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EVALUATION OF ANTIOXIDANT POTENTIAL AND ANTI- INFLAMMATORY ACTIVITIES OF *Cleistanthus collinus* (Roxb.) Benth.ex Hook. f.

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ABSTRACT

This study investigated the antioxidant and anti-inflammatory activities of *Cleistanthus collinus* leaf, bark, and fruit extracts. Different solvent extracts (methanol, ethyl acetate, chloroform, petroleum ether, and water) were assessed at concentrations of 100-500 µg mL^{-1}. Antioxidant activity was assessed by using DPPH, FRAP, H_2O_2 scavenging, metal chelating, and reducing power assays, while total phenolic and flavonoid contents were quantified spectrophotometrically. The anti-inflammatory potential was evaluated through inhibition of albumin denaturation, anti-proteinase activity, and hypotonicity-induced hemolysis assays. Results revealed that the methanolic extract of *C. collinus* fruit had highest phenolic content (253.1 mg GAE g^{-1}) and exhibited the most potent antioxidant effects in several assays. Based on the IC_{50} values, fruit-based methanol extract had highest antioxidant values in DPPH assay (271.69 ± 0.65 µg mL^{-1}), H_2O_2 assay (284.41 ± 2.61 µg mL^{-1}), and metal chelating assay (305.94 ± 1.72 µg mL^{-1}), however, bark methanol extract had substantially lower IC_{50} value in FRAP assay (363.80 ± 4.04^{-1} g mL^{-1}). Leaf methanolic extract had highest flavonoid content (164.65 mg RU g^{-1}). Anti-inflammatory activity was highest in fruit methanol extract, with hemolysis inhibition reaching up to 30.21%. The study suggests that *C. collinus* extracts hold promise as natural sources of antioxidants and anti-inflammatory agents for therapeutic use.

Keywords: Antioxidants**,** *Cleistanthus collinus,* DPPH, FRAP, hemolysis, phytomedicinet, total phenolic content

INTRODUCTION

Reactive oxygen species (ROS) contain unpaired electrons which are highly reactive and can lead to the oxidative stress in cells, resulting in numerous health issues like cancer, cellular damage, aging, and neurological disorders. ROS levels can increase by external factors like pollution, ultraviolet radiation, alcohol consumption, smoking, and exposure to certain chemicals (Agarval *et al.*, 2011). When ROS production is excessive, the body's natural antioxidants get depleted so weaken its defence. Antioxidant enzymes, like catalase, superoxide dismutase, and peroxidase, along with dietary antioxidants such as β-carotene and vitamins C and E, play a crucial role in maintaining the cellular health by scavenging these free radicals (Rahman, 2007). However, when ROS production surpasses the body's antioxidant capabilities, an imbalance may occur leading to oxidative stress (Gao *et al.,* 2019). Thus finding safe and effective antioxidants is essential to protect body against oxidative stress.

Plants are among the most promising natural sources of antioxidants, as they contain a variety of secondary metabolites with antioxidant properties (Aragona *et al.,* 2018). These metabolites include phenols, flavonoids, and terpenoids, which are known for their immunomodulatory effects (Gangwar *et al.,* 2014). In fact, most of the antioxidants obtained through diet are derived from

plant-based foods rich in phenolic and flavonoid compounds (Lee *at al.,* 2014). The compounds possessing hydroxyl groups enhance antioxidant activity by directly contributing to the redox processes and acting as electron donors (Bendary *et al.,* 2013). In biological systems, phenolic and flavonoid compounds play a protective role by reducing oxidative stress and preventing the oxidation of macromolecules (Pereira *et al.,* 2007).

Inflammation, the body's response to harmful stimuli, is a complex biological process that includes the localized accumulation of plasma, fluid, and blood cells, along with protein denaturation, increased vascular permeability, and alterations in cell membranes (Ferrero-Miliani *et al.,* 2006). This response, primarily driven by chemical mediators released by injured or migrating cells, helps in removing the harmful agents and support tissue repair (Chandra *et al.,* 2012). Inflammation can either be acute or chronic, and while non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used to manage it, they can have adverse side effects (Kim *et al.,* 2016). Consequently, there is increasing interest in natural anti-inflammatory agents, because many phytochemicals from plants have the ability to reduce inflammation with fewer side effects (Pan *et al.,* 2009). Epidemiological studies support the health benefits of polyphenols and flavonoids in diet, suggesting an inverse relationship between their intake and the risk of chronic conditions, including cancer and cardiovascular disease (Garcia-Lafuente *et al.,* 2009).

Cleistanthus collinusi (Roxb.) Benth. ex Hook. f., a toxic tree from Euphorbiaceae family, is native to semi-arid regions in central and southern India, as well as parts of Malaysia and Africa. This plant is especially dangerous due to the presence of toxic compounds like diphylline lactone and its glycosides, cleistanthin A and B (Ranjith and Arivudainambi, 2020). Despite its toxicity, *C. collinus* has significant ethnomedicinal applications. Traditionally, alcoholic preparations made from its aerial parts are used to treat gastrointestinal ailments, clean infected wounds, and address fungal and skin diseases. The plant is valued for its astringent, antitumor, antiseptic, and anti-fungal properties (Remya *et al.,* 2018). The phytochemistry of *C. collinus* is diverse with active compounds like furfuranoid lignans, arylnaphthalide lignans and their glycosides, and pimarane diterpenes (Misbah and Alok, 2022). Perusal of literature has revealed that there is lack of comprehensive study on antioxidant potential of *C. collinus* bark and fruit extracts, hence the present study was aimed to analyse it's *in vitro* antioxidant and anti-inflammatory activities. Several extracts of plant were assessed using DPPH, FRAP, H_2O_2 scavenging, metal chelating, and reducing power assays, and determine its phenolic and flavonoid contents. Additionally, the anti-inflammatory properties of various extracts were evaluated to explore the therapeutic potential of *C. collinus* as a natural source of antioxidants and anti-inflammatory agents.

