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Research Article

Isolation and Characterization of Phenolic Derivative (Flavonoid) from *Crassocephalum crepidioides* with Antiangiogenic, Anti-inflammatory and Antioxidant Activities

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ABSTRACT

A phenolic compound was successfully isolated from the methanolic extract of the aerial parts of *Crassocephalum crepidioides*. Structural elucidation was carried out using spectroscopic techniques, including UV-vis, IR, mass spectrometry, and nuclear magnetic resonance (NMR). Based on the combined spectral data, the compound was identified as 3-(dimethylamino)-2-hydroxy-5-(3,5,7-trihydroxychroman-2-yl)benzaldehyde. The bioactivity of the isolated compound was evaluated for antiangiogenic, anti-inflammatory, and antioxidant properties. In the shell-less chorioallantoic membrane (CAM) assay, the compound exhibited significant inhibition of angiogenesis. Furthermore, it demonstrated inhibitory activity against phospholipase A_2 enzyme derived from *Daboia russelii* (Russell's viper), suggesting potential anti-inflammatory effects. The compound also displayed notable antioxidant activity, supporting its therapeutic potential in conditions associated with oxidative stress and aberrant angiogenesis. These findings warrant further investigation into its pharmacological applications.

Introduction

The plant *Crassocephalum crepidioides* (CC), which belongs to the family of Asteraceae, is an erect, annual, and juicy herb generally seen in hot and humid provinces. The herb is commonly known as bushy head or red flower rag leaf. CC plant is used in many cultures as food and medicine.[1] The plant is reported to have antitumour,[2] anti-inflammatory,[3] antimicrobial,[4] and antidiabetic activities.[5]

The blood vessel formation from pre-existing vasculature is called angiogenesis, which has a critical role in many physiological events, including wound healing and embryonic development.[6] However, dysregulated angiogenesis is also associated with numerous pathological conditions such as rheumatoid arthritis, certain immune disorders, and cancer. In oncology, angiogenesis is particularly significant, as the sprouting of new blood vessels within tumors ensures an adequate supply of oxygen and micro nutrients required for the proliferation of neoplastic cells. Primary tumors often invade the blood and lymphatic systems, facilitating metastasis to distant sites.[7] The essential role of angiogenesis in sustaining tumor growth has prompted the development of therapeutic strategies aimed at selectively inhibiting the

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formation of tumor-associated microvasculature.[8] The potential of antiangiogenic therapy was demonstrated in preclinical studies, where tumor-bearing mice treated with angiogenesis inhibitors such as endostatin and angiostatin exhibited marked suppression of tumor growth.[9] These findings have intensified efforts to identify novel angiostatic agents capable of specifically inhibiting endothelial cell proliferation and migration, thereby preventing neovascularization.[10] Recently, considerable attention has turned toward the identification of dietary phytochemicals with antiangiogenic properties, which may serve as potential chemopreventive or therapeutic agents in cancer management.[11]

Inflammation is an inborn defence mechanism to remove the microbe or the extraneous particles and to protect the tissue from damage. Inflammation is characterized by dilatation of blood vessels, outpouring of plasma, and release of certain cytokines, and migration of leukocytes through the blood vessels. Further, it leads to multiplication of blood vessels, fibroblasts and more connective tissue formation. The enzyme phospholipases A_2 (PLA_2) hydrolyses the Sn2-ester bond of phospholipids present in the plasma membrane, and releases arachidonic acid (AA) and lysophospholipid (LP). The released arachidonic acid enters two different pathways. In the cyclooxygenase pathway, it produces prostaglandins and thromboxanes. In the lipoxygenase pathway, it produces leukotrienes and lipoxins. The platelet-activating factor (PAF) is synthesized using lysophospholipid as a precursor; these molecules cause inflammation under normal physiological conditions.[12] Many findings highlight the importance of PLA_2 in inflammatory conditions, inhibitions of the PLA_2 enzyme by dietary molecules is a potential remedy in many inflammatory disease states.

Antioxidants, both endogenous and exogenous, are compounds that either prevent the formation of harmful oxidants or neutralize them post-formation, thereby interrupting the oxidative chain reactions typically initiated by these species.[13] *In-vivo*, the generation of free radicals such as superoxide anion (O_2^-), nitric oxide (NO•), and reactive oxygen species like hydrogen peroxide is a continuous physiological process.[14] Increasing attention is being directed toward natural free radical scavengers derived from plants. These phytochemicals can function as nutraceuticals, contributing significantly to human health promotion and disease prevention. [15]

In the present study we have isolated a phenolic compound from CC aerial parts and evaluate its biological activity. Phytochemical analysis of the extract revealed a high concentration of phenolic compounds, flavonoids, ascorbic acid, total sugars, and vitamin C. In addition to these, other bioactive constituents previously reported in CC include alkaloids, diterpenoid lactones, glycosides, steroids, sesquiterpenoids, aliphatic compounds, and polysaccharides. [16]

