coleus malabaricus* Benth. (*Plectranthus malabaricus* Benth.) by Dr. M. U. Sharief, Scientist E and Head of office Botanical Survey of India, Coimbatore, and the voucher specimen records are BSK 01 and BSK 02 (No: BSI/SRC/5/23/2019/TECH/308). The plant materials *Coleus zeylanicus* and *Coleus*

amboinicus for the study were procured from cultivars near Cherthala and authenticated by Prof P. Jayaraman, Plant Anatomy Research Centre, Thambanam, Chennai, with voucher specimen numbers PARC/2021/4845 and PARC/2021/4846 respectively.

2.2. Extraction of plant material by successive solvent extraction.

Fresh plant materials were washed thoroughly with water to remove adhering soil, freed from other plant materials, and finally chopped into small pieces. The drying was carried out in the shade for 20 days. The dried plant material was powdered using a mechanical grinder to get a fine powder. The powdered plant materials (30 gm) were extracted in a soxhlet extractor using solvents of increasing polarity (petroleum ether, ethyl acetate, and ethanol) for 6-8 hours, and the solvent was separated by distillation [11]. The dried, semisolid extract was stored in an airtight container in a refrigerator for further use. Merck grade/analytical grade solvents and reagents were used for the study.

2.3. Phytochemical screening of extracts.

The petroleum ether, ethyl acetate, and alcoholic extracts of CM 1, CM 2, CZ, and CA were screened by qualitative chemical tests to identify the presence of various classes of phytoconstituents like carbohydrates, proteins, and amino acids, glycosides, alkaloids, flavonoids, tannins and phenolic compounds, saponins, sterols, and triterpenoids using standard procedure [11-13].

2.4. Estimation of phytoconstituents

2.4.1. Estimation of total phenolic content

Total phenolic content was estimated using the Folin-Ciocalteu method, using gallic acid as the standard. From the stock solution (1 mg/ml), working solutions of gallic acid in concentrations of 20, 40, 60, 80, and 100 µg/ml were prepared and transferred to tubing (each in triplicates) and made up to 1ml with distilled water. 1 ml of alcoholic extract of CM1, CM2, CZ, and CA at a concentration of 500 µg/ml was used for the estimation. The standard and samples were mixed well with 5 ml Folin-Ciocalteu Reagent (1:10 dilution), kept for 5 minutes, and then treated with 4 ml 20% Na₂CO₃ solution. After 30 minutes, absorbance was measured against a blank at 760 nm. The total phenol content for each extract was derived from the gallic acid calibration curve and expressed as gallic acid equivalent (GAE) [14].

2.4.2. Estimation of total flavonoid content.

An aluminum chloride colorimetric assay was used to measure total flavonoid content. From the standard quercetin stock solution (1000 µg/ml), a working solution of quercetin containing 20, 40, 60, 80, and 100 µg/ml quercetin. The alcoholic extracts of CM1, CM2, CZ, and CA were tested at 500 µg/ml concentration. Add 4 ml water and 0.3 ml of 5% sodium nitrite solution to all the test tubes and mix thoroughly. After 5 minutes, 0.3 ml of 10% aluminum chloride solution was added, and at the 6th minute, 2 ml of 1M sodium hydroxide was added. The total volume of the contents was then made up to 10 ml with distilled water and once again mixed well, and the absorbance was measured against the blank at 415 nm. The blank was prepared without the addition of aluminum chloride solution. The total phenol

content for each extract was derived from the calibration curve of standard quercetin and expressed as quercetin equivalent (QE) [15].

2.5. Antioxidant activity.

2.5.1. Nitric oxide radical scavenging assay.

The nitric oxide radical scavenging assay of the alcoholic extract of CM1, CM2, CA, and CZ was determined using a slightly modified method described previously [16,17]. Different concentrations (5, 10, 20, 40, 80, and 160 µg/ml) of the test samples and standard were taken. A volume of 0.5 ml of 10 mM sodium nitroprusside in phosphate-buffered saline was added and incubated at 25°C for 180 mins. The tubes were mixed with an equal volume of freshly prepared Griess reagent (Griess reagent was prepared by mixing equal amounts of 1% sulphanilamide in 2.5% phosphoric acid and 0.1% naphthyl ethylenediamine dihydrochloride in 2.5% phosphoric acid immediately before use). The absorbance was measured at 546 nm using a spectrophotometer. Gallic acid was used as the positive control. The percentage inhibition of the test sample and standard was calculated using the equation,

$$\text{Percentage inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

Percentage inhibition was plotted against concentration, and from the graph, IC₅₀ was calculated.

