Natural Resources for Human Health
eISSN: 2583-1194

Original Research

Received 08 May 2026
Revised 10 June 2026
Accepted 05 July 2026

Natural Resources for Human Health 2026, Vol. 6 (4s): 01–19

KEYWORDS:
Chamabainia cuspidata, Phytochemicals, Quercetin, Anti-inflammatory activity

Exploration of Phytoconstituents and In-Vitro Pharmacological Activities of Chamabiania Cuspidata Leaves from Himalayan Regions

Panshul Sharma[*1], Nitin Verma[1]

[1]Chitkara School of Pharmacy Chitkara University, Solan Himachal Pradesh, India-174103

ABSTRACT:

Background: Inflammation remain significant global health challenges, necessitating the exploration of safer plant-based therapeutic agents. *Chamabainia cuspidata*, a Himalayan medicinal plant has been traditionally used but lacks comprehensive scientific validation.

Objectives: The present study aimed to investigate the phytochemical composition and evaluate the in-vitro anti-inflammatory potential of *Chamabainia cuspidata* leaves.

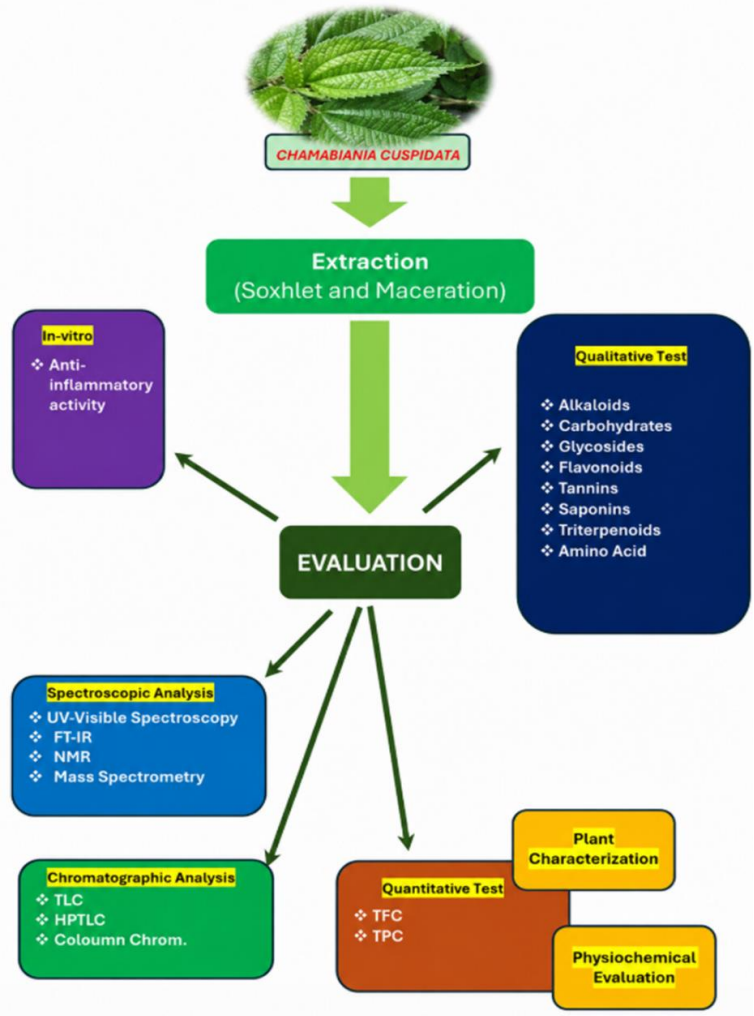
Methods: Ethanolic extracts were subjected to pharmacognostical, physicochemical, and phytochemical evaluation following WHO guidelines. Chromatographic profiling was performed using TLC and HPTLC with quercetin as a marker compound. Anti-inflammatory activity was assessed using protein denaturation and membrane stabilization assays.

Results: The extract demonstrated the presence of major phytoconstituents including alkaloids, glycosides, phenolics, tannins, saponins, triterpenoids and steroids. Total phenolic and flavonoid contents were found to be 17.24 mg GAE g^{-1} and 266.30 mg RE g^{-1}, respectively. The extract exhibited concentration-dependent inhibition of protein denaturation and membrane haemolysis along with significant inhibition of calcium oxalate crystal formation and aggregation.

Conclusion: The findings suggest that *Chamabainia cuspidata* possesses moderate anti-inflammatory supporting its traditional use and highlighting its promise as a source of bioactive compounds for therapeutic applications.

Natural Resources for Human Health
eISSN: 2583-1194

Graphical Abstract



1. INTRODUCTION

Medicinal plants have been extremely important in healthcare since ancient times when early civilization like Indus valuable were created by the Indus valley. The Ayurveda, Unani and Siddha systems of traditional medicine have a long history of utilizing plant-based medicine to treat diseases. One of the most biodiverse countries is India having some 45,000 plant species, about 15,000 of which have known medicinal actions. An estimate of 2000 species is used solely in Ayurveda and other traditional systems are also heavily reliant on herbs. Medicines based on plants obtain various parts of the plant, such as leaves, roots, stems, bark, flowers, and seeds & also plant exudates gums or resins. These are natural resources that are still used to promote both the traditional and modern healthcare practices **[1, 2]**. Medicinal plants are the main source of medication utilized globally with a higher percentage in developing nations, where a senior percentage of the population relies on using herbs as the first healthcare choice. In contemporary medicine, numerous drugs are based on the compounds in plants or are based on them **[3]**. The pharmacological rich Indian flora is spread throughout various parts of the country like the Eastern Himalayas, Western Ghats and the Andaman and Nicobar Islands. Though just about 3000 species of plants are officially listed as being used medicinally, traditional healers use many more. Himachal Pradesh, a province in the Western Himalayas is especially identified with a wide range of medicinal plant resources owing to, its unique climatic and ecological conditions. Nonetheless, overexploitation, loss of habitats and climate change pose threats to these useful plant species and hence the need to protect and scientifically prove their value **[4]**.

In recent years the growing interest in herbal medicines has been on their being safer and cheaper than synthetic drugs. These natural remedies have been popularly employed in treating diseases like inflammation, metabolism and chronic illnesses. Modern methods of analysis have led to better insights into plant bioactive compounds of plant origin and their mode of action. Though important, various medicinal plants have not been explored scientifically. One such plant that has limited phytochemical and pharmacological information is *Chamabainia cuspidata (CC)* commonly referred to as Hayata, a Himalayan plant **[5]**. This uncertainty in terms of scientific proof demonstrates the important gap in research. Inflammation is an important biological mechanism linked to a number of chronic conditions whereas urolithiasis is the formation of calcium oxalate crystals in the urinary system. Compounds which act as natural inhibitors of these processes are of great therapeutic interest. Thus, the goal of this work is to evaluate phytochemical properties of *CC* leaf, along with anti-inflammatory and anti-urolithiatic properties in-vitro **[6-8]**.

2. MATERIALS AND METHODS

2.1 Collection of the samples

CC leaves data was gathered from Bali Chowki from Himachal Pradesh (31.68425° N, 77.285498° E). The collected plant parts were identified by Mr. Kumar Ambrish, Scientist "F" Botanical survey of India, Solan (Accession No: 743_Date: 29-07-2024). After authentication, the plant leaves were thoroughly cleaned, dried, and ground into a fine powder for further analysis.