MATERIALS AND METHODS

All the experiments including phytochemical extraction, antioxidant, and anti-inflammatory assays were conducted in the Department of Botany, Sri Venkateswara University in the year 2024.

Plant material collection and extraction of phytochemicals

Leaves, bark, and fruits of *Cleistanthus collinus* were collected from Gopavaram village in Maredumilli forest area, Andhra Pradesh (India) [17˚22'09.16" N 81˚47'48.42" E]. After cleaning, the plant materials were shade-dried, pulverized and extracted by Soxhlet apparatus using various solvents (petroleum ether, chloroform, ethyl acetate, water and methanol). The concentrated extracts, dried under vacuum at 60°C, were evaluated for antioxidant and anti-inflammatory activities.

Total phenolic (TPC) and total flavonoid content (TFC) estimation

The total phenolic content was determined by Folin-Ciocalteu method (Siddiqui *et al.,* 2007) while total flavonoid content was determined by colorimetric technique (Zhishen *et al.*, 1999).

Antioxidant activities screening

Following the established protocols for DPPH assay (Mensor *et al.*, 2001), FRAP assay (Lim *et al.*,

2013), H_2O_2 scavenging assay (Ruch *et al.,* 1989), metal chelating assay (Dinis *et al.*, 1994) and reducing power action (Oyaizu, 1986) were employed to evaluate the free radical scavenging activities of plant extract at the concentrations of 100, 200, 300, 400, and 500 μg mL^{-1}.

Anti-inflammatory activities screening

The albumin denaturation, anti-proteinase activity, and hypotonicity-induced hemolysis assays were conducted following the standard protocols of Govindappa *et al.* (2011), Juvekar *et al.* (2009) and Slowing *et al.* (2009), respectively.

Statistical analysis

The experiment was conducted in a completely randomized design and each treatment replicated three times. For *in vitro* antioxidant experiments, one-way ANOVA test followed by a Tukey's test ($p < 0.01$) was performed to compare the IC_{50} values of various fractions across the multiple antioxidant assays. The data collected were presented as mean ± standard deviation. MS Excel for Windows version 7 was used to calculate the linear regression coefficient (R^2) between phenolic and flavonoid contents and antioxidant capacity. The probability of $p \leq 0.05$ was regarded to be statistically significant.

RESULTS AND DISCUSSION

Total phenolic contents (TPC)

Phenolic substances are the plant elements with redox characteristics that contribute to the anti-oxidant action. The hydroxyl groups present in plant-based extracts aid in the elimination of free radicals. Table 1 shows TPC of *C. collinus* leaf, bark, and fruit extracts. The calibration curve was prepared by recording the absorbance at various gallic acid doses (Fig. 1A). The total phenolic content of extracts was extrapolated by using calibration curve regression equation ($Y = 0.008x + 0.201$; $R^2 = 0.997$) and expressed in terms of mg equivalent gallic acid (GAE) g^{-1} dry mass (Fig. 1A). The total phenolic content of *C. collinus* fractions ranged from 253.02 ± 3.5 to 20.1 ± 0.7 mg GAE g^{-1} in a descending order of CFM > CBM > CLM > CLC > CFE > CBE > CBC > CFC > CFP > CBP > CLP > CLE > CFA > CLA > CBA (Table 1). The methanolic extracts had maximum phenolic content (243.02 ± 3.1 mg GAE g^{-1} in CLM, 248.02 ± 3.2 mg GAE g^{-1} in CBM, and 253.02 ± 3.5 mg GAE g^{-1} in CFM). Contrarily, the aqueous extracts exhibited lowest phenolic levels in leaf

Table 1: Total phenolic and flavonoid contents in the numerous extracts of *C. collinus*

Plant extracts	Total phenols [mg GAE g^{-1} dry extract]	Total flavonoids [mg RU g^{-1} dry extract]
Leaf aqueous extract (CLA)	20.29 ± 0.90^{a}	155.45 ± 2.20^{c}
Leaf petroleum ether extract (CLP)	65.75 ± 1.20^{b}	55.45 ± 0.57^{a}
Leaf ethyl acetate extract (CLE)	57.79 ± 1.20^{b}	94.54 ± 1.10^{b}
Leaf chloroform extract (CLC)	139.61 ± 2.30^{c}	52.01 ± 0.57^{a}
Leaf methanol extract (CLM)	243.02 ± 3.10^{d}	164.65 ± 2.20^{d}
Bark aqueous extract (CBA)	20.10 ± 0.70^{a}	69.25 ± 0.57^{b}
Bark petroleum ether extract (CBP)	82.79 ± 1.20^{b}	62.35 ± 0.90^{b}
Bark ethyl acetate extract (CBE)	125.97 ± 2.30^{c}	40.51 ± 0.57^{a}
Bark chloroform extract (CBC)	104.38 ± 2.10^{b}	89.94 ± 1.20^{b}
Bark methanol extract (CBM)	248.02 ± 3.20^{d}	157.75 ± 2.30^{c}
Fruit aqueous extract (CFA)	28.25 ± 0.57^{a}	54.31 ± 0.70^{a}
Fruit petroleum ether extract (CFP)	84.79 ± 1.20^{b}	33.62 ± 0.57^{a}
Fruit ethyl acetate extract (CFE)	127.11 ± 2.10^{c}	89.94 ± 0.60^{b}
Fruit chloroform extract (CFC)	102.11 ± 1.10^{b}	50.86 ± 0.90^{a}
Fruit methanol extract (CFM)	253.02 ± 3.50^{d}	147.41 ± 2.10^{c}