2.5.2. Hydroxyl radical scavenging assay.

The hydroxyl radical was generated by the Fe³⁺-ascorbate-EDTA-H₂O₂ system (Fenton reaction). The reaction mixture contained 0.8 mL of phosphate buffer solution (50 mmol L⁻¹, pH 7.4), 0.2 mL of extractives/standard at different concentrations (10, 20, 40, 80, 160, 320 µg/mL), 0.2 mL of EDTA (1.04 mmol L⁻¹), 0.2 mL of FeCl₃ (1 mmol L⁻¹) and 0.2 mL of 2-deoxy-D-ribose (28 mmol L⁻¹). The mixtures were kept in a water bath at 37°C, and the reaction was started by adding 0.2 mL of ascorbic acid, AA (2 mmol L⁻¹), and 0.2 mL of H₂O₂ (10 mmol L⁻¹). After incubation at 37°C for 1 h, 1.5 mL of cold thiobarbituric acid, TBA (10 g L⁻¹), was added to the reaction mixture, followed by 1.5 mL of HCl (25 %). The mixture was heated at 100°C for 15 min and then cooled down with water. The absorbance of the solution was measured at 532 nm using a spectrophotometer. Quercetin was used as a positive control. The hydroxyl radical scavenging capacity was evaluated by the percentage inhibition of 2-deoxy-D-ribose oxidation on hydroxyl radicals [18,19].

The percentage of hydroxyl radical scavenging activity was calculated according to the following formula,

$$\text{Percentage inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

2.6. Anti-inflammatory study.

2.6.1. Protein denaturation assay.

Different concentrations (50, 100, 200, 400, 800, and 1600 µg/mL) of alcoholic extracts of CM1, CM2, CA, and CZ and reference standard (Diclofenac sodium) and control (without standard/test sample) were made up to 4 mL of phosphate buffer solution (0.2 M, pH 7.4). 1

mL of 1mM albumin solution in phosphate buffer was mixed with all the tubes and incubated at 37°C in an incubator for 15 minutes. Denaturation was induced by keeping the reaction mixture at 60°C in a water bath for 15 minutes. After cooling, the turbidity was measured at 660 nm [20, 21]. The percentage inhibition of denaturation was calculated using the following formula,

$$\text{Percentage inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

2.6.2. Lipoxygenase inhibition assay.

Lipoxygenase inhibition activity of alcoholic extracts of CM1, CM2, CZ, and CA was assayed according to the method described by Gunathilake et al. (2018) [22] with slight modifications. The reaction mixture consists of sodium phosphate buffer 160 µl of 100 mM (pH 8.0), 10 µl of plant extract with various concentrations (50, 100, 200, 400, 800, and 1600 µg/mL), and 20 µl of soybean lipoxygenase solution (167 U/ml) are mixed and incubated at 25°C for 10 min. The reaction was initiated by adding 10 µl linoleic acid substrate (10 mmol). Diclofenac sodium was used as a reference drug. The absorbance was measured at 234 nm. Control was prepared by omitting the addition of plant extracts. All the reactions were performed in triplicates. The percentage of lipoxygenase inhibition was calculated using,

$$\text{Percentage inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

2.7. Statistical analysis.

The results were expressed as mean±SD and analyzed statistically with analysis of variance (ANOVA) at a significance level of p<0.05, and pairwise comparison was done using the Tukey's test with version 27 of Statistical Package for Social Sciences (SPSS) (SPSS Inc., Chicago, IL, USA).

3. Results and Discussion

3.1. Phytochemical screening.

The results of the phytochemical screening of petroleum ether, ethyl acetate, and alcohol extracts of CM1, CM2, CZ, and CA are shown in Table 1. Petroleum ether extract of CM1, CM2, CZ, and CA showed positive tests for steroids and terpenoids. Both ethyl acetate and ethanol extracts gave positive tests for carbohydrates, alkaloids, amino acids and proteins, steroids and terpenoids, phenolic compounds, and tannins and flavonoids.

Table 1. Phytochemical profiling of plant extracts.

Phyto constituents	CM 1			CM2			CZ			CA		
	P.E	E.A	Eth	P.E	E.A	Eth	P.E	E.A	Eth	P.E	E.A	Eth
Alkaloids	-	+	++	-	+	++	-	+	+	-	+	+
Flavonoids	-	+	++	-	+	+++	-	+	+++	-	+	++
Phenolic compounds and tannins	-	+	++	-	+	+++	-	+	++	-	+	++
Terpenoids and Steroids	+++	+	++	+++	-	-	+++	-	-	+++	-	-
Glycosides	-	-	-	-	-	-	-	-	-	-	-	-
Saponins	-	-	-	-	-	-	-	-	-	-	-	-

Key: (+++) - Highly present, (++) -Moderately present, (+) -present, (-) -Absent

Still, a high presence of phytoconstituents, especially phenolic compounds and tannins, is seen in the alcoholic extract. So, the alcoholic extract was selected for further *in-vitro* pharmacological studies.

3.2. Estimation of phytoconstituents.

3.2.1. Estimation of total phenol content.

The total phenol content in the alcoholic extract of CM1, CM2, CZ, and CA was estimated by a simple colorimetric method based on the reaction of phenolic compounds with Folin-Ciocalteu reagent. Gallic acid was used as the standard compound. The estimation is based on the reaction of phosphotungstic (H_3PWO_{40}) and phosphomolybdic acid ($H_3PMO_{12}O_{40}$) with phenolic compounds from the extracts resulting in a mixture of blue oxides (W_8O_{23} & Mo_8O_{23}) [23]. The absorbance values obtained at different concentrations of gallic acid (20, 40, 60, 80, and 100 $\mu\text{g/ml}$) were used for constructing the calibration curve. The result is expressed as Gallic acid equivalents. The total phenolic content of the extracts was calculated from the regression equation of the calibration curve ($y = 0.0104x + 0.0422$, $R^2 = 0.9985$) and expressed as mg gallic acid equivalents (GAE) per gram of extract (mg/g). The results are presented in Table 2.