2.2 Plant extract preparation

The dried leaves of *CC* were manually reduced into fine powder with the help of a mechanical grinder. Solvents were used to extract the bioactive phytoconstituents in the form of powdered material using two methods Soxhlet extraction and maceration. Regarding Soxhlet extraction, the powdered sample was put in a thimble and extracted in ethanol using a Soxhlet apparatus **[9]**. The solvent was left to boil so that the solvent would continue to evaporate and condense in order to percolate through the plant materials many times. This was done to provide an efficient soluble compound extraction and this was repeated until the solvent is clear which is an indication of completion **[10]**. A rotary vacuum evaporator was used to concentrate the obtained extract to eliminate the excess solvent and provide a crude extract. The maceration technique was done by soaking the powdered leaves in ethanol or aqueous solution (24-72 hrs) and stirring it periodically to maximize the relation between the solution and the extract. When this was done, we filtered the mixture in order to get the residue and the extract. A rotary evaporator was then used to concentrate the filtrate which was then kept in airtight containers at 4^{o}C until analysis **[11]**

2.3 Plant Characterization

2.3.1 Macroscopical Characterization

The *CC* had their fresh leaves studied on visual observations to note the macroscopic features. Such parameters as colour, odour, taste, texture, size and overall morphology were incorporated into the evaluation. The breath of the leaves was found to be green in colour having typical scent and flavour. They had ovate to lanceolate form with serrated edges and acute tip. The paper was a bit scratched with the presence of tiny hairs **[12]**.

2.3.2 Microscopical Characterization

Sectioning: Microscopic observation of *CC* leaves was conducted to examine the detailed anatomical and surface characteristics required to determine identifying and standardization of leaves. The study employed FESEM to perform the analysis which helps see microstructural features in high resolution **[13]**.

Leaf Microscopy: In the case of leaf microscopy, properly prepared samples were observed on using the various magnifications. Observations made showed unique epidermal cells, stomata and surface appendages. Stomata were very prominent and diagnosed as diacytic type which had subsidiary cells perpendicular to the guard cells. Existence of crystal cells and vascular elements on the leaf surface also depict the tissue being structurally organized. Furthermore, the rhizome-like structures were also observed on the surface of the leaves that were linked to the pollen grains **[14]**.

Powder Microscopy: Microscopy was done on powdered dried leaf material which was finely powdered. Characteristic features were observed in the powdered sample as fragmented cellular structures, microcrystalline components, calcium oxalate

crystals, plant wax and isolated trichomes. These are microscopic diagnostic characters that are important in authentication, quality control and pharmacognostic standardization of the plant material **[15]**.

2.4 Physicochemical Evaluation

To monitor the quality, purity and consistency of the crude drug, physicochemical analysis of powdered leaf material of *CC* was conducted in agreement with standard procedures as suggested by the WHO guidelines. The measured parameters were total ash, soluble ash in water, insoluble ash in water, extractive values of various solvents, water content, pH, foaming index and the foreign organic matter. The presence of any inorganic residues and impurities was measured by determining ash values **[16]**. The level of solvency of the solvents like water, chloroform, ether and ethanol were determined to estimate the content of active constituents in solvents. The moisture content was also quantified in order to know the stability and vulnerability of the plant material to microbial infestation and pH was also registered in order to know the chemical nature of it. Other parameters like foaming index and foreign organic matter were also measured to analyse the presence of saponins and extraneous material, respectively. These physicochemical constants are required as the basic criteria of identification, standardization and control of quality of the material of *CC* leaf matter **[17]**.

2.5 Qualitative phytochemical screening

Preliminary phytochemical screening of *CC* ethanolic extract was conducted using standard chemical tests to detect alkaloids, glycosides, flavonoids, tannins, phenolics, saponins and triterpenoids, confirming the presence of major bioactive secondary metabolites **[18]**.

2.5.1 Test for Alkaloids

The extract was dissolved in a known amount of dilute hydrochloric acid and then filtered to remove insoluble particles. The filtrate was split into three and the three portions treated separately with Hager reagent with the reagent of Dragendroff and that of Mayer. Alkaloids were confirmed by the development of a yellow colour precipitate with the Hager reagent, orange-brown precipitate with the Dragendroff reagent and cream-coloured precipitate with Mayer reagent. Such reactions are evidence of the creation of insoluble complexes between the alkaloids and the reagents **[19, 20]**.

2.5.2 Test for Carbohydrates

Molisch test was conducted by placing the extract in a test tube with enough drops of Molisch reagent were added to it and then carefully Concentrated sulfuric acid was introduced along the side of the test tube, resulting in the formation of a violet ring at the interface was a sign that showed the presence of carbohydrates. To work on the reduction of sugars, the extract was heated using suitable reagents which led to the development of a coloured precipitate. Hydrolysates of the extract detected non-reducing sugars which were then tested again **[21, 22]**.

2.5.3 Test for Glycosides

This extract was hydrolysed using the dilute acid and extracted using the chloroform. The chloroform layer was broken off and subjected to ammonia solution. Glycosides were identified in the presence of a pink or reddish colouration. Further confirmation was done with concentrate sulfuric acid and aqueous sodium hydroxide as they yielded typical colour changes **[23, 24]**.

2.5.4 Test for Flavonoids

The alkaline reagent was added on the extract to produce the yellow colouration. When dilute acid was added, the colour faded away which was evidence of the presence of flavonoids. Additional identification was done by testing the extract using concentrated sulfuric acid and ferric chloride solution that generated specific colour changes that were characteristic of flavonoid compounds **[25, 26]**.

2.5.5 Test for Tannins

A part of the extract was mixed with gelatine solution and the precipitate was formed; this was an indication of the presence of the tannins. Also, when sodium hydroxide solution of 10 per cent was added, the solution turned a distinct colour adding more confirmation to the presence of tannins as the solution could form complexes with proteins and alkali **[27, 28]**.

2.5.6 Test for Saponins

In the foam test, distilled water was equitably stirred in a graduated cylinder. The fact that stable and persistent froth was formed and lasted a few more minutes showed the presence of saponins which are characterized to have surfactant properties **[29, 30]**.

2.5.7 Test for Triterpenoids

Chloroform was added to the extract, followed by careful addition of concentrated sulfuric acid along the right side of the test tube. The formation of a reddish-brown coloration at the interface confirmed the reaction which proved the occurrence of triterpenoids in the reaction of unsaturated compounds with sulfuric acid **[31, 32]**.

2.5.8 Test for Proteins and Amino Acids

In the case of amino acids, the extract was incubated with Ninhydrin reagent and heated, producing a violet tint. In the case of proteins, the extract was prepared with the addition of the reagent suggested by Millon which gave a reddish colouration during heating, validating the existence of proteinaceous substances **[33, 34]**.

2.5.9 Test for Phenolic Compounds

The extract was reacted with iodine solution and ferric chloride solution respectively. The formation of typical colour changes e.g. blue, green or black colouration was indicative of phenolic compounds as a result of their capacity to be complexed with metal ions **[35]**.

2.5.10 Test for Phytosterols

Salkowski test and Hesse test were used to detect phytosterols. Salkowski test entailed the use of a chloroform and concentrated sulfuric acid on the extract, creating a change in colour. The presence of phytosterols was confirmed in a test conducted by Hesse in which there were particular colour reactions **[36]**.

2.5.11 Test for Steroids

The identification of steroids was achieved by adding sulphuric acid to the extract and chloroform and then the added acid was used to titrate. Colour changes typical of steroidal compounds like greenish or bluish colouration appeared and were used to test the presence of steroidal compounds **[37]**.

2.6 Quantitative phytochemical analysis

2.6.1 Total phenolic content (TPC) determination

The TPC of the ethanolic extract was determined using the Folin-Ciocalteu colorimetric method. *CC.* A 0.2 mL sample of the plant extract was taken, and 2.5 mL of Folin-Ciocalteu reagent was added, then gently mixed to initiate the reaction. The mixture was left to sit for about 5 minutes. Subsequently, 2 mL of 7.5% sodium carbonate solution was added to develop colour. The total volume was adjusted to 7 mL with distilled water. The mixture was incubated in the dark at 25°C for approximately 90 minutes to ensure thorough interaction between phenolic compounds and the reagent. After incubation, absorbance was measured at 760 nm using a UV-Visible spectrophotometer. A calibration curve was created using gallic acid standards in the range of 20-100 µg/mL. The phenolic content was then expressed as milligrams of gallic acid equivalents (GAE) per gram of dried plant extract. **[38]**.