The values are mean± SD (n = 3). The values superscripted by different letters in the same column significantly differ from each other ($P \leq 0.01$)

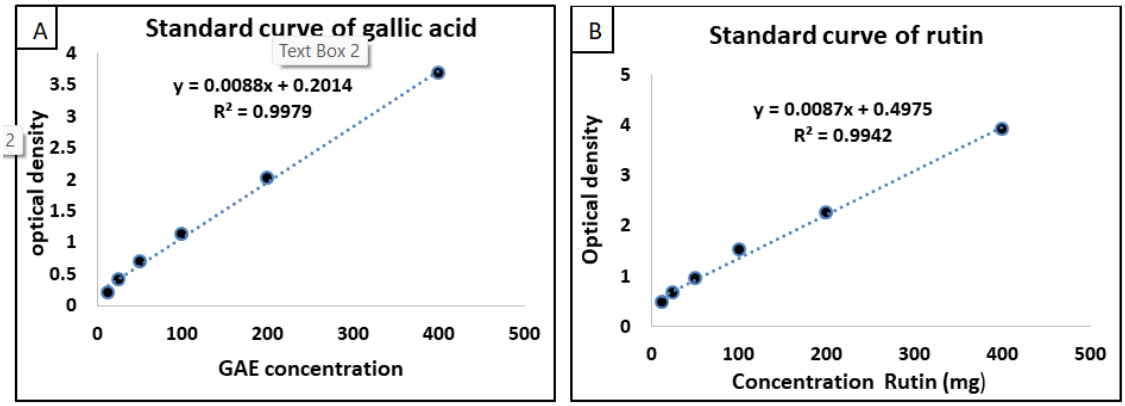

Fig. 1: Standard curves for assessing the total phenolic and total flavonoid contents in extracts: A) The standard curve of gallic acid, and B) Standard curve of rutin

(20.29 ± 0.9 mg GAE g^{-1}), bark (20.1 ± 0.7 mg GAE g^{-1}), and fruit extracts (28.25 ± 0.57 mg GAE g^{-1}), and moderate in all other solvent extracts.

Total flavonoid contents (TFC)

The flavonoid content of selected plant-based extracts was measured quantitatively by employing aluminium chloride in a colorimetric approach. The overall flavonoid concentration of extracts was calculated by assuming the equation for regression of calibration curve (Y = 0.008x + 0.497; R_2 = 0.994) and expressed as mg rutin equivalent (RU) g^{-1} dry mass (Fig. 1B). TFC followed the same pattern as TPC. The methanol extract had higher flavonoid content (164.65 ± 2.2 mg RU g^{-1} dried extract) in leaf, (157.75 ± 2.30 mg RU g^{-1} dried extract) in bark and (147.41 ± 2.1 mg RU g^{-1} dried extract) in fruit, while petroleum ether had lowest flavonoids (33.62 ± 0.57 mg RU g^{-1} dry extract) in fruit followed by ethyl acetate (40.51 ± 0.57 mg RU g^{-1} dried extract) in bark and chloroform (52.01 ± 0.57 mg RU g^{-1} dried extract) in leaf (Table 1). The flavonoids in chloroform bark and ethyl acetate fruit extracts were same (89.94 ± 1.2 mg RU g^{-1} dry extract). The descending order of total flavonoid content was CLM > CBM > CLA > CFM > CLE > CBC = CFE > CBA > CBP > CLP > CFA > CLC > CFC > CBE > CFP (Table 1).

Phenols and flavonoids have diverse chemical and biological roles, like protection against pathogens, UV radiation, and scavenging free radicals (Soobrattee *et al.,* 2005). Among *C. collinus* extracts, methanolic leaf, bark, and fruit extracts had highest phenolic content, while aqueous extracts had lowest values. Phenolic compounds significantly enhanced Fe^{3+} to Fe^{2+} reduction, H_2O_2 to H_2O conversion, and Fe^{2+} chelation (Ruch *et al.*, 1989). DPPH, FRAP, H_2O_2 scavenging, metal chelating, and reducing power assays revealed that methanolic extracts had highest activity, indicating a positive correlation between phenol and flavonoid levels and antioxidant capacity (Holasova *et al.*, 2002; Aljadi and Kamaruddin, 2004). The antioxidant activity was higher in fruit and bark extracts than in leaf extracts. Chaphalkar *et al.* (2017) reported high phenolic and flavonoid content in *Phyllanthus emblica*, a relative of *C. collinus*, which supports the antioxidant potential of *C. collinus* extracts, while petroleum ether and aqueous extracts showed less antioxidant activity.