Materials And Methods

Ethyl acetate, chloroform, n-Hexane, acetone, methanol, silica gel G (for TLC and column chromatography), DPPH, NBT, gallic acid, thiobarbituric acid (TBA), potassium hydroxide, EDTA, ascorbic acid, phenazine methosulfate (PMS), ferric chloride ($FeCl_3$), deoxyribose, sulfanilamide, 1-naphthyl ethylenediamine dihydrochloride, hydrogen peroxide were purchased from SRL, Mumbai, India. *Vipera russelli* venom was acquired from IRULA snake catchers (Madras). All reagents used were of analytical grade. Fertilized eggs were procured from the local shop, Mysore.

Preparation of Leaf and Stem Powder and Extraction

Fresh aerial parts of CC were collected from Hunsur, India. The leaves and stems were separated, thoroughly cleansed, shade-dried, crushed into powder, and stored in an airtight container until further use. A 40 g quantity of the parched powder was subjected to serial extraction utilizing a soxhlet device. The extraction was performed using highly non-polar hexane, chloroform, ethyl acetate, acetone, and polar methanol, followed by an aqueous extract.

Measurement of complete phenolic contents

The total amount of phenolic content in the extract was measured using the reported method[17] with minor changes, using gallic acid as the compound of reference. The total phenolic content was computed and articulated as milligrams of gallic acid per gram of dried powder.

Estimation of flavonoids

Flavonoids were estimated by the Woisky *et al.* method. Aluminium chloride favours acid-stable complexes either with the C-4 keto group or with the hydroxyl groups at C-3 or C-5 of flavones and flavanols. Further, it creates acid-sensitive complexes with ortho-dihydroxyl groups present on either of the two rings of flavonoid structures. Quercetin is employed as a reference criterion for the quantification of total flavonoid content, owing to its well-characterized chemical reactivity and distinctive structural characteristics. Thus, a sequence of quercetin standard solutions at diverse concentrations was prepared to construct a calibration curve for subsequent analytical determinations. The aluminium chloride method was used from the protocol illustrated by Woisky *et al.*[18] with minor changes. A standard calibration curve was established utilizing quercetin.

Detection of α- tocopherol

The α-tocopherol (vit E) content was estimated using the approach defined by Kivçak and Mert[19] here α- tocopherol reducing ferric to ferrous ions. The produced ferrous ions form an orange-red colour with 2,2′-dipyridyl, which can be determined by a spectrophotometer.

Measurement of carbohydrates

The complete carbohydrate present in the extracts was assessed as per the reported method.[20] Plant extract

in various aliquots, along with glucose as a reference in the range of (0–100 µg), were made up to 1000 µL with distilled water. To this, 1000 µL of 5% phenol and 5000 µL of concentrated H_2SO_4 were added. The absorbance of the orange-red colour produced was read at 520 nm.

Determination of ascorbic acid

The total quantity of ascorbic acid was calculated by Das Gupta *et al.*[21] using pure ascorbic acid (0–16 µg) as the standard.

Separation of Bioactive Molecules from *C. crepidioides,* Purification of the Methanol Extract

A total of 40 g of dried powder of CC was taken for sequential extraction using a soxhlet device. Various solvents, which have increased polarity, were used in the following order: n-hexane, chloroform, ethyl acetate, acetone, and methanol. The resulting extracts were evaluated for antiangiogenic activity. Among them, the methanol extract exhibited the highest antiangiogenic property and was further concentrated using a rotavapor. Analysis of the methanol extract by analytical TLC showed three separate spots. The crude methanol extract was further fractionated by column chromatography employing a glass column (22 × 1.3 cm) sealed with silica gel G for column chromatography (100–200 mesh), which was pre-equilibrated with chloroform. Elution was executed using chloroform: methanol mixtures in the ratios 9:1, 8:2, and 7:3 to get three fractions, F1, F2, and F3. These fractions were further scrutinized using analytical TLC using a solvent system of methanol: chloroform: hexane (1:1:0.5). A single spot was observed in each fraction upon exposure to iodine vapours, showing compound purity. The compounds in the F1 and F2 fractions degraded subsequently; only F3 compound was stable. It was then subjected to spectral characterization.

Analysis of Purified Bioactive Compound by Spectroscopic Methods

UV spectroscopy

A UV spectrum of the purified bioactive molecule was noted using a Hitachi spectrophotometer at RT. The scanning was executed in the wavelength range between 200 and 700 nm. For the investigation, 1-mg of the pure compound was dissolved in 1-mL of methanol and employed as the sample.