2.6.2 Total flavonoid content (TFC) determination

The total flavonoid content of the ethanolic extract of the CC was determined using the aluminium chloride colorimetric method. Briefly, 0.5 mL of the plant extract was mixed with 0.15 mL of 5% sodium nitrite ($NaNO_2$) and 0.15 mL of 10% aluminium chloride ($AlCl_3$). The reaction mixture was left to stand for 5 minutes to allow sufficient interaction. Then, 2 mL of 4% sodium hydroxide (NaOH) was added, and the total volume was adjusted to 5 mL with distilled water. The mixture was thoroughly mixed and incubated to develop the colour fully. The absorbance was measured at 510 nm using a UV-Visible spectrophotometer, compared against a reagent blank. A calibration curve was prepared for rutin concentrations ranging from 20 to 100 µg/mL. Results were expressed as milligrams of rutin equivalents (RE) per gram of dried plant extract [39].

2.7 Chromatographic analysis

2.7.1 Thin Layer Chromatography

To study the pattern of separation of the constituents that are found in *CC* extract, the test was done using the thin layer chromatographic analysis. Silica gel 60 F_{254} pre-coated plates with a thickness of 0.2 mm were selected as the adsorbent medium. Using fine capillary tubes, the preferred amounts of the sample solution were mixed onto the plate in discrete spots and left to dry in ambient conditions. To achieve development, the plates were moved into a chromatographic modulus that had a solvent system that had been equilibrated. Capillary movement in the stationary phase, in turn, allowed the solvent front to move upwards, allowing the sample components to migrate differently. Distribution of compounds was controlled by their different affinities to the adsorbent layer as well as their differing other-solubility in the mobile phase. Different polarities in components produced distinct migration patterns that resulted in a resolution of the components across the plate under controlled conditions **[40, 41]**.

2.7.2 High-Performance Thin Layer Chromatography (HPTLC) analysis

CC extract was characterized by HPTLC. The stationary phase was made up of aluminium plates pre-coated with silica gel 60 F_{254}. Standard quercetin and sample solutions (2-10 µL) were used as bands with an automatic applicator. The mobile stage comprised toluene: ethyl acetate: formic acid: methanol (2.5:1.5:0.5:0.5). The plate development was performed under controlled conditions in a pre-saturated chamber. Plates were dried after the development and subjected to a densitometric scan at 254 nm and 366 nm. Peaks were noted on a chromatograph and the Rf values were compared to the standard. The Rf values of quercetin and its peak features also matched with those of the sample, leading to the confirmation of the presence of quercetin in the sample **[42]**.

2.8 Isolation of bioactive compounds

Isolation of bioactive compounds in *CC* has done through silica gel column chromatography. As the stationary phase, a glass column (250 × 30 mm) filled with silica gel (60-120 mesh) was used. The column was first washed using acetone, dried and packed under wet process of slurry. Ethanolic extract, 1-2 g of the extract was absorbed onto the column. Elution was done with a solvent system of toluene and ethyl acetate (8.2:1.8, v/v) under isocratic conditions. Fractions were gathered in sequence, concentrated and TLC analysed. The visualization was performed (UV light: 254 and 365 nm and visible light) and phytochemical tests were assessed to detect active constituents **[43]**.

2.9 Spectroscopic analysis

2.9.1 UV-Visible Spectroscopy

An analysis of the isolated fraction of *CC* was done using a UV spectrophotometer (Shimadzu UV-1700). The sample was scanned with a wavelength between 200-800 nm and the peaks of the absorption were noted. The spectra obtained were informative about the presence of conjugated systems and chromophoric groups **[44]**.

2.9.2 Infrared (FT-IR) Spectroscopy

FT-IR analysis was carried out to identify the functional groups that were present in the fraction that had been isolated. Potassium bromide (KBr) was added to the sample to a fine powder and pressed into a pellet. An IR spectrometer was utilized to discern characteristic functional groups by obtaining FT-IR spectrum with the assistance of Thermo Nicolet Model 6700 spectrometer in a scanning range of 400-4000 cm^{-1} **[8, 45]**.

2.9.3 Nuclear Magnetic Resonance (NMR) Spectroscopy

The NMR spectroscopy was performed to further clarify the feature structure of the compounds found in the isolated fraction of *CC*. A JNM EC-500 NMR spectrometer was used to carry out the analysis. The resulting spectra were very specific and the environment of the hydrogen and carbon in the molecule **[34]**.

2.9.4 Mass Spectrometry

Mass spectrometric analysis was performed on a microTOF-Q system to detect the mass of the different compounds found in the fraction isolated. The solution was the ionization of molecules and subsequent separation based on the mass-to-charge ratio (m/z) that allowed determining the molecular mass and structural information **[24]**.

2.10 Biological Activity

2.10.1 Anti-inflammatory activity

2.10.1.1 Protein denaturation assay

The anti-inflammatory effect was determined by modified bovine serum albumin (BSA) denaturation procedure. Tri's buffer was used to make a 0.4% BSA solution (pH adjusted to 6.4). BSA solution (1 mL) was combined with varying extract concentrations (50-250 µg/mL). Aspirin was the standard and solvent was a control. The mixtures of the reactions were heated at 72 ^{o}C in 10 minutes and cooled in 20 minutes. The turbidity was measured at 660 nm using a spectrophotometer. Inhibition of the denaturation of proteins was calculated as percent inhibition against control and IC_{50} was determined by graphical differentiation of the inhibition verses concentration **[46, 47]**.

2.10.1.2 Membrane stabilization assay

Membrane stabilizing activity was determined by the analysis of the fluid hypotonic solution at the expense of the human erythrocytes. Bloods were taken in EDTA tubes and centrifuged to get erythrocytes washed and reconstituted as 40 % suspension. The reagent mixture was placed with 0.5 mL of RBC suspension and 5 mL of hypotonic solution in extract concentration (50-250 µg/mL) or aspirin normal. Incubations lasted 10 minutes after which the samples were centrifuged and The absorbance of the supernatant was measured at 540 nm. The inhibition percentage of hemolysis was determined and the IC_{50} values were acquired out of the inhibition curve **[48-50]**.

3. RESULTS

The WHO recognizes medicinal plants as valuable sources of therapeutic agents in traditional medicine. However, proper identification and standardization are crucial for guaranteeing their quality, safety, and effectiveness. Therefore, pharmacognostical evaluation plays a crucial role in authenticating *CC*.

3.1 Plant Characterization

3.1.1 Macroscopical evaluation

The plant exhibited greenish leaves with a characteristic odour and taste. The surface was rough in texture. Leaves were elongated, ovate to elliptic in shape with sharply serrated margins, measuring approximately 10-20 cm in length. The stem was slender and ascending in nature. The flowers were greenish-red, sessile and measured about 1-2 cm in length. These macroscopic characteristics are important for the identification and authentication of *CC* (Table 1).