Antioxidant activity

The antioxidant activity of *C. collinus* leaf, bark, and fruit extracts and their derived fractions were examined using five different techniques.

DPPH assay: The antioxidant capacity of *C. collinus* leaf, bark, and fruit was assessed by DPPH-based free radical scavenging test, and its reducing power was estimated by using the concentration showing 50% inhibition (IC_{50}) values, or the quantity necessary to eliminate 50% free radicals of DPPH. Several extract's radical scavenging actions are raised in a concentration-related way. By using DPPH scavenging test approach, methanol extracts of leaf, bark, and fruit of *C. collinus* showed greater antioxidant capacity. Ascorbic acid has an IC_{50} value of 218.26 ±0.2 µg mL^{-1}. The methanol extract had the most potent DPPH radical-scavenging potential, yielding an IC_{50} value of

271.69 ± 0.65 µg mL^{-1} in fruit, followed by 304.87 ± 0.06 µg mL^{-1} in bark and 336.74 ± 1.09 µg mL^{-1} in leaf which was fairly similar to standard ascorbic acid. In contrast, petroleum ether extract possessed lowest DPPH radical-scavenging capability with IC_{50} value of 1760.2 ± 17.50 µg mL^{-1} in bark, followed by IC_{50} values of 1157.0 ± 12.11 µg mL^{-1} in leaf and 962.74 ± 16.52 µg mL^{-1} in fruit. An equal antioxidant activity was observed in ethyl acetate bark and fruit extracts (422.73 ± 1.25 and 422.45 ± 0.74 µg mL^{-1}, respectively). Ethyl acetate exhibited modest antioxidant activity with 470.25 ± 5.07 µg mL^{-1} in leaf, 422.73 ± 1.25 µg mL^{-1} in bark, and 422.45 ± 0.74 µg mL^{-1} in fruit. The DPPH radical scavenging action of *C. collinus* extracts and their resultant fractions can be classified as CFM > CBM > CLM > CFE > CBE > CLE > CFC > CBC > CLC > CLA > CFP > CLP > CFA > CBA > CBP (Table 2).

FRAP assay: The capacity of substances to convert Fe^{3+}/ferricyanide combination to Fe^{2+} ferrous form is used to predict antioxidant activity. The ferric ion radical scavenging potential of *C. collinus* extracts and their resultant fractions may be classified as CBM > CFM > CFE > CLE > CBE > CLM > CBC > CFC > CLC > CFP > CBP > CLP > CFA > CBA> CLA (Table 2). The methanolic extract demonstrated strongest FRAP radical-scavenging activity with IC_{50} value of 363.80 ± 4.04 µg mL^{-1} in bark, followed by 373.68 ± 4.23 µg mL^{-1} in fruit. But ethyl acetate fruit extract demonstrated higher antioxidant potentiality (457.39 ± 6.80 µg mL^{-1}) subsequently followed by leaf (498.77 ± 2.64 µg mL^{-1}) and bark extract (517.11 ± 1.61 µg mL^{-1}) than methanolic leaf extract (529.38 ± 4.20 µg mL^{-1}), which is comparable to normal ascorbic acid (224.2 ± 2.21 µg mL^{-1}). In contrast, water extract had least FRAP radical-scavenging activity with IC_{50} value of 2111.36 ± 66.26 µg mL^{-1} in leaf, followed 2061.15 ± 59.23 µg mL^{-1} in bark and 1816.78 ± 53.53 µg mL^{-1} in fruit. Chloroform-based extract exhibited modest antioxidant activity of 1073.08 ± 15.24 µg mL^{-1} in leaf, 768.33 ± 5.70 µg mL^{-1} in bark, and 1051.33 ± 4.51 µg mL^{-1} in fruit.

H_2O_2 scavenging action

Methanolic extract showed most potent H_2O_2 radical-scavenging action with IC_{50} of 284.41 ± 2.61 µg mL^{-1} in fruit, 313.40 ± 3.20 µg mL^{-1} in bark and 403.74 ± 4.20 µg mL^{-1} in leaf, which was somewhat similar to standard ascorbic acid (127.49 ± 2.54 µg mL^{-1}) (Table 2). In contrast, water extract had least H_2O_2 scavenging activity with IC_{50} value of 1723.23 ± 34.92 µg mL^{-1} in bark, 1454.44 ± 12.12 µg mL^{-1}

Table 2: Radical scavenging activities (IC_{50} values) of *C. collinus* along with standard ascorbic acid