Table 2. Estimation of phytoconstituents.

Plant material	Total Phenolic Content (mg/gm GAE)	Total flavonoid Content (mg/gm QE)
CM 1	67.992 \pm 2.5	75.176 \pm 2.35
CM 2	81.047 \pm 1.5	74.196 \pm 6.235
CZ	38.511 \pm 5.13	126.55 \pm 2.27
CA	33.34 \pm 0.192	52.134 \pm 56

Values are expressed as mean \pm SD of the triplicate experiment.

3.2.2. Estimation of total flavonoid content.

Flavonoids easily chelate with metal ions to form complex compounds due to their peculiar structure, and the formed compounds exhibit exceptional spectrophotometric properties. The aluminum chloride colorimetric method determined the flavonoid content based on forming aluminum flavonoid complexes in alkaline conditions, which was monitored spectrophotometrically at a wavelength of 510 nm [24]. Quercetin was used as a standard compound. The absorbance values obtained at different concentrations of quercetin (20, 40, 60, 80, and 100 $\mu\text{g/ml}$) were used for constructing the calibration curve. The total flavonoid content of the extracts was calculated from the regression equation of the calibration curve ($y = 0.0017x + 0.0522$, $R^2 = 0.9951$) and expressed as mg quercetin equivalent (QE) per gram of extract (mg/g). The results are given in Table 2.

3.3. Antioxidant activity.

3.3.1. Nitric oxide radical scavenging activity.

Chronic exposure to nitric oxide and reactive oxygen species will lead to the development of various carcinomas and inflammatory conditions [25]. Incubation of solutions of sodium nitroprusside in phosphate buffer saline resulted in linear time-dependent nitrite production, which was reduced by the standard quercetin and ethanolic extracts of CM1, CM2, CZ, and CA. This may be due to the antioxidant principles in the extract, which compete with

reactive free radicles. The percentage nitric oxide radical scavenging activity of standard quercetin and the plant extracts at concentrations 5,10, 20, 40, 80, and 160 µg/ml was performed in triplicate, and the results were expressed as mean ± standard deviation. The percentage of free radical scavenging was plotted against the concentration of the extract, as shown in Figure 1. The IC₅₀ values of CM1, CM2, CA, and CZ were found to be 88.09 µg/ml, 78.55 µg/ml, 103.25 µg/ml and 91.49 µg/ml, and the standard drug quercetin was 17.12 µg/ml (Table 3 and Figure 1). The results were analyzed using the analysis of variance (ANOVA) test, and pairwise comparisons were made using Tukey’s test. The analysis showed a statistically significant difference at 1% significance level ($p < 0.01$). All extracts exhibited good activity at a concentration greater than 80 µg/ml. Among the extracts, maximum activity was shown by CM2.

Table 3. Nitric oxide radical scavenging activity.

Concentration (µg/ml)	Quercetin % inhibition	CM1 % inhibition	CM 2 % inhibition	CZ % inhibition	CA % inhibition
5	20.56 ± 0.58	4.37 ± 0.51**	6.02 ± 0.60**	3.01 ± 0.52**	1.55 ± 0.52**
10	30.67 ± 0.30	8.99 ± 0.52**	10.69 ± 1.32**	7.58 ± 0.68**	6.12 ± 0.44**
20	55.61 ± 0.30	21.82 ± 1.54**	25.42 ± 1.02**	19.78 ± 2.34**	16.91 ± 1.78**
40	81.81 ± 0.30	31.69 ± 0.58**	36.55 ± 1.10**	30.09 ± 0.51**	26.20 ± 0.91**
80	87.50 ± 0.51	52.74 ± 1.54**	59.84 ± 1.24**	49.53 ± 1.57**	43.75 ± 0.51**
160	93.48 ± 0.51	79.53 ± 2.26**	84.29 ± 0.65**	78.85 ± 1.40**	71.85 ± 0.38**

Values are expressed as mean±SD of the triplicate experiment. **indicates a statistically significant difference at a 1% level of significance ($p < 0.01$).

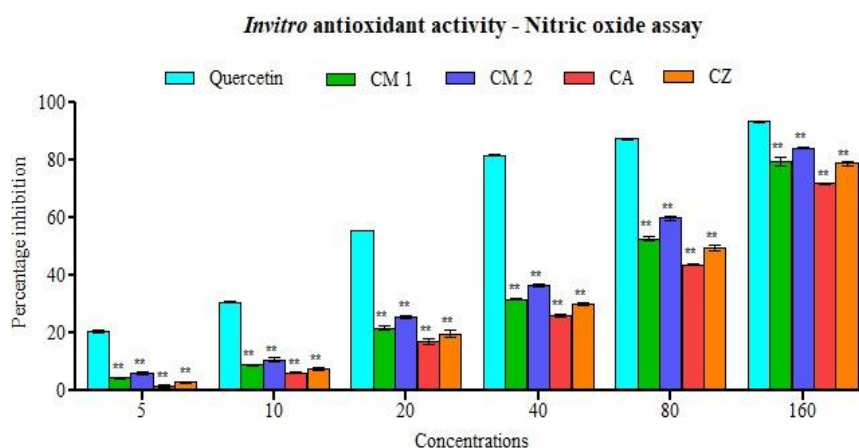


Figure 1. Nitric oxide radical scavenging assay of plant extracts and standard quercetin. Data expressed as Mean±SD (n=3, $p < 0.01$) for all tested dosages