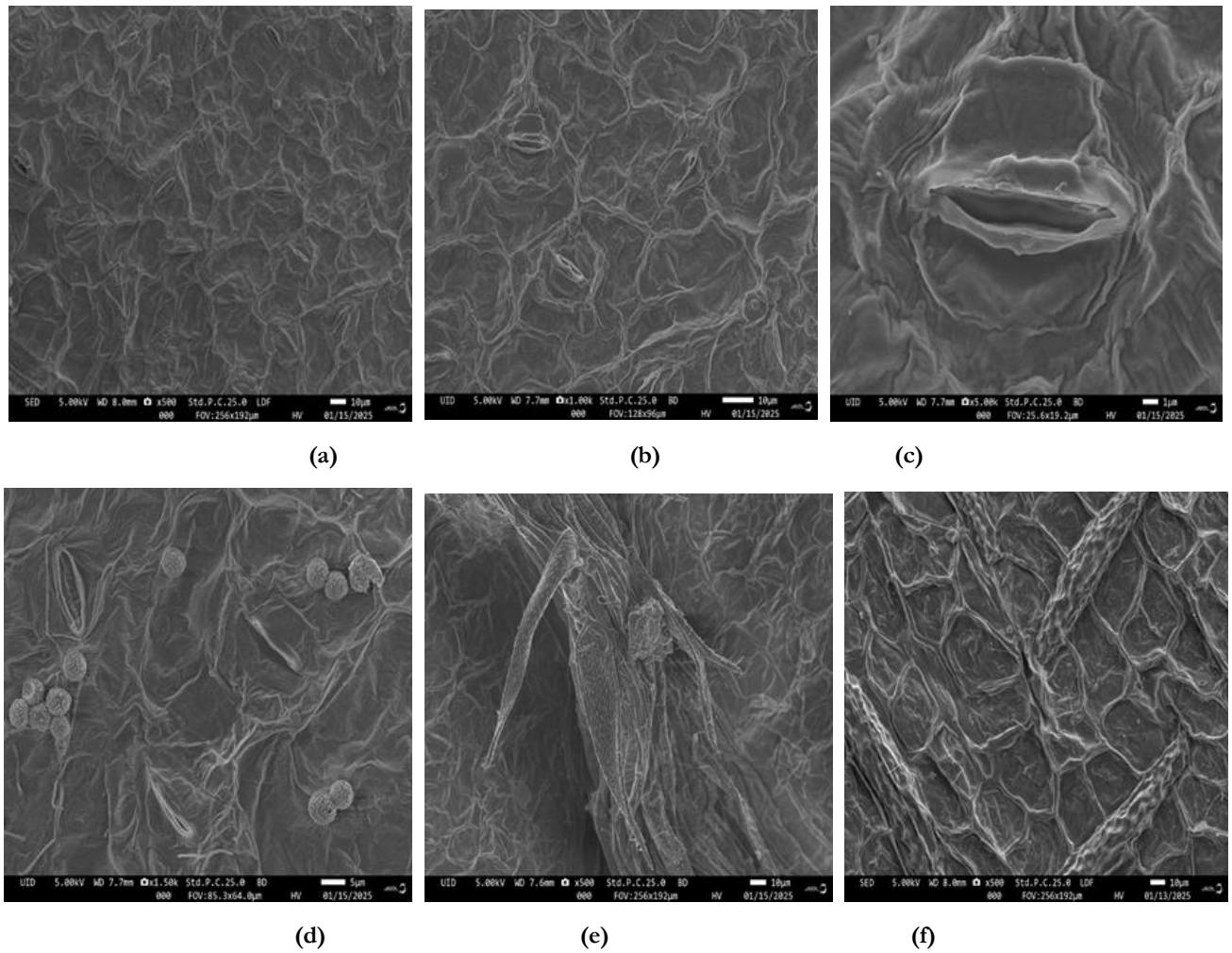
Table 1: Macroscopical Parameters of *CC*

]	**Observation**
Colour	Greenish
Odour	Characteristics
Taste	Characteristics
Texture	Rough
Length	10-20 cm long
Leaves	Leaves are long ovate and elliptic; margins are acutely serrate
Flowers	Flowers are greenish red, sessile, 1-2 cm long
Stem	Stems are slender, ascending

3.1.2 Microscopical evaluation

Leaf: Microscopical examination of *CC* leaves using FESEM revealed distinct surface characteristics, including epidermal cells, stomata, trichomes and associated pollen grains. The stomata were identified as diacytic type with subsidiary cells arranged perpendicular to the guard cells. Detailed imaging at different magnifications confirmed the presence of trichomes and crystal-containing cells on the leaf surface. These features contribute to the anatomical identification of the plant material (shown in Fig. 1).

Powder: Powder microscopy of *CC* showed the presence of characteristic structures such as fragmented epidermal cells, trichomes, calcium oxalate crystals, pollen grains and rhizome-like structures. These diagnostic components observed in the powdered drug is useful for authentication and quality evaluation of the plant (shown in Fig. 2).



(a) **(b)** **(c)**

(d) **(e)** **(f)**

Fig 1: Samples of leaf surfaces showing (a) Chamabiania leaf surface (b) Stomata are seen (c) Single diacytic stomata (d) Crystals cells (e) Rhizomes present in leaf (f) Vascular bundles

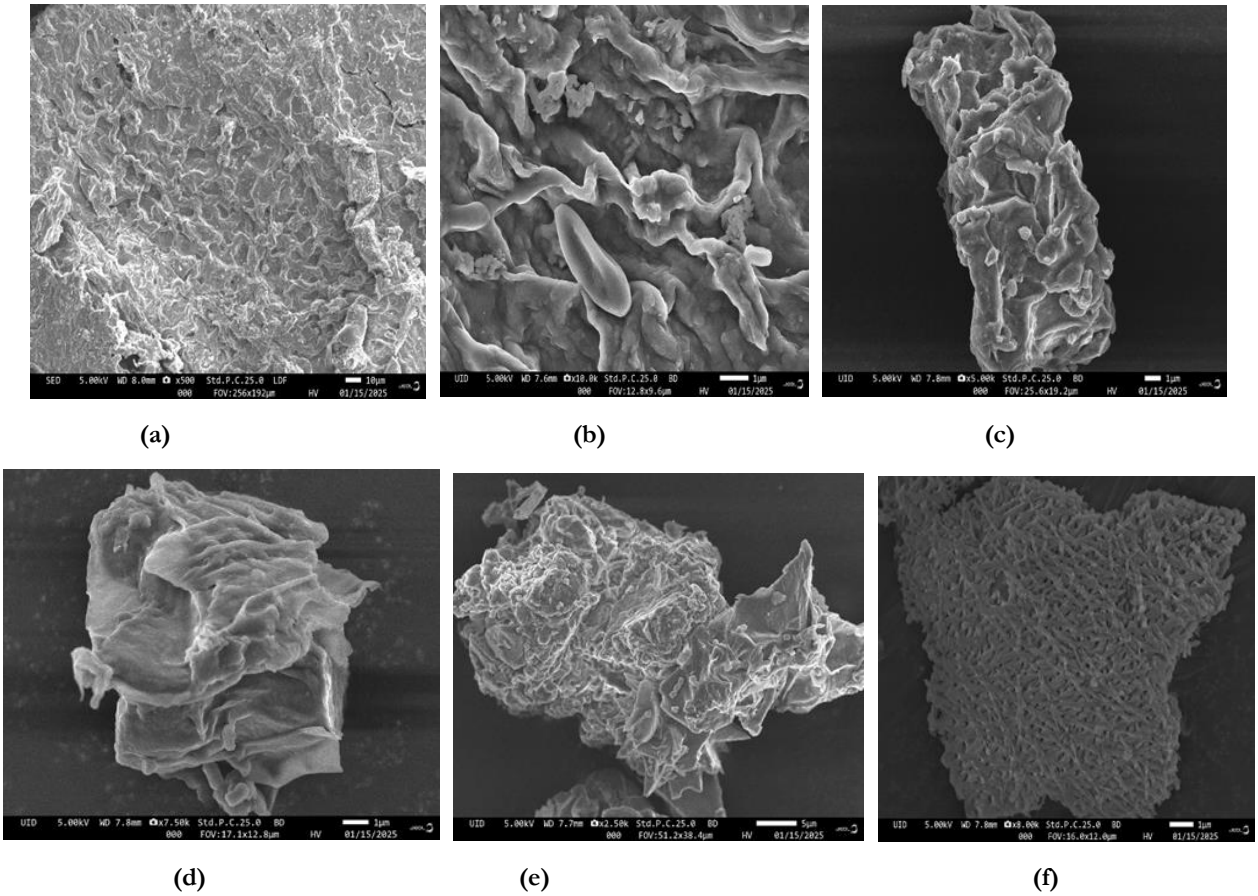


Fig 2: Samples of powdered drug showing (a) fracture surface (b) cellular structures (c) microcrystalline cellulose (d) crystal cell (e) calcium oxalate cell (f) plant wax seen

3.2 Physicochemical Evaluation

The physicochemical parameter values are useful for Assessing the quality and purity of the raw drug (Table 2).