Plant extracts	IC_{50} values of radical scavenging activity (mg mL^{-1})			
	DPPH	FRAP	H_2O_2 scavenging	Metal chelating activity
CLA	901.2 ± 10.7^{d}	2111.4 ± 66.2^{f}	1454.5 ± 12.1^{e}	1257.9 ± 5.1^{e}
CLP	1157.0 ± 12.1^{d}	1367.6 ± 15.3^{e}	1401.0 ± 10.1^{e}	1085.9 ± 8.0^{d}
CLE	470.2 ± 5.0^{b}	498.8 ± 2.6^{b}	673.1 ± 3.2^{b}	533.8 ± 5.3^{b}
CLC	850.9 ± 6.7^{c}	1073.0 ± 15.2^{d}	1004.9 ±17.0^{d}	778.1 ± 9.5^{c}
CLM	336.7 ± 1.0^{a}	529.3 ± 4.2^{b}	403.7 ± 4.2^{b}	422.6 ± 1.4^{b}
CBA	1439.7 ± 3.0^{e}	2061.1 ± 59.2^{f}	1723.2 ± 34.9^{f}	984.9 ± 4.2^{d}
CBP	1760.2 ± 17.5^{f}	1606.2 ± 13.6^{f}	1262.7 ± 24.6^{e}	916.6 ± 4.3^{d}
CBE	422.7 ± 1.2^{b}	517.1 ± 1.6^{b}	530,0 ± 6.2^{b}	528.0 ± 1.0^{b}
CBC	772.7 ± 8.3^{c}	768.3 ± 5.7^{c}	715.6 ± 9.6^{c}	557.0 ± 0.7^{b}
CBM	304.8 ± 0.06^{a}	363.8 ± 4.0^{a}	313.4 ± 3.2^{a}	354.8 ± 2.6^{a}
CFA	1424.6 ± 16.8^{e}	1816.7 ± 53.5^{f}	1277.3 ± 34.0^{d}	695.6 ± 5.6^{b}
CFP	962.7 ± 16.5^{d}	1605.0 ± 10.8^{f}	819.8 ± 14.9^{c}	778.9 ± 0.4^{c}
CFE	422.4 ± 0.7^{b}	457.3 ± 6.8^{b}	441.7 ± 5.6^{b}	466.4 ± 0.9^{b}
CFC	746.1 ± 4.9^{c}	1051.3 ± 4.5^{d}	706.2 ± 9.5^{c}	459.4 ± 3.8^{b}
CFM	271.7 ± 0.6^{a}	373.6 ± 4.2^{a}	284.4 ± 2.6^{a}	305.9 ± 1.7^{a}
Ascorbic acid	218.0 ± 0.2^{a}	224.0 ± 2.2^{a}	127.0 ± 2.5^{a}	134.0 ± 4.2^{a}

All the values shown are mean standard deviation (n = 3). a-f superscripts that are significantly distinct ($p \leq 0.01$). CLA = Leaf aqueous extract; CLP = Leaf petroleum ether extract); CLE = Leaf ethyl acetate extract; CLC = Leaf chloroform extract; CLM = Leaf methanol extract; CBA = Bark aqueous extract; CBP = Bark petroleum ether extract; CBE = Bark ethyl acetate extract; CBC = Bark chloroform extract; CBM = Bark methanol extract; CFA = Fruit aqueous extract; CFP = Fruit petroleum ether extract; CFE = Fruit ethyl acetate extract; CFC = Fruit chloroform extract; and CFM = Fruit methanol extract

mL^{-1} in leaf and 1277.35 ± 34.09 µg mL^{-1} in fruit. Ethyl acetate based extract showed modest antioxidant activity with IC_{50} value of 673.12 ± 3.24, 530.08 ± 6.25 and 441.77 ± 5.63 µg mL^{-1} in leaf, bark and fruit. Antioxidant activity of different extracts followed the order of CFM > CBM > CLM > CFE > CBE > CLE > CFC > CBC > CFP > CLC > CBP > CFA > CLP > CLA > CBA.

Metal chelating action

Metal chelating activity along with DPPH, FRAP, and H_2O_2 radical scavenging tests, rose with increase in extract concentration from 100 to 500 µg mL^{-1}. Ascorbic acid had an IC_{50} value of 134.73 ± 4.2 µg mL^{-1}. The highest antioxidant activity was found in the methanol extract of fruit (IC_{50}: 305.94 ± 1.72 µg mL^{-1}), followed by bark extract (IC_{50}: 354.80 ± 2.69 µg mL^{-1}) and leaf extract (IC_{50}: 422.62 ± 1.41 µg mL^{-1}). The proportion of free radical inhibition by multiple concentrations of methanol samples was lower than that of the standard, ascorbic acid. The lowest IC_{50} values were observed in leaf water extract (1257 ± 5.15 µg mL^{-1}), followed by leaf petroleum ether extract (1085.99 ± 8.07 µg mL^{-1}) and bark water extract (984.98 ± 4.19 µg mL^{-1}). The metal chelating capability of *C. collinus* extracts and ascorbic acid declined in the following sequence, respectively: CFM > CBM > CLM > CFC > CFE > CBE > CLE > CBC > CFA > CFP = CLC > CBP > CBA > CLP > CLA.

Reducing power assay

Dose dependence curve (Table 3) shows the relative reducing capabilities of leaf, bark, and fruit extracts, and their derived fractions (100-500 µg mL^{-1}). The sequence of reducing power of extracts was in the ascending order: CBM > CFM > CLM > CFE > CBE > CFC > CLE > CBC > CLC > CFP > CLP > CFA > CBP > CLA > CBA. Significantly higher reducing power (3.01 ± 0.02) was evident in CBM fraction, followed by- CFM (2.9±0.02) and CLM (2.52±0.02) at 500 µg mL^{-1} concentration. The extract's reducing power in all cases rose with sample concentration, as their antioxidant activity increased.