3.2.2. Hydroxyl radical scavenging activity.

Hydroxyl radicals are the major active oxygen species causing lipid peroxidation and enormous cell damage, leading to the pathogenesis of various human diseases. Hydroxyl radical scavenging activity was measured by the ability of the different concentrations of standard quercetin and alcoholic extract of CM1, CM2, CZ, and CA to scavenge the hydroxyl radicals generated by the Fe³⁺-ascorbate-EDTA-H₂O₂ system (Fenton reaction). The percentage hydroxyl radical scavenging activity of standard quercetin and the plant extracts at concentrations 10, 20, 40, 80, 160, and 320 µg/ml was performed in triplicate, and the results were expressed as mean ± standard deviation (Table 4). The percentage of free radical scavenging was plotted against the concentration of the extract, as shown in Figure 2. The IC₅₀ values of the given test samples CM1, CM2, CA, and CZ and the standard drug quercetin were found to be 92.51 µg/ml, 80.21 µg/ml, 111.23 µg/ml, and 104.29 µg/ml and 23.23 µg/ml,

respectively. The hydroxyl radical scavenging activity of the standard and plant extracts was evident at all concentrations and correlated well with increasing concentrations. All plant extracts exhibited good hydroxyl scavenging activity at 80, 160, and 320 (g/ml concentrations. Maximum activity was shown by CM2, followed by CM1, CZ, and CA (Figure 2).

Table 4. Nitric oxide radical scavenging activity.

Concentration (µg/ml)	Quercetin % inhibition	CM 1 % inhibition	CM 2 % inhibition	CZ % inhibition	CA % inhibition
10	23.19 ± 0.38	2.66 ± 0.56**	4.38 ± 0.84**	1.54 ± 0.61**	1.42 ± 0.39**
20	52.79 ± 0.53	10.24 ± 0.39**	13.03 ± 0.48**	5.16 ± 0.52**	6.54 ± 0.56**
40	67.03 ± 0.52	25.25 ± 1.02**	28.22 ± 0.68**	23.02 ± 0.32**	21.55 ± 0.45**
80	78.70 ± 0.46	51.89 ± 1.09**	54.99 ± 0.71**	50.51 ± 0.93**	49.18 ± 0.75**
160	87.60 ± 0.34	64.45 ± 1.00**	68.89 ± 1.10**	59.68 ± 0.66**	58.30 ± 0.46**
320	92.85 ± 0.45	77.15 ± 0.46**	80.72 ± 0.32**	75.34 ± 0.46**	72.76 ± 0.71**

Values are expressed as Mean±SD of the triplicate experiment. **indicates a statistically significant difference at a 1% level of significance ($p < 0.01$).

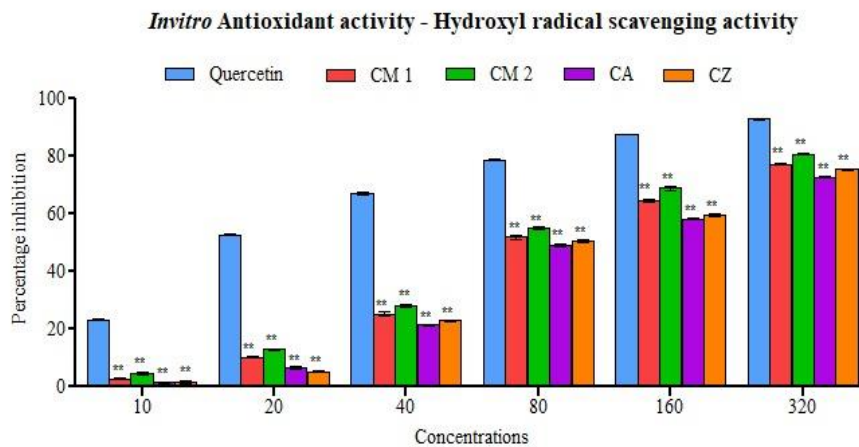


Figure 2. Hydroxyl radical scavenging assay of plant extracts and standard quercetin. Data expressed as Mean±SD (n=3, $p < 0.01$) for all tested dosages.

3.4. *In vitro* anti-inflammatory activity studies.

The *in vitro* anti-inflammatory activity of alcoholic CM1, CM2, CZ, and CA extract was screened by protein denaturation and lipoxygenase inhibition assay using standard diclofenac sodium.