Table 2: Physicochemical Parameters of CC

Sr. No.	Parameters	Observations
1.	Ash Value	7.5%
	Water soluble ash value	8%
	Acid insoluble ash value	9%
2.	**Extractive Values**	
	For Water	2%
	For Chloroform	3%
	For Ether	4%
	For Ethanol	1%
3.	Foaming Index	0.24
4.	pH	9-12
5.	Moisture Content	11%

6.	**Heavy Metal analysis**	**(mg/kg)**
	Zinc	36.59
	Iron	831.18
	Manganese	80.04
	Copper	11.39
	Nickel	2.53
	Cadmium	0.45
	Lead	1.09
	Arsenic	2.84
7.	**Foreign Organic Matters**	0-1%

3.3 Qualitative phytochemical screening

Preliminary qualitative phytochemical evaluation was done on the *CC* alcoholic extracts to identify the occurrence of principal phytoconstituents like alkaloids, carbohydrates, glycosides, flavonoids, tannins, phenolic compounds, saponins, terpenoids, proteins and steroids (shown in Table 3).

Table 3: Phytochemical Evaluation of CC

Tests	**Test Name**	**Alcoholic Extract**
Test for alkaloids	Hager's	
	Dragendorff's	
	Mayer's reagent	+
Test for Carbohydrates	Molisch	
	Reducing sugars	-
	Non-reducing sugars	
Test for Glycosides	Borntrager's	+
	Conc. H_2SO_4	
	Aqueous NaOH	
Test for Flavonoids	Alkaline reagent	
	Conc. H_2SO_4	-
	$FeCl_3$	
Test for Tannins	Gelatin	
	10% test	+
Test for Saponin	Foam	
Test for Triterpenoids	Salkowski	
Test for Proteins and Amino acids	Ninhydrin	-

	Millon's	
Test for Phenolic Compounds	Iodine	+
	$FeCl_3$	-
Test for Phytosterols	Salkowski	
Test for Steroids	Hesse's response	+

3.4 Quantitative phytochemical analysis

TPC

The standard (gallic acid) of different concentrations was added to the calibration graph and the linear regression formula. y = 0.005x + 0.003 was obtained with regression coefficient R^2 = 0.9994 (shown in Fig.3). The ethanolic extract of *CC* was dried and a calibration curve was made using that extract. The TPC was found using that calibration curve and it has express as mg GAE / gram of extract. The TPC value ethanol extract of the *CC* was found to be 17.24 mg GAE g^{-1} of extract, corresponding to an absorbance value of 0.2969 ± 0.003.

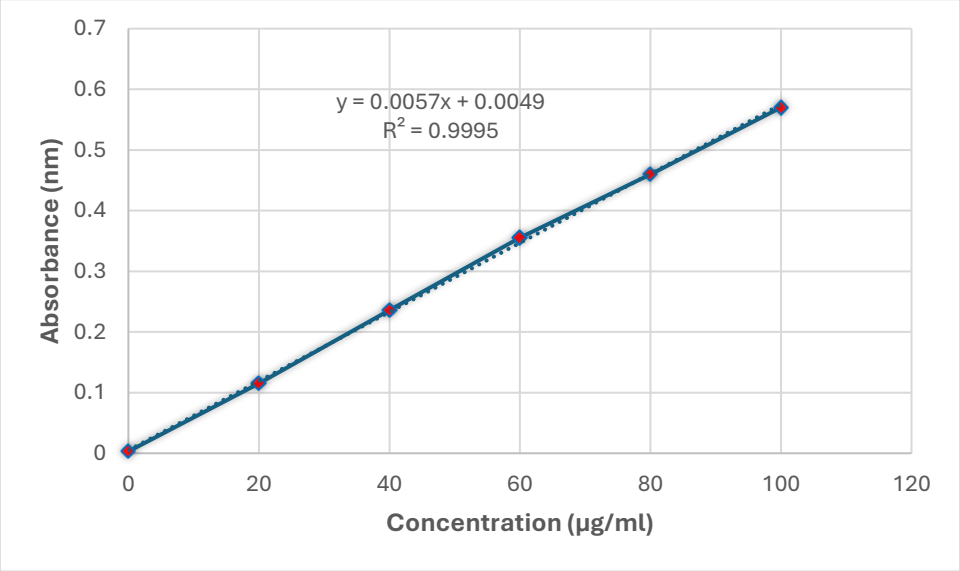


Fig. 3: Calibration curve of gallic acid for determination of TPC in ethanolic extract of *CC*.

TFC

Standard calibration curve has developed by different conc. of rutin as the linear regression equation y = 1.3225x + 0.0379 & regression coefficient R^2 = 0.996 (shown in Fig. 4). The calibration curve was used to obtain the TFC of ethanolic extract of *CC* and this was expressed as mg of RE/g of extract. TFC value of ethanolic extract the CC was discovered to be 266.30 mg GAE g^{-1} of extract, corresponding to an absorbance value of 0.8039 ± 0.003.

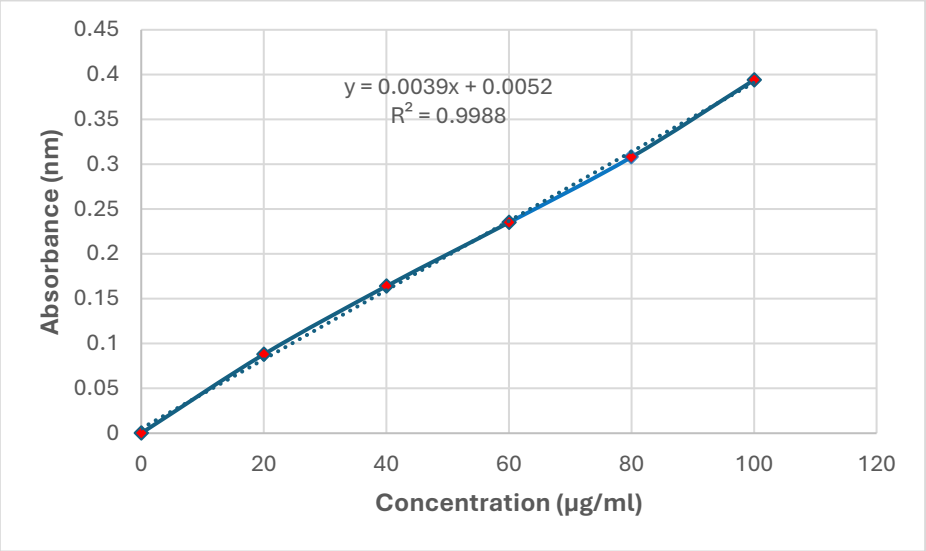


Fig. 4: Calibration curve of rutin for determination of TFC in ethanolic extract of *CC*.

3.5 Chromatographic fingerprinting

3.5.1 Thin Layer Chromatography

TLC analysis of ethanolic extract of *CC* has performed by various solvent systems. Toluene: Ethyl acetate (7.8 : 2.2) provided the best separation and was selected as the optimal mobile phase. Under UV light (254 and 365 nm) about 16 spots with Rf values ranging from 0.06-0.98 were observed that indicating multiple phytoconstituents with different polarities suitable for further isolation studies (shown in Table 4).

Table 4: TLC Analysis of Ethanolic Extract of *CC*.

Solvent System	Spot No.	Colour at 365 nm	Colour at 254 nm	Rf Value
Toluene: Ethyl acetate (7.8 : 2.2)	1	Pink	Dark Green	0.06
	2	Pink Fluorescence		0.12
	3	Fluorescence	Light Green	0.20
	4	Fluorescence		0.28
	5	Fluorescence	Green	0.36
	6	Purple	Green	0.46
	7	Light Yellow	Light Green	0.58
	8	Pink	Light Green	0.62
	9	Purple	Green	0.68
	10	Fluorescence	Light Green	0.70
	11	Blue	Light Green	0.74
	12	Pink	Light Green	0.80
	13	Fluorescence	Green	0.82
	14	Blue	Light Green	0.90
	15	Pink	Green	0.92
	16	Light Pink	Green	0.98

3.5.2 HPTLC

HPTLC analysis of the ethanolic extract of *CC* was carried out using quercetin as the reference marker compound. Chromatographic profiles were recorded at 254 nm and 366 nm and compared with the quercetin standard. The standard exhibited a characteristic peak at an Rf value around 0.67. The plant extract showed prominent peaks with Rf values close to 0.64 to 0.69, corresponding to the standard.