Table 3: Reducing power of *C. collinus* extracts using different solvents at various concentrations

Plant extracts	Concentrations in µg mL^{-1} (Od values mean ± SD)				
	100	200	300	400	500
CLA	1.25 ± 0.00^{a}	1.29 ± 0.01^{a}	1.35 ± 0.01^{a}	1.37 ± 0.00^{a}	1.42 ± 0.02^{b}
CLP	1.36 ± 0.01^{a}	1.39 ± 0.00^{a}	1.47 ± 0.01^{b}	1.57 ± 0.01^{b}	1.63 ± 0.02^{c}
CLE	1.58 ± 0.01^{b}	1.64 ± 0.02^{c}	1.95 ± 0.01^{d}	2.17 ± 0.02^{e}	2.26 ± 0.01^{e}
CLC	1.53 ± 0.00^{b}	1.58 ± 0.01^{b}	1.67 ± 0.02^{c}	1.82 ± 0.01^{d}	1.96 ± 0.00^{d}
CLM	1.65 ± 0.00^{c}	1.83 ± 0.01^{d}	2.01 ± 0.00^{e}	2.36 ± 0.02^{e}	2.52 ± 0.02^{e}
CBA	1.23 ± 0.02^{a}	1.28 ± 0.01^{a}	1.32 ± 0.00^{a}	1.36 ± 0.01^{a}	1.39 ± 0.02^{a}
CBP	1.34 ± 0.01^{a}	1.42 ± 0.02^{b}	1.48 ± 0.01^{b}	1.57 ± 0.00^{b}	1.59 ± 0.01^{b}
CBE	1.63 ± 0.02^{c}	1.71 ± 0.01^{c}	1.86 ± 0.02^{d}	2.28 ± 0.00^{e}	2.37 ± 0.01^{e}
CBC	1.51 ± 0.00^{b}	1.59 ± 0.01^{b}	1.68 ± 0.01^{c}	1.84 ± 0.00^{d}	1.98 ± 0.02^{d}
CBM	1.89 ± 0.01^{d}	2.24 ± 0.01^{e}	2.51 ± 0.01^{e}	2.87 ± 0.01^{e}	3.01 ± 0.02^{f}
CFA	1.28 ± 0.00^{a}	1.35 ± 0.01^{a}	1.49 ± 0.01^{b}	1.49 ± 0.00^{b}	1.62 ± 0.02^{c}
CFP	1.45 ± 0.02^{b}	1.57 ± 0.00^{b}	1.63 ± 0.01^{c}	1.69 ± 0.02^{c}	1.76 ± 0.00^{c}
CFE	1.65 ± 0.01^{c}	1.75 ± 0.01^{c}	1.98 ± 0.01^{d}	2.26 ± 0.02^{e}	2.41 ± 0.00^{e}
CFC	1.67 ± 0.01^{c}	1.79 ± 0.00^{c}	1.90 ± 0.02^{d}	2.12 ± 0.00^{e}	2.30 ± 0.01^{e}
CFM	1.90 ± 0.00^{c}	2.14 ± 0.01^{e}	2.43 ± 0.00^{d}	2.79 ± 0.02^{e}	2.90 ± 0.02^{e}
Ascorbic acid	2.41 ± 0.01^{e}	2.72 ± 0.02^{e}	3.12 ± 0.05^{f}	3.12 ± 0.05^{f}	3.97 ± 0.01^{f}

All the values shown are mean standard deviation (n = 3). a-f superscripts that are significantly distinct ($p \leq 0.01$). CLA = Leaf aqueous extract; CLP = Leaf petroleum ether extract); CLE = Leaf ethyl acetate extract; CLC = Leaf chloroform extract; CLM = Leaf methanol extract; CBA = Bark aqueous extract; CBP = Bark petroleum ether extract; CBE = Bark ethyl acetate extract; CBC = Bark chloroform extract; CBM = Bark methanol extract; CFA = Fruit aqueous extract; CFP = Fruit petroleum ether extract; CFE = Fruit ethyl acetate extract; CFC = Fruit chloroform extract; and CFM = Fruit methanol extract

In present study methanol-, ethyl acetate-, and chloroform-based extracts had higher antioxidant activities than petroleum ether and water extracts and were positively related to TPC. Vennila *et al*. (2013a) worked on *C. collinus* silver nanoparticles of leaf extracts (AgNPs) and reported 43.5-57.2% inhibition. The DPPH-scavenging ability of Ag-doped tin oxide nanoparticles rises as the

ratio climbs from 33.5 to 80% (Vennila *et al.,* 2013b). Kanipandian *et al.* (2014) also noticed that AgNPs have 20 to 69% scavenging rate at concentrations of 50 to1000 µg mL^{-1}. The anti-oxidant activities of five *Phyllanthus* species were examined by Kumaran and Karunakaran, (2007) who reported antioxidant activities ranging from 38.67 to 87.24% in *P. amaras* and *P. deblis*, respectively, at 25 µg mL^{-1} concentration. Perusal of literature reveals that no work has so far been done on bark and fruit extracts, and in present study, fruit and bark extracts outperformed leaf extract. *C. collinus* has a high concentration of phenolic compounds, and its methanolic fractions effectively scavenge hydrogen peroxide and neutralize it by donating electrons. Kadoo and Badere (2020) showed peroxidase and catalase activities of *C. collinus* on cucumber and chili plants. Chaphalkar *et al*. (2017) found hydro-alcoholic bark extract of *Phyllanthus emblica* (IC_{50} value, 188.80 μg mL^{-1}) had better antioxidant activity than ascorbic acid (IC_{50} value, 177.7 μg mL^{-1}) in H_2O_2 radical scavenging assay. *C. collinus* showed IC_{50} values of 313.4 ± 3.2 μg mL^{-1} in methanolic bark extracts. The hydrogen peroxide scavenging activities of five *Phyllanthus* species were (used @ 10 µg mL^{-1} of their methanol extracts: *P. debilis* (45.83%), *P. urinaria* (33.33%), *P. virgatus* (30.83%), *P. maderaspatensis* (28.33%), and *P. amarus* (20.83%) (Slowing *et al.,* 2009).