Mass spectroscopy

Mass of the isolated compound was acquired using an HP 1100 MSD Series device provided with an electrospray ionization source. The study was conducted in positive ionization mode with a flow rate of 200 µL/min using a C-18 column for a running time of 40 minutes.

Nuclear magnetic resonance spectroscopy

Nuclear magnetic resonance spectra were documented on a Bruker Avance spectrometer functioning at 100 MHz for ^{13}C nuclei and 400 MHz for ^{1}H. The chemical shifts of both proton and carbon spectra were referenced to the internal standard tetramethylsilane (TMS).

Inhibition of angiogenesis by the shell-less chorioallantoic membrane assay

Angioinhibitory outcome of the isolated flavonoid from CC was validated using a shell-less chorioallantoic membrane assay. The experiment was carried out according to the method of Rekha, N.D *et al.*[22] The surface of the fertilized hens' eggs was sterilized with 70% ethanol and incubated at 37°C in a humidified incubator. After 5 days of incubation, the shells of the eggs were removed carefully and the contents were released into a hammock; the whole procedure was carried out in a laminar air flow cabinet. The hammock was kept back into incubator. On the 6th day, a paper disc containing 100 µg of flavonoid was placed upon the proliferating blood vessels (on the chorioallantoic membrane) and further incubated. Results were observed the next day.

Anti-inflammatory Activity

Inhibition of phospholipase A2

V. russelli venom was used as a source of phospholipase A2 and was assayed by the indirect haemolytic method (Boman and Kaletta's method).[23] Here, egg yolk, phosphate-buffered saline and packed human erythrocyte, was mixed in the ratio of 1: 8:1 (v/v). About 1-mL of this solution was used as substrate and incubated with the enzyme (70 µg) for 30 minutes at room temperature. The reaction was terminated with the addition of 9 mL of ice-cold phosphate-buffered saline; the reaction mixture was centrifuged at 4°C for 10 minutes at 1500 rpm. The extent of haemoglobin released in the supernatant due to lysis of erythrocytes was noted at 540 nm. The lysis of erythrocytes by the addition of 9 mL of distilled water was considered as positive control (100%). The assay was also carried out with different concentrations of flavonoids.

Determination of Antioxidant Activity

Scavenging assay for DPPH radical

The scavenging capacity of DPPH radical was measured by the method designed by Scherer and Godoy.[24] About 1-mL of 0.3% DPPH solution prepared in methanol was combined with various amounts of flavonoid isolated from CC. The content was incubated at RT for 20 minutes. The absorbance of the resultant solution was obtained at 517 nm. As a control, ascorbic acid was used. The capacity of the flavonoid as a radical scavenger was represented as the EC_{50} value, described as the quantity of flavonoid needed to scavenge 50% of the DPPH radicals.

Scavenging assay for hydroxyl radical

Phenolic derivative (flavonoid) of different aliquots, EDTA (1-mM), 10 mM deoxyribose, $FeCl_3$ (5 mM), H_2O_2, including 5 mM of vitamin C in neutral buffer (50 mM, pH 7.2), was incubated for 50 minutes at room temperature.[25] 5% TCA was used to stop the reaction and the resultant outcome was noted by the reaction with 1-mL of 0.2% TBA in a hot water bath for 20 min. The absorbance was estimated at 530 nm using a proper blank. Ascorbic acid was employed as a standard reference, and radical scavenging capacity was described as an EC_{50} value.

DNA protection assay

The protective consequence of the phenolic derivative from *CC* against hydroxyl radical-induced DNA impairment was evaluated using a DNA protection assay.[26] The reaction mixture consisted of 5 µL of calf thymus (CT) DNA, 5 µL of Fenton's reagent (Composed of 0.03M H_2O_2, 0.05M ascorbic acid ,and 0.08M FeCl3), and variable amounts of phenolic derivatives (0, 5, and 10 µg/mL) (Table 5). The total volume was adjusted to 20 µL with water. The content was kept for incubation at room temperature up to 20 minutes. After incubation, 2 µL of 0.25% bromophenol blue dye was added to every sample. The reaction contents (30 µL) were later loaded onto a 1% agarose gel (prepared by boiling 1 g of agarose in 100 mL of TAE buffer, and 5 µL of ethidium bromide was added when the temperature of the agarose was 55°C.) and subjected to electrophoresis at 90 V for 1-hour. DNA bands were observed by keeping the gel on a trans-illuminator.