The peak table indicated quercetin-like peaks with area values ranging from 592.8 to 8004.6 AU, confirming the presence of quercetin in the extract. These findings validate the occurrence of flavonoid constituents in *CC* (shown in Fig. 5).

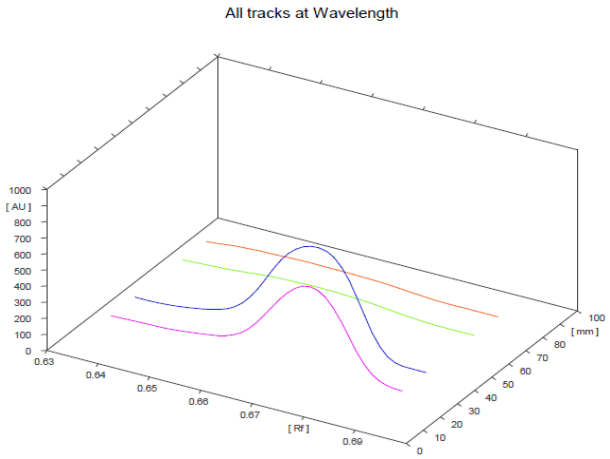


Fig. 5: 3D overlay chromatogram of rutin standard and CC extract at different wavelengths.

3.6 Isolation of compounds

The silica gel and Toluene :Ethyl Acetate (7.8 : 2.2) column chromatography of ethanolic extract of *CC* provided 10 fractions (A-J) (shown in Table 5). Fraction F which was obtained in larger quantity was brownish in colour and was subjected to further purification and analysis.

Table 5: Fraction collected from Column Chromatography of *CC* extract

Sr. No.	**Eluent Composition**	**Fraction Collected**	Observation
1	Toluene : Ethyl Acetate (7.8 : 2.2)	(A)	White coloured mixture of compound
2		(B)	Creamy coloured mixture of compound
3		(C)	Very light yellowish coloured mixture of compound
4		(D)	Greenish coloured mixture of compound
5		(E)	Light greenish coloured mixture of compound
6		(F)	Light yellowish coloured mixture of compound
7		(G)	Yellowish coloured mixture of compound
8		(H)	White coloured mixture of compound
9		(I)	Brownish coloured mixture of compound
10		(J)	White coloured mixture of compound

3.7 Spectroscopic characterization

3.7.1 UV-Visible Spectroscopy

The spectrum obtained from the isolated fraction using UV-Visible spectroscopy of *CC* exhibited characteristic absorption maxima in the range of 250-370 nm, indicating the presence of conjugated aromatic systems. These peaks correspond to $\pi \rightarrow \pi^*$ transitions typically associated with flavonoid compounds, supporting the presence of quercetin-like structures (shown in Fig.6a).

3.7.2 FT-IR Spectroscopy

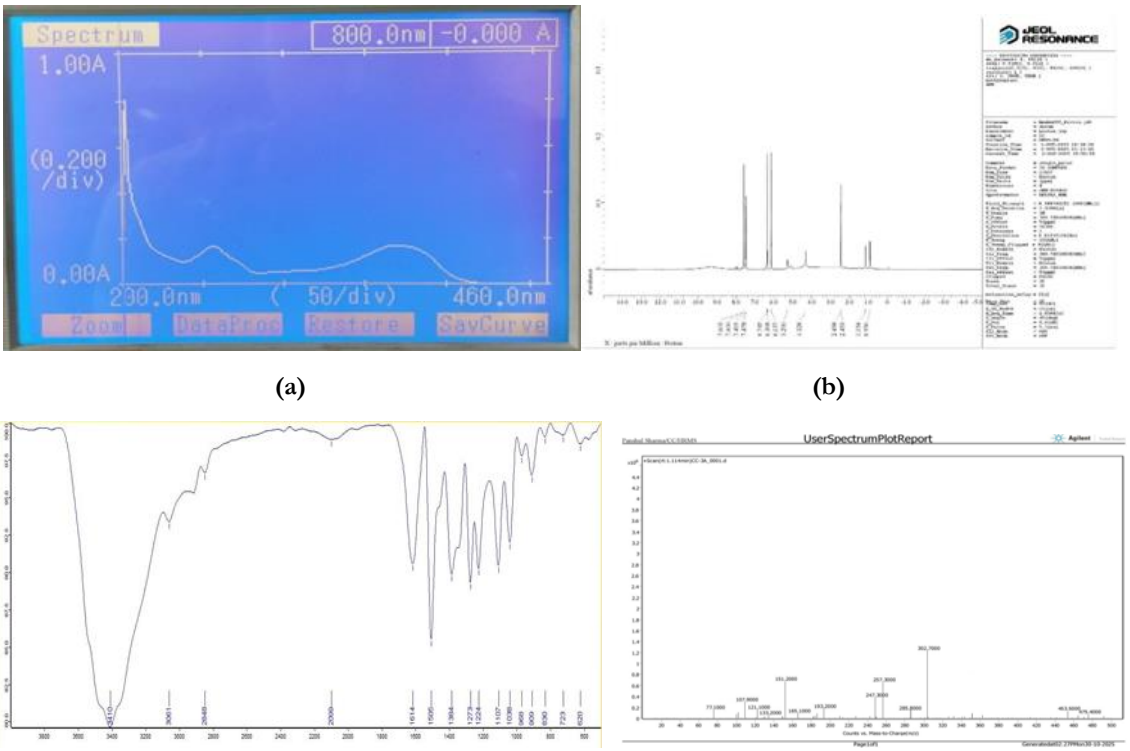
FT-IR analysis showed a A broad absorption band near 3400 cm^{-1} signifies O-H stretching in phenolic groups. Peaks near 2920 cm^{-1} indicated C-H stretching while bands around 1600-1650 cm^{-1} confirmed C=C stretching of aromatic rings. Additional peaks between 1000-1300 cm^{-1} represented C-O stretching, indicating phenolic and flavonoid structures (shown in Fig.6c).

3.7.3 ^{1}H-NMR Spectroscopy

The ^{1}H-NMR spectrum revealed aromatic proton signals typically appear between 6.0 and 8.0 ppm, indicating the presence of flavonoid rings. Signals in lower regions corresponded to aliphatic protons. The absence of strong sugar proton signals suggested a non-glycosidic flavonoid structure, supporting quercetin-type compounds (shown in Fig.6b).

3.7.4 Mass Spectrometry

Mass spectrometric analysis the molecular ion peak observed was approximately at m/z 302, indicating the molecular weight weight of quercetin. The fragmentation pattern further supported the presence of a flavonoid aglycone, confirming quercetin as a major bioactive compound in *Chamabainia cuspidate* (shown in Fig.6d).



(a) **(b)**

(c) **(d)**

Fig. 6: Spectra of (a) U.V (b) ^{1}H-NMR (c) FTIR (d) Mass

3.8 Anti-inflammatory activity

Ethanolic extract of *CC* inhibited protein denaturation in a concentration dependable way (50-250 µg/ml). The percentage inhibition was 36.06% to 63.49% and with IC_{50} of 153.309 µg/ml. Aspirin used as the standard drug showed stronger activity with an IC_{50} of 20.326 µg/ml, showing effectiveness of the plant extract as anti-inflammatory potential which result in the determination of successful anti-inflammatory activity of plant extract. It was tested by the human RBC method where the stabilization of membranes by the *CC* extract was calculated. The extract had concentration dependent inhibition of hemolysis at 50-250 µg/ml where percentage inhibition was varying between 38.14% to 61.48%. The resultant IC_{50} was 151.724 µg/ml which is a moderate membrane stabilizing activity. Comparatively, of the usual drug aspirin increased activity was indicated by an IC_{50} value of 11.845 µg/ml (shown in Table 6 and 7). According to these findings, the extract has a moderate anti-inflammatory effect via erythrocyte membrane stabilization.