Anti-inflammatory activity screening

Inhibition of albumin denaturation: The radical scavenging activities of all the test extracts demonstrated a concentration-dependent increase. The methanolic extract of *C. collinus* fruit exhibited highest inhibition (71.14 ± 2.23%), while the aqueous leaf showed lowest (16.42 ± 0.76%) at a concentration of 250 µg mL^{-1}. Diclofenac sodium, used as a standard, displayed an IC_{50} value of 149.92 ± 6.2 µg mL^{-1}. The methanol fruit extract showed a significant activity with an IC_{50} value of 160.84 ± 7.62 µg mL^{-1}, followed by 174.82 ± 6.16 µg mL^{-1} in bark and 274.74 µg mL^{-1} in leaf. Conversely, the aqueous and petroleum ether extracts exhibited lowest inhibition. Ethyl acetate- and chloroform-extracts displayed modest antioxidant activity. The hierarchy of anti-inflammatory activity was observed with fruit having highest, followed by bark, and leaf exhibiting the lowest activity. The albumin denaturation inhibition for *C. collinus* extracts and their respective fractions can be arranged as CFM > CBM > CLM > CFE > CLE > CBE > CLC > CFA > CLP > CFC > CFP > CBP > CBC > CBA > CLA (Table 4). These findings suggest that the methanol fractions of fruit and bark hold promise for the development of plant-based anti-inflammatory drugs.

Table 4: Anti-inflammatory activities (IC_{50} values) of *C. collinus* leaf, fruit and bark extracts

	Anti-proliferative activities (IC_{50} values) of *C. collinus* (mg mL^{-1})		
Plant extracts	Albumin denaturation	Proteinase inhibition	Hemolytic activity
CLA	851.31 ± 32.7^{e}	863.83 ± 34.2^{e}	1359.53 ± 44.9^{e}
CLP	499.31 ± 15.1^{c}	662.92 ± 22.9^{d}	1014.18 ± 41.6^{d}
CLE	351.82 ± 10.5^{b}	358.06 ± 10.6^{b}	725.23 ± 36.2^{c}
CLC	410.54 ± 14.7^{c}	490.83 ± 16.5^{c}	963.18 ± 39.6^{d}
CLM	274.74 ± 10.2^{b}	315.23 ± 9.12^{b}	762.88 ± 29.12^{c}
CBA	801.44 ± 30.3^{e}	658.85 ± 23.5^{d}	912.31 ± 34.9^{d}
CBP	735.34 ± 32.0^{d}	551.05 ± 15.8^{c}	608.81 ± 24.6^{c}
CBE	349.70 ± 12.2^{b}	361.45 ± 11.6^{b}	581.59 ± 16.2^{b}
CBC	738.22 ± 38.3^{d}	550.77 ± 16.7^{c}	713.35 ± 27.6^{c}
CBM	174.82 ± 6.16^{a}	285.84 ± 11.2^{b}	541.35 ± 17.12^{b}
CFA	434.29 ± 16.8^{c}	606.89 ± 21.3^{c}	1275.58 ± 42.32^{e}
CFP	701.22 ± 18.5^{d}	756.66 ± 30.2^{d}	1104.60 ± 42.9^{d}
CFE	329.24 ± 11.7^{b}	427.45 ± 12.8^{c}	573.83 ± 20.6^{b}
CFC	655.34 ± 24.9^{d}	402.88 ± 14.5^{c}	787.43 ± 26.5^{c}
CFM	160.84 ± 7.6^{a}	195.52 ± 7.2^{a}	429.14 ± 19.12^{b}
Diclofenac sodium	149.92 ± 6.2^{a}	171.22 ± 6.8^{a}	218.05 ± 10.34^{a}

All the values shown are mean standard deviation (n = 3). a-e superscripts that are significantly distinct ($p \leq 0.01$). CLA = Leaf aqueous extract; CLP = Leaf petroleum ether extract); CLE = Leaf ethyl acetate extract; CLC = Leaf chloroform extract; CLM = Leaf methanol extract; CBA = Bark aqueous extract; CBP = Bark petroleum ether extract; CBE = Bark ethyl acetate extract; CBC = Bark chloroform extract; CBM = Bark methanol extract; CFA =

Fruit aqueous extract; CFP = Fruit petroleum ether extract; CFE = Fruit ethyl acetate extract; CFC = Fruit chloroform extract; and CFM = Fruit methanol extract

Anti-proteinase activity: The extracts displayed anti-proteinase activity in a concentration-dependent manner, and their efficacy at higher concentrations was comparable to that of standard drug. The inhibition in protein denaturation by leaf, fruit, and bark extracts fell within the range of 3.8 to 56.57% at concentrations ranging from 50 to 250 µg mL^{-1}. Fruit extracts exhibited a significantly higher ($p < 0.05$) level of inhibition as compared to the studied bark and leaf extracts. The order of inhibition among the extracts was CFM > CBM > CLM > CLE > CBE > CFC > CFE > CLC > CBP = CBC > CFA > CBA > CLP > CFP > CLA. The IC_{50} obtained from methanol extract of fruit was 195 µg mL^{-1}, while IC_{50} value for diclofenac sodium was 171.22 ± 6.84 µg mL^{-1}.