Scavenging of nitric oxide radical

Sodium nitroprusside was used to generate nitric oxide (NO) radical and measured using the Griess reaction.[27] Sodium nitroprusside (5 mM) spontaneously produces nitric oxide; oxygen reacts with nitric oxide to produce nitrite ions. Griess reagent was employed to detect nitrite ions. Scavengers of nitric oxide compete with oxygen, thereby reducing the formation of nitrite. Here in the assay, sodium nitroprusside (5 mM) in phosphate buffer saline was combined with variable concentrations of phenolic derivative and incubated at room temperature for 2 hours. Further, the reaction mixture was added with Griess reagent and the optical density was measured at 546 nm using a double-beam UV-vis spectrophotometer. AA was employed as a positive control. Scavenging activity of the nitric oxide radical was computed and described as an EC_{50} value, denoting the concentration needed to scavenge 50% of nitric oxide formation.

Scavenging assay of superoxide radical

The capacity of the flavonoid to scavenge the superoxide radical was determined according to the Nishikimi *et al*. method.[28] The reaction contents having various concentrations of 20 to 100 µg of phenolic derivative, 0.01M of PMS, 0.001M of NBT and 0.01M NADH in neutral buffer (0.1 M, pH 7.2) were kept at room temperature for 30 minutes. The colour reaction was documented at 560 nm by a spectrophotometer. AA was used as a reference, and radical scavenging capacity was expressed as an EC_{50} value.

Statistical Evaluation

Results were conveyed as mean ± SEM, EC_{50} values were calculated by using Graph pad prism, using non-linear regression with two parameters.

RESULTS

Yield from Different Extracts

The weight of the solid mass of different solvent extracts is given in Table 1. Among the extracts, maximum yield was found in the extract of methanol, followed by ethyl acetate, chloroform, n-hexane, and acetone extracts. The high yield in methanol extract appears to be because of the presence of ascorbic acid, phenolic derivatives and other hydrophilic molecules.

Biochemical Constituents of *C. crepidoides*

Table 2 exemplifies the relative composition of diverse bioactive constituents across various solvent extracts. Phenolics were recognized as the most plentiful bioactive compounds, with a total content of 15.23 mg/g. The highest concentration was observed in the methanol extract (9.04 ± 1.2 mg), followed by the acetone extract (4.19 ± 1.4 mg). These two extracts also demonstrated relatively high levels of phenolic derivatives (flavonoids) and ascorbic acid. α -tocopherol were detected in minute quantities, with 0.22 ± 1.1 mg in the chloroform extract and 0.05 ± 1.5 mg in the hexane extract. The presence of these active molecules is likely to add greatly to the plant's observed bioactivities.

Spectral Analysis

UV-visible spectrum

The phenolic derivative (flavonoid) exhibited maximum absorption at 250.5 nm in UV-visible spectra confirm conjugation and aromatic systems.

IR spectroscopy

A wide peak at 3300 cm^{-1} indicates the presence of OH stretching. A peak at 1342 cm^{-1} confirms the presence of

Table 1: Weight of different solvent extracts

Extracts	*Yield (in g)*
HEGC	0.9
CEGC	1.02
EAEGC	1.6
AEGC	0.24
MEGC	4.09

Table 2: Proximate constitutes of various extracts

Extracts of *C. crepidoides*	Carbohydrates	Phenolics	Flavonoids	Ascorbic acid	α- tochopherol
	Values are expressed in mg/gm.				
n-hexane	1.4 ± -1.6	-	-	-	0.05 ± 1.5
Chloroform	1.2 ± 1.5	-	-	-	0.22 ± 1.1
Ethyl acetate	2.14 ± 1.2	2 ± 1.5	1.2 ± 1.6	-	-
Acetone	2.0 ± 1.3	4.19 ± 1.4	4.01 ± 2.3	2.05 ± 2.1	-
Methanol	4.15 ± 1.8	9.04 ± 1.2	5.02 ± 1.4	4.32 ± 1.8	-
Total	10.89	15.23	10.23	6.37	0.27

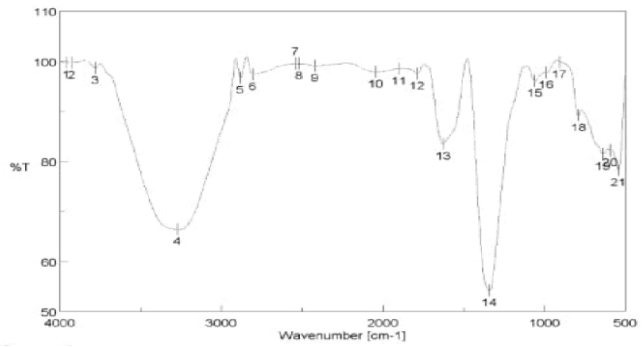

Fig. 1: IR spectra of the isolated compound

C-O stretching and a narrow peak at 1700 cm^{-1} indicates the occurrence of C=O stretching (Fig. 1 and Table 3)

LCMS: Liquid chromatography- Mass spectroscopy (Fig. 2)

m/z observed = 368.7915

m/z calculated= $[M^{+}+Na]$ = 368.0746

^{1}H-NMR (Fig. 3) and (Table 4)

δ 2.98 indicates the presence of alkyl protons (N-CH_3 protons)

δ 6.6-7.8 indicates the presence of Aromatic protons

δ 9.7 indicates the presence of aldehydic proton

^{13}C-NMR (Fig. 4)

δ 40 indicates the presence of Alkyl carbon (N-CH_3)

δ 110 to 140 indicates the presence of aromatic carbon.