Table 6: Protein denaturation activity of Aspirin and *CC*

Aspirin			***CC***		
Concentration (µg/ml)	**Absorbance**	**% Inhibition**	**Concentration (µg/ml)**	**Absorbance**	**% Inhibition**
25	0.554	50.667	**50**	0.718	36.064
50	0.448	60.106	**100**	0.649	42.208
75	0.372	66.874	**150**	0.571	49.154
100	0.287	74.443	**200**	0.483	56.990
125	0.204	81.834	**250**	0.41	63.490
Control	1.123		**Control**	1.123	
IC_{50}	20.326		**IC_{50}**	153.309	

Table 7: Membrane stabilization activity of Aspirin and *CC*

Aspirin			***CC***		
Concentration (µg/ml)	**Absorbance**	**% Inhibition**	**Concentration (µg/ml)**	**Absorbance**	**% Inhibition**
25	0.512	52.941	**50**	0.673	38.143
50	0.443	59.283	**100**	0.607	44.209
75	0.381	64.981	**150**	0.581	49.816
100	0.329	69.761	**200**	0.419	55.790
125	0.251	79.930	**250**	0.419	61.488
Control	1.088		**Control**	1.088	
IC_{50}	11.845		**IC_{50}**	151.724	

4. DISCUSSION

The current paper critically assessed the phytochemicals and anti-pharmacological potentials of *CC* leaf which was found in the western Himalayan region. Early phytochemical screening assurance indicated the existence of various repeated bioactive

compounds include alkaloids, glycosides, phenolics, tannins, saponins, triterpenoids, and steroids have diverse biological actions. Chromatographic and spectroscopic determinations also corroborated these results and TLC and HPTLC profiling demonstrated that there were numerous compounds with different Rf values and the presence of flavonoid markers like quercetin were confirmed. The quantitative. The study demonstrated that the extract had a high TPC: 17.24 mg GAE g^{-1} and TFC: 266.30 mg RE g^{-1}, suggesting the availability of potent antioxidant phytoconstituents. It is also well known that these compounds can neutralize reduces free radicals and lowers oxidative stress which is of a key role in inflammation.

Assays assessing protein denaturation and membrane stabilization were conducted to evaluate the anti-inflammatory activity of the extract. The extract itself showed concentration-effectual block of the protein denaturing (36.06-36.49%) and erythrocyte haemolysis (38.14-61.48%) with higher IC_{50} values (153.309 µg/ml, 151.724 µg/ml, respectively) than aspirin. This indicates the extract has moderate anti-inflammatory effect which is not as good as the standard drug. The membrane stabilization effect is evidence of the power of the extract to its defence of the lysosomal membranes, thus averting an escape of the inflammatory mediators.

5. CONCLUSION

This study showed that the ethanolic extract of CC leaves is rich in bioactive compounds, especially phenolics and flavonoids, which play a role in its biological effects. The extract exhibited moderate anti-inflammatory activity through inhibition of protein denaturation and stabilization of erythrocyte membranes. Although less potent than standard drugs such as aspirin and cystone, the findings suggest indicating that the plant may serve as a natural therapeutic agent. Further studies including isolation of active compounds, in-vivo evaluation and clinical validation are required to confirm its efficacy and safety.

ABBREVIATIONS

CC: *Chamabainia cuspidata;* ***BSA:*** *Bovine Serum Albumin;* ***CaOx:*** *Calcium Oxalate;* ***COM:*** *Calcium Oxalate Monohydrate;* ***FESEM:*** *Field Emission Scanning Electron Microscopy;* ***FT-IR:*** *Fourier Transform Infrared Spectroscopy;* ***GAE:*** *Gallic Acid Equivalent;* ***HPTLC:*** *High-Performance Thin Layer Chromatography;* ***IC_{50}:*** *Half Maximal Inhibitory Concentration;* ***MS:*** *Mass Spectrometry;* ***NaCl:*** *Sodium Chloride;* ***NaOH:*** *Sodium Hydroxide;* ***NMR:*** *Nuclear Magnetic Resonance;* ***RBC:*** *Red Blood Cells;* ***RE:*** *Rutin Equivalent;* ***TFC:*** *Total Flavonoid Content;* ***TLC:*** *Thin Layer Chromatography;* ***TPC:*** *Total Phenolic Content;* ***UV:*** *Ultraviolet;* ***WHO:*** *World Health Organization.*

AUTHORS' CONTRIBUTIONS

PLANT AUTHENTICATION AND IDENTIFICATION

The plant material was collected from Himachal Pradesh, India and authenticated by a qualified taxonomist. The collected plant parts were identified by Mr. Kumar Ambrish, Botanical survey of India, Solan (Accession No: 743).

AVAILABILITY OF DATA AND MATERIALS

All data generated in the study are provided in the manuscript. No extra data is available.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not Applicable.

FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial or not for profit sectors.

CONSENT FOR PUBLICATION

All authors have read and approved the final manuscript.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

REFERENCES

[1] Yuan H, Ma Q, Ye L, Piao G. The traditional medicine and modern medicine from natural products. *Molecules*. 2020;25(3):1–18. https://doi.org/10.3390/molecules25030432

[2] Newman DJ, Cragg GM. Natural products as sources of new drugs over the nearly four decades. *J Nat Prod*. 2020;83(3):770–803. https://doi.org/10.1021/acs.jnatprod.9b01285

[3] Atanasov AG, Zotchev SB, Dirsch VM, Supuran CT. Natural products in drug discovery: advances and opportunities. *Nat Rev Drug Discov*. 2021;20(3):200–216. https://doi.org/10.1038/s41573-020-00114-z

[4] Petrovska BB. Historical review of medicinal plants' usage. *Pharmacogn Rev*. 2012;6(11):1–5. *(Foundational reference retained due to citation relevance)* https://doi.org/10.4103/0973-7847.95849

[5] Bhardwaj A, Kaur M, Sharma A. Ethnopharmacological review of Himalayan medicinal flora with therapeutic potential. *J Ethnopharmacol*. 2022;284:114734. https://doi.org/10.1016/j.jep.2021.114734

[6] Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, et al. Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget*. 2018;9(6):7204–7218. *(Mechanistic standard paper)* https://doi.org/10.18632/oncotarget.23208

[7] Khan SR, Pearle MS, Robertson WG, Gambaro G, Canales BK, Doizi S, et al. Kidney stones. *Nat Rev Dis Primers*. 2021;7:54. https://doi.org/10.1038/s41572-021-00294-y

[8] Gulcin İ. Antioxidants and antioxidant methods: an updated overview. *Arch Toxicol*. 2020;94:651–715. https://doi.org/10.1007/s00204-020-02689-3

[9] Azwanida NN. A review on the extraction methods use in medicinal plants, principle, strength and limitation. Medicinal & Aromatic Plants. 2015;4:196. DOI: https://doi.org/10.4172/2167-0412.1000196

[10] Dai J, Mumper RJ. Plant phenolics: extraction, analysis and their antioxidant properties. Molecules. 2010;15(10):7313–7352. DOI: https://doi.org/10.3390/molecules15107313

[11] Harborne JB. Phytochemical methods: a guide to modern techniques of plant analysis. Phytochemistry. 1998;49(1):3–14. DOI: https://doi.org/10.1016/S0031-9422(98)00035-1

[12] Ogunmefun SA, et al. Morphometrical, phytochemical and microscopical characterization of medicinal plants. *Discover Applied Sciences*. 2025. https://doi.org/10.1007/s44372-025-00287-0.