Effect on hemolysis

The ability to inhibit hemolysis was observed in all extracts, displaying a concentration-dependent pattern. Methanolic extracts exhibited highest inhibition, followed by ethyl acetate- and chloroform-based extracts. The inhibition ranged from 2.46 to 30.31%. Fruit and bark extracts demonstrated significantly higher ($p < 0.05$) hemolysis inhibition levels as compared to leaf extracts. Notably, the aqueous extract of leaves exhibited lowest inhibition (11.24 ± 0.75%) among the 15 extracts at a higher concentration (250 µg mL^{-1}), with an IC_{50} value of 1359.53 µg mL^{-1}. It is noteworthy that all the extracts showed lower inhibition in hemolytic activity as compared to protein denaturation and proteinase inhibitory activities. The order of inhibition among extracts was CFM > CBM > CFE > CBE > CBP> CBC > CLE > CLM > CFC > CBA > CLC > CLP > CFP > CFA > CLA.

IC_{50} values of antioxidant activity concerning TPC and TFC

Except for the reducing power and DPPH assays, there was a significant but positive correlation among TPC and IC_{50} values of reducing power activity, hydrogen peroxide radical scavenging, FRAP metal chelating tests, and DPPH scavenging activities (R^2 = 0.756, 0.665, 0.5936, 0.548, and 0.441, respectively). TPC and TFC had a strong correlation with reducing power assay (R^2 = 0.756 & 0.263) and DPPH assay (R^2 = 0.441 & 0.213, respectively) (Table 5).

Table 5: Correlations between phenol & flavonoid contents and IC_{50} values of antioxidant activities

IC_{50} of different assays	Correlation (R^2)	
	Total phenolic content	Total flavonoid content
DPPH radical scavenging activity	0.441*	0.213*
Ferric reducing antioxidant power	0.593*	0.110
Hydrogen peroxide scavenging activity	0.665*	0.135
Metal chelating assay	0.548*	0.063
OD of reducing power assay at 500 µg mL^{-1} concentration	0.756*	0.263*

* indicates significance at $P \leq 0.05$.

Medicinal herbs are a primary source of treatment for inflammatory conditions, especially in developing countries (Mbendana *et al.,* 2019). Mostofa *et al.* (2017) demonstrated that methanolic extracts of *P. niruri* leaves markedly reduced inflammation in rat paw edema models induced by carrageenan, with varying efficacy at doses of 100, 200, and 400 mg kg^{-1}. Specifically, 400 mg kg^{-1} dose nearly eliminated inflammatory cell infiltration as compared to the carrageenan control group. Similarly, the methanolic extract of *Phyllanthus fraternus* @ 300 µg mL^{-1} has shown 72.23% inhibition in protein denaturation, highlighting its potential to alleviate inflammation by inhibiting prostaglandins and leukotrienes (Sarkar *et al.,* 2017).

Euphorbia thymifolia's ethanolic extract demonstrated significant anti-inflammatory effects at 100 mg kg^{-1}, comparable to Indomethacin in reducing carrageenan-induced rat paw edema (Nagaraju *et al.,* 2012). Additionally, *Euphorbia hirta* has shown notable anti-inflammatory potential, with its water extract achieving a nine-fold decrease in fluorescence intensity in a protein denaturation assay, surpassing synthetic drugs such as paracetamol and Combiflam (Arun *et al.,* 2023). Atul *et al.* (2013) showed concentration-dependent inhibition of erythrocyte hemolysis and protein denaturation by hydroalcoholic extract of *P. fraternus* with an IC_{50} value of 52 µg mL^{-1} for albumin denaturation. Similarly, *Phyllanthus emblica* exhibited significant inhibitory effects against hypotonicity-induced hemolysis and protein denaturation with IC_{50} value of 101.08 µg mL^{-1} as compared to the quercetin's IC_{50} of 58.62 µg mL^{-1} (Sharif *et al.,* 2023). These findings signify the

potential of methanolic and hydroalcoholic extracts of *E. thymifolia* as potent anti-inflammatory agents, suggesting these solvents are optimal for extracting bioactive compounds. Further research and clinical trials are required to fully understand their therapeutic potential and safety.

Conclusion: Replacing synthetic antioxidants with natural ones could benefit human health, as plant extracts contain compounds capable of neutralizing free radicals. This study revealed that the methanolic extracts of *Cleistanthus collinus* are rich in phenolic and flavonoid compounds and show strongest antioxidant activity among the tested solvents. The methanolic extract demonstrated substantial antimitotic effects, with ethyl acetate extracts showing moderate activity. Further research into the specific antioxidant and antimitotic compounds in these extracts, along with *in-vivo* studies, could confirm their potential for managing ROS-related diseases.

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