Using the above spectral details, the structure of the isolated molecule may be predicted as 3-(dimethylamino)-2-hydroxy-5-(3,5,7-trihydroxychroman-2-yl)benzaldehyde (Fig. 5).

Antioxidant Activities

Scavenging assay for DPPH radical

Antioxidant activity of flavonoid in scavenging DPPH radical was moderate (EC_{50} value 66 ± 1.1 µg/mL) in comparison with the reference compound AA (EC_{50} value 61 ± 1.6 µg/mL), as revealed by Fig. 6.

Assay for hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity of a flavonoid was noted as (EC_{50} value 40.37 ± 1.32 µg/mL) when compared to that of the reference compound AA (EC_{50}value of 38.34 ± 0.9 µg/mL) as shown in Fig. 7.

Table 3: Stretching frequency observed for the proposed structure.

S. No.	*Wavenumber*	*Functional group*
1.	3300 cm^{-1}	OH stretching
2.	1700 cm^{-1}	C=O stretching
3.	1342 cm^{-1}	C-O stretching

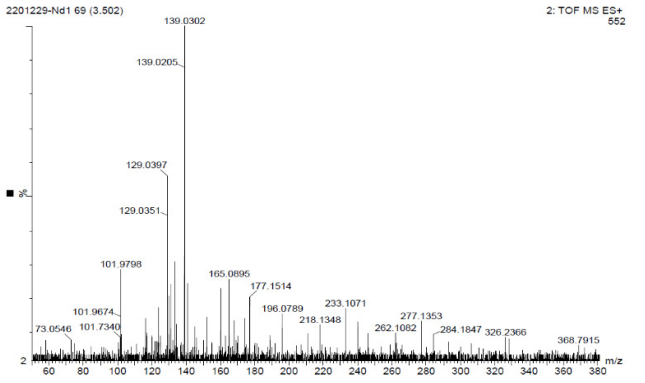

Fig. 2: LCMS spectra of the isolated compound

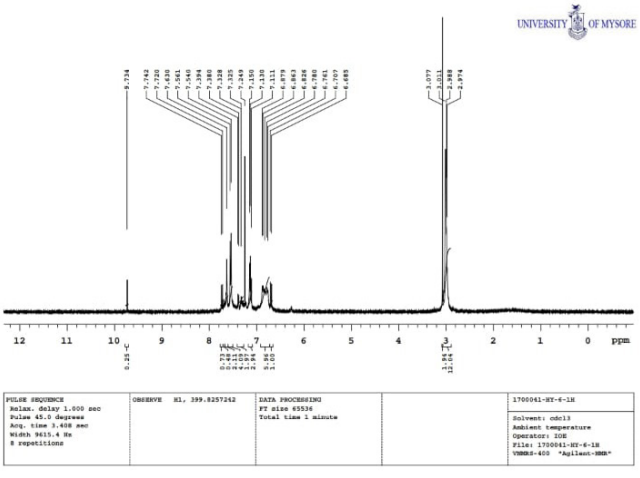

Fig. 3: Spectra of the isolated compound- ^{1}H-NMR

Table 4: ^{1}H-NMR Chemical shift observed for the proposed structure

S. No.	*Chemical shift (δ)*	*Proton type*
1.	2.98	N-methyl
2.	6.6–7.8	Aromatic
3.	9.7	Aldehyde

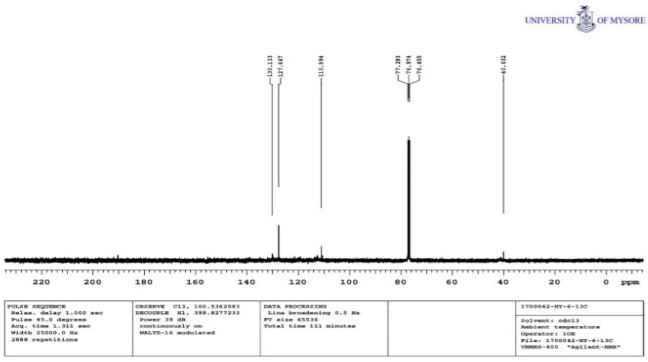

Fig. 4: Spectra of the isolated compound- ^{13}C-NMR

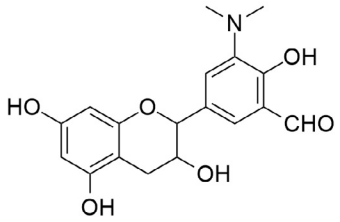

Fig. 5: Structure of the isolated molecule (Flavonoid)