3.4.1. Protein denaturation assay.

The denaturation of tissue proteins may be the cause behind the production of auto-antigens in certain arthritic diseases. So, it may be said that tissue protein denaturation is a marker for inflammatory and arthritic diseases and agents that can prevent protein denaturation, therefore, would be, and agents that can prevent protein denaturation are, therefore, possible candidates for anti-inflammatory drug development [20]. All data obtained were expressed as mean ± standard deviation. The IC₅₀ values of the alcoholic extracts CM1, CM2, CA, and CZ and reference standard Diclofenac sodium were found to be 273.39 µg/mL, 244.39 µg/mL, 330.98 µg/mL, and 313.68 µg/mL and 70.45 µg/mL, respectively. The present findings of the anti-inflammatory study by protein denaturation assay exhibited a concentration-dependent inhibition of protein denaturation by both the extracts (CM1, CM2, CZ, and CA) and standard drug, Diclofenac sodium (Table 5 and Figure 3). Significant anti-inflammatory activity

comparable to standard diclofenac was noticed at concentrations of 800 µg/mL and 1600 µg/ml. The activity is in the order CM2 > CM1 > CZ >CA.

Table 5. Protein denaturation assay.

Concentration (µg/ml)	Diclofenac %	CM 1 %	CM 2 %	CZ %	CA %
50	35.69 ± 0.91	13.39 ± 0.14**	13.00 ± 2.02**	6.19 ± 1.96**	11.87 ± 2.04**
100	59.53 ± 0.48	23.04 ± 1.34**	26.91 ± 1.19**	22.45 ± 1.58**	22.16 ± 1.42**
200	73.15 ± 0.18	38.13 ± 0.98**	45.37 ± 2.41**	33.59 ± 1.13**	29.19 ± 1.67**
400	81.03 ± 0.08	62.30 ± 0.68**	63.62 ± 0.44**	58.67 ± 0.70**	56.41 ± 0.84**
800	86.41 ± 0.12	81.03 ± 0.085**	83.42 ± 0.13**	77.60 ± 0.13**	73.84 ± 0.30**
1600	92.87 ± 0.01	86.84 ± 0.16**	87.98 ± 0.02**	86.35 ± 0.15**	85.17 ± 0.05**

Values are expressed as Mean±SD of the triplicate experiment. ** indicates a statistically significant difference at a 1% significance level ($p < 0.01$).

Invitro Anti-inflammatory activity - Protein Denaturation Assay

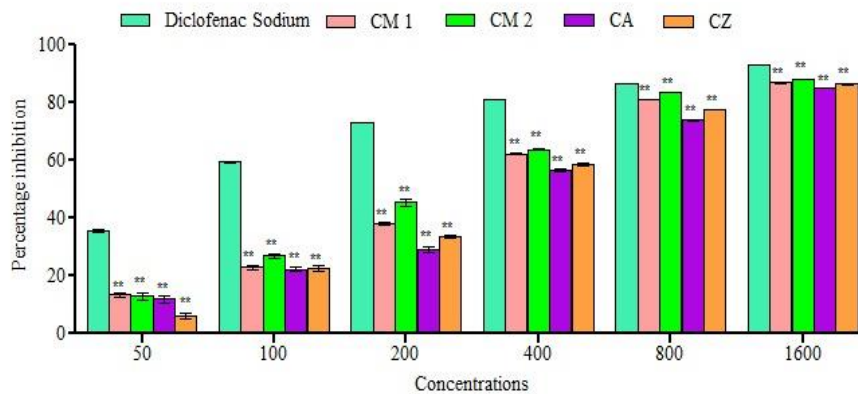


Figure 3. Protein denaturation assay of plant extracts and standard diclofenac sodium. Data expressed as Mean±SD (n=3, $p < 0.01$) for all tested dosages.

3.4.2. Lipoxygenase inhibition assay.

The anti-inflammatory mechanism may include a series of processes in which arachidonic acid metabolism has a prominent role. Upon getting appropriate stimulation from neutrophils, arachidonic acid is cleaved from the membrane phospholipids and converted to leukotrienes and prostaglandins, the mediators of inflammation, through lipoxygenase and cyclooxygenase pathways. The inhibition of lipoxygenase can reduce leukotrienes, thereby producing an anti-inflammatory effect [26,27]. In the study, the lipoxygenase inhibition was monitored as the absorbance increased at 234 nm. All experimental results were expressed as mean ± SD.

Table 6. Lipoxygenase inhibition.

Concentration (µg/ml)	Diclofenac %	CM 1 %	CM 2 %	CZ %	CA %
50	62.11 ± 1.84	27.14 ± 7.73**	45.90 ± 1.20**	28.63 ± 7.23**	30.50 ± 8.21**
100	86.17 ± 0.23	63.01 ± 2.53**	63.24 ± 1.46**	55.35 ± 2.98**	55.88 ± 3.13**
200	91.12 ± 0.11	76.88 ± 0.549**	76.66 ± 0.70**	74.55 ± 0.67**	66.77 ± 1.42**
400	94.98 ± 0.04	83.21 ± 0.34**	85.19 ± 0.18**	84.34 ± 0.29**	77.03 ± 0.58**
800	97.01 ± 0.01	87.58 ± 0.16**	91.44 ± 0.12**	90.82 ± 0.14**	84.26 ± 0.30**
1600	97.58 ± 0.01	90.49 ± 0.13**	94.52 ± 0.03**	94.28 ± 0.05**	87.60 ± 0.19**

Values are expressed as Mean±SD of the triplicate experiment. ** indicates a statistically significant difference at a 1% level of significance ($p < 0.01$).