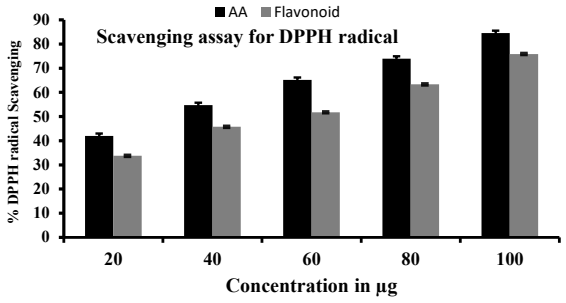

Fig. 6: Scavenging assay of DPPH radical by flavonoid and ascorbic acid

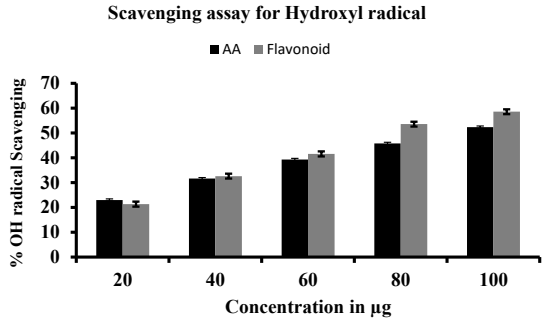

Fig. 7: Scavenging assay for hydroxyl radical by flavonoid from *C. crepidioides*

DNA protection assay

The DNA protection assay reveals the capacity of the flavonoid to protect the calf thymus (CT) DNA against hydroxyl (OH°) radicals, which damage the DNA. Fenton's reagent, when exposed to CT DNA, results in DNA strand breaks because of the generation of hydroxyl radical. CT DNA incubated using different concentrations of flavonoid (5, 10 µg) was protected from damage (Fig. 8). The protecting effect of flavonoid on CT DNA is mainly because of the scavenging ability of hydroxyl radicals.

Assay for nitric oxide radical scavenging activity

Nitric oxide radical scavenging activity of flavonoid appears to be moderate with (EC_{50} value 54 ± 0.8 µg/mL) in contrast to that of the reference standard AA (EC_{50} value 50 ± 1.23 µg/mL) as shown in Fig. 9.

Scavenging assay for superoxide radical

Flavonoid proved insignificant in scavenging superoxide radicals by having an EC_{50} value of 75.91 ± 1.1 µg/mL when compared to AA with an EC_{50} value of 53.61 ± 0.8 µg/mL, as illustrated in Fig. 10.

It is noticed that, though flavonoid from *CC* showed scavenging activity of DPPH, hydroxyl, nitric oxide and superoxide radical, based on the T-test analysis, there was no statistically significant difference ($p > 0.05$) in the EC_{50} value between standard ascorbic acid and the flavonoid. Therefore, we fail to reject the null hypothesis. The two-tailed *p-value* equals 0.3909.

Anti-inflammatory Activity (*In-vitro*)

Indirect haemolytic assay/PLA2 Inhibitory assay

With an increase in the concentration of flavonoid, the inhibition of PLA_2 of *V. russeli* also increased, with an EC_{50} value of 31.77 ± 1.3 µg/mL. Inhibition appears to be dose-dependent, as depicted in Fig. 11.

Angiogenic assay

The inhibition of vasculature by flavonoid is as displayed in Fig. 12. The investigation of antiangiogenic activity of flavonoid, a bioactive compound from the methanol extract of *C. crepidiodes* exhibiting a noticeable decrease in the formation of blood vessels around the disc loaded with the flavonoid when compared to a control, which reveals the potency of flavonoid in regulating angiogenesis. Though the antiangiogenic effect of 3-(dimethylamino)-2-hydroxy-5-(3,5,7-trihydroxychroman-2-yl)benzaldehyde was proved using *in-vivo* CAM assay, further confirmation requires experiments using animal models.