The IC₅₀ values of the alcoholic extracts CM1, CM2, CZ, CA, and reference standard diclofenac sodium were found to be 76.57 µg/mL, 42.43 µg/mL, 88.31 µg/mL, 95.69 µg/mL, and 3.71 µg/mL, respectively (Table 6 and Figure 4). The analysis showed statistically significant differences at a 1% level of significance ($p < 0.01$).

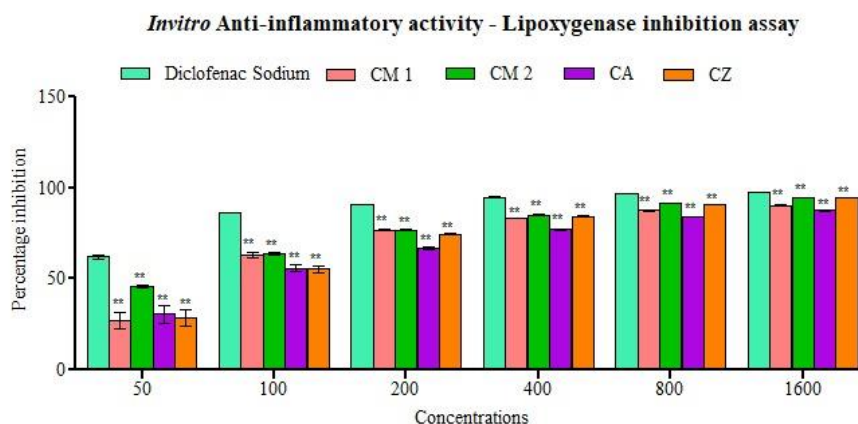


Figure 4. Lipoxygenase inhibition of plant extracts and standard diclofenac sodium. Data expressed as Mean ± SD (n=3, $p < 0.01$) for all tested dosages.

3.5. Discussion.

The plant species, *Coleus malabaricus* Benth., morphotype of *Coleus malabaricus* Benth., *Coleus zeylanicus* (Benth.) L.H. Cramer and *Coleus amboinicus* Lour. were selected based on their ethnomedicinal use in treating infectious diseases, inflammation, and disorders due to oxidative cell damage [28,29]. The antioxidant and anti-inflammatory activities of the alcoholic extract were examined using *in vitro* methods. The ethanol extract was selected for the study because the phytochemical screening showed the maximum presence of phytoconstituents in the alcoholic extract. Also, the plants *Coleus zeylanicus* (Benth.) and *Coleus amboinicus* are used in the indigenous systems of medicine for treating inflammatory conditions and infectious diseases [30].

The antioxidant activity of alcoholic extracts of CM1, CM2, CZ, and CA were evaluated by nitric oxide and hydroxy radical scavenging assay. The phytochemical analysis in the current study revealed the presence of high phenolic and flavonoid content in these plants. There is a correlation between the total phenolic and flavonoid content and antioxidant, anti-inflammatory, and anticancer activities. Phenolic compounds are well known for their antioxidant capacity, and their presence makes these plants potent antioxidant and anti-inflammatory agents. The antioxidant and anti-inflammatory properties of *Coleus amboinicus* and *Coleus zeylanicus* were well established by various *in vitro* and *in vivo* studies [28-30]. Even though it is used as an anti-inflammatory agent for curing muscle pain [10], the activity of *Coleus malabaricus* needs to be proven clinically. So, the study aimed to investigate the anti-inflammatory activity of *Coleus malabaricus* on a comparative basis with the activity of these indigenous plants, *Coleus amboinicus* and *Coleus zeylanicus*. The determination of antioxidant activities of the plant extracts was also included in the study on the assumption that free radicals and reactive oxygen species are implicated in the inflammation cascade.

The results of antioxidant studies showed that alcoholic extract of the plants under investigation has remarkable antioxidant activity and is directly linked to the concentration of the extracts. CM2 showed the highest antioxidant activity in the nitric oxide and hydroxyl radical scavenging assays. At high concentrations, activity was comparable to the standard

drug. The results of the anti-inflammatory activity of ethanol extract of CM1, CM2, CZ, and CA by protein denaturation assay and lipoxygenase inhibition assay exhibited a dose-dependent enhancement similar to standard diclofenac sodium. There is a direct correlation between total phenol and flavonoid content and antioxidant activity. The plant *Coleus malabaricus* (morphotype) showed the highest phenolic content and antioxidant and anti-inflammatory activities.

4. Conclusions

The study results have provided a scientific basis for the traditional use of *Coleus malabaricus* Benth. as an anti-inflammatory agent. The alcoholic extracts of CMI and CM2 showed greater antioxidant and anti-inflammatory activity compared to the traditional medicinal plants *Coleus zeylanicus* and *Coleus amboinicus*. The activities can be well correlated with the high content of phenolic and flavonoid compounds. This work deserves further in vivo studies, isolation, and characterization of the active constituents before future applications.

Funding

This research was funded by the State Board of Medical Research, grant number S46/2021.

Acknowledgments

The authors are thankful to Dr. Suresh Rathinasamy, Greensmed Labs, Chennai for the institutional support in carrying out the *in vitro* studies and Mr. Vipin Xavier and Mrs. Ancy for their support in the statistical work.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Karrat, L.; Abajy, M.Y.; Nayal, R. Investigating the anti-inflammatory and analgesic properties of leaves ethanolic extracts of *Cedrus libani* and *Pinus brutia*. *Heliyon* **2022**, *8*, e09254, <https://doi.org/10.1016/j.heliyon.2022.e09254>.
2. Borquaye, L.S.; Laryea, M.K.; Gasu, E.N.; Boateng, M.A.; Baffour, P.K.; Kyeremateng, A.; Doh, G. Anti-inflammatory and antioxidant activities of extracts of *Reissantia indica*, *Cissus cornifolia* and *Grosseria vignei*. *Cogent Biol.* **2020**, *6*, 1785755, <https://doi.org/10.1080/23312025.2020.1785755>.
3. Pirentos, S.; Panagiotopoulos, A.; Bariotakis, M.; Daskalakis, V.; Lionis, C.; Sourvinos, G.; Karakasiliotis, I.; Kampa, M.; Castanas, E. From traditional ethnopharmacology to modern natural drug discovery: A methodology discussion and specific examples. *Molecules* **2022**, *27*, 4060, <https://doi.org/10.3390/molecules27134060>.
4. Nurzyńska-Wierdak, R. Phenolic Compounds from New Natural Sources—Plant genotype and ontogenetic variation. *Molecules* **2023**, *28*, 1731, <https://doi.org/10.3390/molecules28041731>.
5. Liu, J.-Z.; Zhang, C.-C.; Fu, Y.-J.; Cui, Q. Comparative analysis of phytochemical profile, antioxidant and anti-inflammatory activity from *Hibiscus manihot* L. flower. *Arab. J. Chem.* **2022**, *15*, 103503, <https://doi.org/10.1016/j.arabjc.2021.103503>.
6. Kasote, D.M.; Katyare, S.S.; Hegde, M.V.; Bae, H. Significance of Antioxidant Potential of Plants and its Relevance to Therapeutic Applications. *Int. J. Biol. Sci.* **2015**, *11*, 982, <https://doi.org/10.7150/ijbs.12096>.

7. Kamal, B.S.; Balakrishnan, B.R. Medicinally important *Coleus Species*, *Coleus Malabaricus* Benth. and many others of family Lamiaceae from the Western Ghats, India – A review. *Int. J. Pharm. Sci. Res.* **2021**, *12*, 2001-2011, [http://dx.doi.org/10.13040/IJPSR.0975-8232.12\(4\).2001-11](http://dx.doi.org/10.13040/IJPSR.0975-8232.12(4).2001-11).
8. Lukhoba, C.W.; Simmonds, M.S.J.; Paton, A.J. *Plectranthus*: A review of ethnobotanical uses. *J. Ethnopharmacol.* **2006**, *103*, 1-24, <https://doi.org/10.1016/j.jep.2005.09.011>.
9. Waldia, S.; Joshi, B.C.; Pathak, U.; Joshi, M.C. The Genus *Plectranthus* in India and Its Chemistry. *Chem. Biodivers.* **2011**, *8*, 244-252, <https://doi.org/10.1002/cbdv.201000048>.
10. Quattrocchi, U: CRC world dictionary of medicinal and poisonous plants: common names, scientific names, eponyms, synonyms, and etymology (5 Volume Set), 1st Edition; CRC Press: **2012**; <https://doi.org/10.1201/b16504>.
11. Murugesan, M.; Balasubramaniam, V.; Arthi, H. Ethno medical knowledge of plants used by Irula tribes, Chengal Combai, the Nilgiris, Tamilnadu. *Anc. Sci. Life* **2005**, *24*, 179-182.
12. Smitha, K. Taxonomic revision and molecular phylogeny of the genus *Plectranthus* L'Hér.(Lamiaceae) in India. Ph.D Thesis, Department of Botany, University of Calicut, Kerala, India, **2018**.
13. Evans, W.C. Trease and Evans' pharmacognosy, 16th Edition; Elsevier Health Sciences: 2009.
14. Shaikh, J.R.; Patil, M. Qualitative tests for preliminary phytochemical screening: An overview. *Int. J. Chem. Stud.* **2020**, *8*, 603-608, <https://doi.org/10.22271/chemi.2020.v8.i2i.8834>.
15. Harborne, J.B. Phytochemical methods: a guide to modern techniques of plant analysis; Chapman and Hall: **1998**; <https://doi.org/10.1007/978-94-009-5570-7>.
16. Maestre-Hernández, A.-B.; Vicente-López, J.-J.; Pérez-Llamas, F.; Candela-Castillo, M.-E.; García-Conesa, M.-T.; Frutos, M.-J.; Cano, A.; Hernández-Ruiz, J.; Arnao, M.B. Antioxidant Activity, total phenolic and flavonoid contents in floral saffron bio-residues. *Processes* **2023**, *11*, 1400, <https://doi.org/10.3390/pr11051400>.
17. Chaudhary, P.; Janmeda, P. Quantification of phytochemicals and *in vitro* antioxidant activities from various parts of *Euphorbia nerifolia* Linn. *J. Appl. Biol. Biotechnol.* **2022**, *10*, 133-145, <https://doi.org/10.7324/JABB.2022.100217>.
18. Lalhminghlu, K.; Jagetia, G.C. Evaluation of the free-radical scavenging and antioxidant activities of Chilauni, *Schima Wallichii* Korth *in vitro*. *Future Sci. OA* **2018**, *4*, FSO272, <https://doi.org/10.4155/fsoa-2017-0086>.
19. Bari, M.W.; Islam, A.; Islam, M.M.; Sultana, M.J.; Afroz, R.; Khan, M.M.R.; Parul, S.S.; Swaraz, A.M.; Hossain, M.I.; Islam, M.A. Determination of *in vitro* antioxidant activity and *in vivo* antineoplastic effects against Ehrlich ascites carcinoma of methanolic extract of *Sphagneticola calendulacea* (L.) Pruski. *Heliyon* **2021**, *7*, e07228, <https://doi.org/10.1016/j.heliyon.2021.e07228>.
20. Richards, A.; Chaurasia, S. Antioxidant activity and reactive oxygen species (ROS) scavenging mechanism of *Eriodictyon californium*, an Edible Herb of North America. *J. Chem.* **2022**, *2022*, 6980121 <https://doi.org/10.1155/2022/6980121>.
21. Perumal, P.; Saravanabhavan, K. Antidiabetic and antioxidant activities of ethanolic extract of *Piper Betle* L. Leaves In Catfish, *Clarias Gariepinus*. *Asian J. Pharm. Clin. Res.* **2018**, *11*, 194-198, <http://dx.doi.org/10.22159/ajpcr.2018.v11i3.22393>.
22. Altir, N.K.M.; Ali, A.M.A.; Gaafar, A.-R.Z.; Qahtan, A.A.; Abdel-Salam, E.M.; Alshameri, A.; Hodhod, M.S.; Almunqedhi, B. Phytochemical profile, *in vitro* antioxidant, and anti-protein denaturation activities of *Curcuma longa* L. rhizome and leaves. *Open Chem.* **2021**, *19*, 945-952, <https://doi.org/10.1515/chem-2021-0086>.
23. Malathi, G.; Vadivelu, J. In-vitro antioxidant, anti-inflammatory and cytotoxic activity of *Cucumis melo* L. of ethanolic extract. *Biomedicine* **2021**, *41*, 439-442.
24. Gunathilake, K.D.P.P.; Ranaweera, K.K.D.S.; Rupasinghe, H.P.V. *In vitro* anti-inflammatory properties of selected green leafy vegetables. *Biomedicines* **2018**, *6*, 107, <https://doi.org/10.3390/biomedicines6040107>.
25. Bancuta, O.R.; Chilian, A.; Bancuta, I.; Ion, R.M.; Setnescu, R.; Setnescu, T.; Gheboianu, A. Improvement of spectrophotometric method for determination of phenolic compounds by statistical investigations. *Rom. Journ. Phys* **2016**, *61*, 1255-1264.
26. Shraim, A.M.; Ahmed, T.A.; Rahman, M.M.; Hijji, Y.M. Determination of total flavonoid content by aluminum chloride assay: A critical evaluation. *LWT* **2021**, *150*, 111932, <https://doi.org/10.1016/j.lwt.2021.111932>.