Discussion

Flavonoids exert antioxidant activity primarily by removing free radicals such as 2,2-diphenyl-1-picrylhydrazyl (DPPH). When a DPPH radical encounters a molecule capable of giving a hydrogen atom or electron,

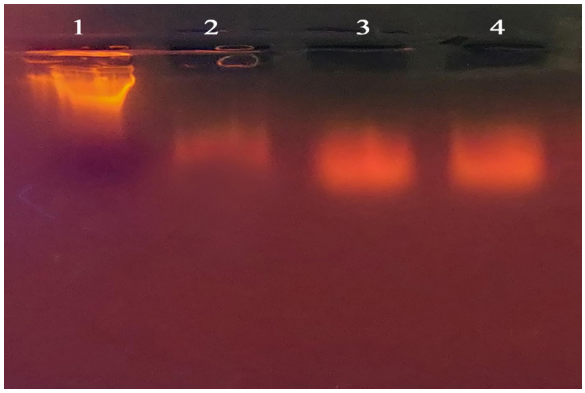

Fig. 8: CT DNA as observed under UV illuminator of gel documentation instrument.

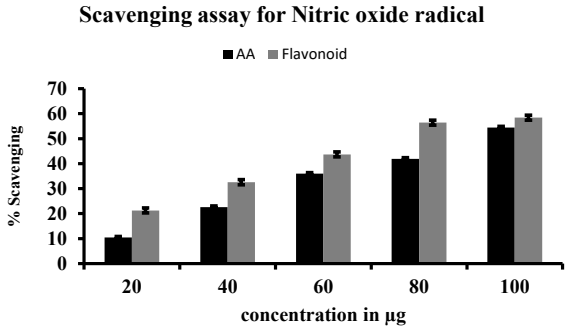

Fig. 9: Scavenging assay for nitric oxide radical by flavonoid from *C. crepidioides*

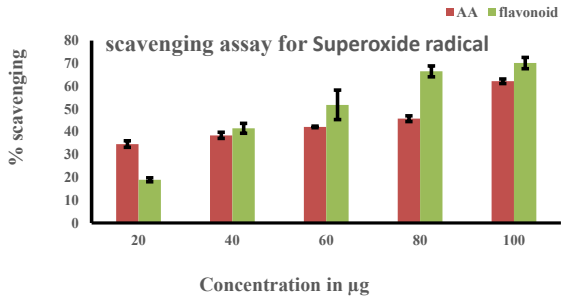

Fig. 10: Scavenging assay for superoxide radical by flavonoid from *C. crepidioides*

it undergoes reduction. Flavonoids possessing many hydroxyl groups on the B-ring generally exhibit strong antioxidant activities.[29] In the present study, the isolated flavonoid demonstrated marginal DPPH radical scavenging activity when compared to ascorbic acid (AA), a standard antioxidant, which may be credited to the presence of only a single hydroxyl group in the B-ring. Structural features of the B and C rings largely determine the hydroxyl radical (•OH) scavenging potential of flavonoids. The existence of a tertiary amine group in the B ring further enhances this activity by stabilizing reactive oxygen species.[30]

Flavonoids also scavenge nitric oxide (NO•) radicals primarily through single electron transfer (SET) and hydrogen atom transfer (HAT) mechanisms. This

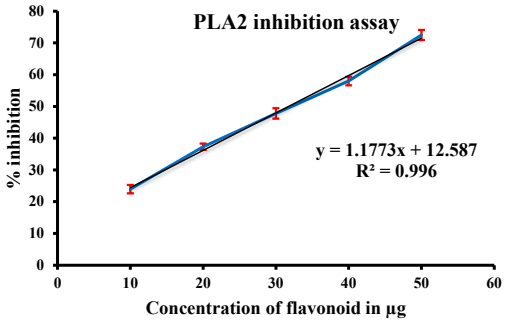

Fig. 11: Inhibition of PLA_2 by a flavonoid from *C. crepidioides*
Note: Values are the average of 3 separate experiments.

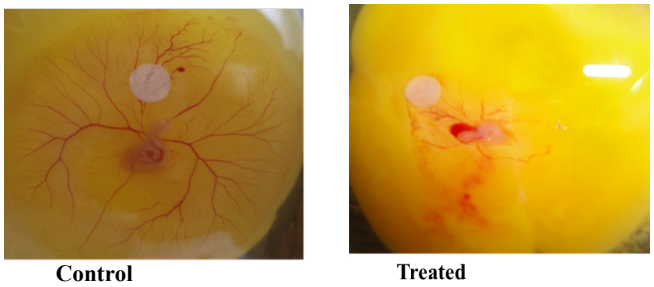

Fig. 12: Shell less CAM assay showing disappearance of vasculature around the disc loaded with flavonoid