27. Jomova, K.; Raptova, R.; Alomar, S.Y.; Alwasel, S.H.; Nepovimova, E.; Kuca, K.; Valko, M. Reactive oxygen species, toxicity, oxidative stress, and antioxidants: chronic diseases and aging. *Arch Toxicol.* **2023**, *97*, 2499-2574, <https://doi.org/10.1007/s00204-023-03562-9>.
28. Lončarić, M.; Strelec, I.; Moslavac, T.; Šubarić, D.; Pavić, V.; Molnar, M. lipoxygenase inhibition by plant extracts. *Biomolecules* **2021**, *11*, 152, <https://doi.org/10.3390/biom11020152>.
29. Barbosa, M.D.; Wilairatana, P.; Leite, GM.; Delmondes, G.D.; Silva, L.Y.; Júnior, S.C.; Dantas, L.B.; Bezerra D.S.; Beltrão, I.C.; Dias, D.D.; Ribeiro-Filho, J. *Plectranthus* species with anti-inflammatory and analgesic potential: A systematic review on ethnobotanical and pharmacological findings. *Molecules.* **2023**, *28*, 15, 5653. <https://doi.org/10.3390/molecules.28155653>.
30. Napagoda, M.; Gerstmeier, J.; Butschek, H.; Lorenz, S.; De Soyza, S.; Qader, M.; Nagahawatte, A.; Wijayarathne, G.B.; Schneider, B.; Svatoš, A.; Jayasinghe, L.; Koeberle, A.; Werz, O. *Plectranthus zeylanicus*: A rich source of secondary metabolites with antimicrobial, disinfectant and anti-inflammatory activities. *Pharmaceuticals* **2022**, *15*, 436, <https://doi.org/10.3390/ph15040436>.
31. Wadikar, D.D.; Patki, P.E. *Coleus aromaticus*: A therapeutic herb with multiple potentials. *J. Food Sci Technol.* **2016**, *53*, 2895-2901, <https://doi.org/10.1007/s13197-016-2292-y>.
32. Arumugam, G.; Swamy, M.K.; Sinniah, U.R. *Plectranthus amboinicus* (Lour.) Spreng: botanical, phytochemical, pharmacological and nutritional significance. *Molecules* **2016**, *21*, 369, <https://doi.org/10.3390/molecules21040369>.