antioxidant property contributes to their beneficial effects in various physiological processes, including the attenuation of inflammation and improvement of endothelial function.[31] Additionally, flavonoids effectively scavenge superoxide anion radicals (O_2^-) via direct electron transfer, converting them into hydrogen peroxide (H_2O_2). This reaction is facilitated by the hydroxyl group at the C3 position of the flavonoid structure, which acts as a hydrogen donor.[32] As the antioxidant activity of the isolated flavonoid molecule is similar to that of ascorbic acid, which relates to specific structural features. Biological activity confirms the presence of a phenolic hydroxyl group, which behaves like the engine of antioxidant activity; it further confirms the presence of conjugated double bonds, and these help stabilize the compound after it has donated an electron, preventing it from becoming a dangerous radical itself. The flavonoid molecules are more stable over time, even at different temperatures, than ascorbic acid, which degrades easily and is temperature sensitive. In this context, an isolated flavonoid has high therapeutic and preservative potential. Flavonoids can inhibit the activity of phospholipase A2 (PLA_2), a key enzyme that begins the inflammatory responses through the release of arachidonic acid and lysophospholipids. They exert this effect by directly binding to the enzyme, potentially altering its conformation or interfering with its active site. *In-silico* studies suggest that the interaction at the C6 position of the flavonoid scaffold plays a critical role in determining its affinity, selectivity, and inhibitory potency toward PLA_2.[33] Diosmin is a

Table 5: Different components loaded into four wells of agarose gel electrophoresis

S. No.	*Components*
1.	20 µg CT DNA+ loading dye
2.	Fenton's reagent + CT DNA (20 µg)
3.	5 µg of flavonoid+ Fenton's reagent + CT DNA
4.	20 µg CT DNA + 10µg of flavonoid +Fenton's reagent + CT DNA

naturally occurring plant flavonoid compound, primarily found in citrus fruits inhibited *Daboia russelii* venom PLA_2s.[34] Morelloflavone, a flavanone- (C-3 C -8″)-flavone biflavonoid, from *Garcinia madruno*, is reported to inhibit the venom PLA_2.[35] Quercetin-3-O-α-rhamnoside is a flavonoid separated from *Euphorbia hirta* and is known to inhibit the PLA_2.[36]

Flavonoids also modulate key signaling pathways involved in angiogenesis. They interfere with the mitogen-activated protein kinase pathway, which controls cellular growth and differentiation, including endothelial cell proliferation. Additionally, they impact the phosphoinositide 3-kinase pathway, which governs cell survival, proliferation, and migration—processes essential to angiogenesis.[37] Tricin, namely 4′,5,7-trihydroxy-3′,5′-dimethoxyflavone, found in navara rice and wheat varities inhibits the proliferation of blood vessels triggered by VEGF by downregulating the signal transduction by VEGFR2 and also by averting the accumulation of HIF-1α in tumor cells. [38-40] Cremastranone is a natural homoisoflavanone that has been isolated from the rhizome of plants belonging to the Asparagaceae and Orchidaceae families. The compound effectively inhibits neovascularization in models by reducing the proliferation of endothelial cells (HUVECs) and showing potent antiangiogenic effects in animal studies. [41] In the present study, the isolated molecule 3-(dimethylamino)-2-hydroxy-5-(3,5,7-trihydroxychroman-2-yl)benzaldehyde is a flavonol with a key biological activity. The fundamental C6-C3-C6 structure allows it to interact with various intracellular and extracellular targets. The presence and position of multiple hydroxyl (-OH) groups are crucial for its antioxidant and anti-inflammatory properties, enabling it to modulate crucial cellular pathways and scavenge reactive oxygen species (ROS). Antiangiogenic activity of the flavonols increased with the lipophilicity of the molecule.[42] The proposed structure has two methyl groups in the B ring, which increase its lipophilicity and thereby its antiangiogenic activity. Lipophilicity allows the molecule cross the cell membranes easily, allowing it to reach intracellular targets like HIF-1α. The –OH group at the 3- position in the C-ring is vital in the proposed structure. It is a distinguishing feature of flavonols like kaempferol and quercetin and is strongly associated with their ability to inhibit kinases like PI3K and VEGFR2. [43]

Conclusion

The results of this study demonstrate that the purified compound separated from the methanolic extract of the aerial parts of *C. crepidioides* possesses significant antioxidant, anti-inflammatory, and antiangiogenic properties. These bioactivities suggest that the plant represents a valuable source of naturally occurring therapeutic molecules with potential pharmacological applications. The results further highlight the promise of plant-based compounds as alternatives or adjuncts to synthetic drugs, offering benefits such as reduced toxicity and better biocompatibility to reduce the burden of snakebite envenomation. Plant-derived molecules may serve as effective adjuncts to conventional antivenoms, addressing issues of cost, availability, and side effects. Further kinetic and *in-silico* studies on PLA_2 inhibition are needed to clarify the underlying mechanisms and pathways involved.

Though the CAM assay reveals the potency of flavonoids to inhibit angiogenesis, the percentage of inhibition cannot be calculated by calculating the microvessel density using an image processor; the results are based on visual observation. Further animal-based experiments need to be carried out to confirm the same.

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