[13] Pandiyan R, Ilango K. Pharmacognostical, physicochemical and phytochemical evaluation of medicinal plant leaves. *J Pharm Pharmacogn Res*. 2022;10(1):160–170. https://doi.org/10.56499/jppres22.110_10.1.160

[14] Pathan AK, Bond J, Gaskin RE. Sample preparation for scanning electron microscopy of plant surfaces. *Methods in Molecular Biology*. 2020;1994:151–160. https://doi.org/10.1007/978-1-4939-9473-1_13

[15] Kumar V, et al. Microscopic investigation and pharmacognostic evaluation of medicinal plant materials. *Microscopy Research and Technique*. 2023. https://doi.org/10.1002/jemt.24316

[16] Geetham PKM, Malarvizhi A. Physicochemical and elemental analysis of plant materials for standardization. *Int J Pharm Sci Res*. 2022;13(3):1349–1354. https://doi.org/10.13040/IJPSR.0975-8232.13(3).1349-54

[17] Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology. 2005;4(7):685-688. DOI: https://doi.org/10.5897/AJB2005.000-3127

[18] Wadood A, Ghufran M, Jamal SB, Naeem M, Khan A, Ghaffar R, et al. Phytochemical analysis of medicinal plants occurring in local area of Mardan.
Biochem Anal Biochem. 2013;2:144. DOI: https://doi.org/10.4172/2161-1009.1000144

[19] Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H. Phytochemical screening and extraction: a review. Int Pharm Sci. 2011;1(1):98-106. DOI: https://doi.org/10.4103/0975-7406.80769

[20] Ncube NS, Afolayan AJ, Okoh AI. Assessment techniques of antimicrobial properties of natural compounds of plant origin. Afr J Biotechnol. 2008;7(12):1797–1806. DOI: https://doi.org/10.5897/AJB07.613

[21] Aiyegoro OA, Okoh AI. Preliminary phytochemical screening and antibacterial properties of *Helichrysum longifolium*. BMC Complement Altern Med. 2010;10:21.
DOI: https://doi.org/10.1186/1472-6882-10-21

[22] Banso A, Adeyemo SO. Phytochemical screening and antibacterial assessment of *Abutilon mauritianum*, *Bacopa monnifera* and *Datura stramonium*.
Biokemistri. 2007;19(2):39–44. DOI: https://doi.org/10.4314/biokem.v19i2.56424

[23] Kokate CK. Practical pharmacognosy techniques for natural product identification. Pharmacognosy Res. 2010;2(3):148-152. DOI: https://doi.org/10.4103/0974-8490.65507

[24] Obadoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the crude extracts of some homeostatic plants in Edo and Delta States of Nigeria.
Global J Pure Appl Sci. 2001;8(2):203–208. DOI: https://doi.org/10.4314/gjpas.v8i2.16033

[25] Wink M. Modes of action of herbal medicines and plant secondary metabolites.
Medicines. 2015;2(3):251–286. DOI: https://doi.org/10.3390/medicines2030251

[26] Harborne JB, Williams CA. Advances in flavonoid research since 1992. Phytochemistry. 2000;55(6):481-504. DOI: https://doi.org/10.1016/S0031-9422(00)00235-1

[27] Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: chemistry, metabolism and structure–activity relationships. J Nutr Biochem. 2002;13(10):572-584. DOI: https://doi.org/10.1016/S0955-2863(02)00208-5

[28] Scalbert A. Antimicrobial properties of tannins. Phytochemistry. 1991;30(12):3875-3883. DOI: https://doi.org/10.1016/0031-9422(91)83426-L

[29] Chung KT, Wong TY, Wei CI, Huang YW, Lin Y. Tannins and human health: a review. Crit Rev Food Sci Nutr. 1998;38(6):421-464. DOI: https://doi.org/10.1080/10408699891274273

[30] Francis G, Kerem Z, Makkar HPS, Becker K. The biological action of saponins in animal systems. Br J Nutr. 2002;88(6):587-605. DOI: https://doi.org/10.1079/BJN2002725

[31] Vincken JP, Heng L, de Groot A, Gruppen H. Saponins, classification and occurrence in plant kingdom. Phytochemistry. 2007;68(3):275-297. DOI: https://doi.org/10.1016/j.phytochem.2006.10.008

[32] Friedman M. Applications of the ninhydrin reaction for analysis of amino acids, peptides and proteins. J Agric Food Chem. 2004;52(3):385-406. DOI: https://doi.org/10.1021/jf030490p

[33] Everette JD, Bryant QM, Green AM, Abbey YA, Wangila GW, Walker RB.
Thorough study of reactivity of various compound classes toward the Folin–Ciocalteu reagent. J Agric Food Chem. 2010;58(14):8139–8144. DOI: https://doi.org/10.1021/jf1005935

[34] Ainsworth EA, Gillespie KM. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. Nat Protoc. 2007;2(4):875-877. DOI: https://doi.org/10.1038/nprot.2007.102

[35] Chang CC, Yang MH, Wen HM, Chern JC.
Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal. 2002;10(3):178–182. DOI: https://doi.org/10.38212/2224-6614.2748

[36] Prior RL, Wu X, Schaich K. Standardized methods for determination of antioxidant capacity and phenolics in foods and dietary supplements. J Agric Food Chem. 2005;53(10):4290–4302. DOI: https://doi.org/10.1021/jf0502698

[37] Wojdyło A, Oszmiański J, Czemerys R.
Antioxidant activity and phenolic compounds in 32 selected herbs. Food Chem. 2007;105(3):940–949. DOI: https://doi.org/10.1016/j.foodchem.2007.04.038

[38] Sherma J, Fried B. Handbook of thin-layer chromatography.
Journal of Liquid Chromatography & Related Technologies. 2003;26(9–10):1645–1650. DOI:10.1016/s0021-9673(96)00967-3

[39] Nyiredy S. Separation strategies of plant constituents—current status.
J Chromatogr B Analyt Technol Biomed Life Sci. 2004;812(1-2):35-51.
DOI: https://doi.org/10.1016/j.jchromb.2004.07.041

[40] Reich E, Schibli A. High-performance thin-layer chromatography for the analysis of medicinal plants. J Chromatogr A. 2007;1154(1-2):94-103. DOI: https://doi.org/10.1016/j.chroma.2007.03.046

[41] Hostettmann K, Marston A. Preparative chromatography techniques: applications in natural product isolation. Phytochem Rev. 2002;1(2):199-207. DOI: https://doi.org/10.1023/A:1026046025733

[42] Sherma J. Review of HPTLC in drug analysis. J AOAC Int. 2000;83(6):1292–1306. DOI: https://doi.org/10.3390/molecules27030975

[43] Waksmundzka-Hajnos M, Sherma J, Kowalska T. Thin-layer chromatography in phytochemistry. J Chromatogr Sci. 2008;46(8):673–676. DOI: https://doi.org/10.1021/ac00225a001

[44] Silverstein RM, Bassler GC. Spectrometric identification of organic compounds. Journal of Chemical Education. 1962 Nov;39(11):546. DOI: https://doi.org/10.1021/ed039p546

[45] Coates J. Interpretation of infrared spectra: a practical approach. Encyclopedia of Analytical Chemistry. 2000. DOI: https://doi.org/10.1002/9780470027318.a5606

[46] Claridge TDW. High-resolution NMR techniques in organic chemistry. Prog Nucl Magn Reson Spectrosc. 2016;96:1-13. DOI: https://doi.org/10.1016/j.pnmrs.2016.08.001

[47] Simmler C, Napolitano JG, McAlpine JB, Chen SN, Pauli GF. Universal quantitative NMR analysis of complex natural products. J Nat Prod. 2014;77(3):684–689. DOI: https://doi.org/10.1016/j.copbio.2013.08.004

[48] Hoffmann ED, Stroobant V. Mass spectrometry principles and applications. Mass Spectrom Rev. 2007;26(1):1-30. DOI: https://doi.org/10.1002/mas.20124

[49] Gross JH. Mass spectrometry: a textbook overview of ionization methods.
Mass Spectrom Rev. 2011;30(1):47–57. DOI: https://doi.org/10.1002/mas.20268

[50] Watson JT, Sparkman OD. Introduction to mass spectrometry. J Am Soc Mass Spectrom. 2007;18(9):1631-1632. DOI: https://doi.org/10.1016/j.jasms.2007.06.010

[51] Mizushima Y, Kobayashi M. Interaction of anti-inflammatory drugs with serum proteins, especially with some biologically active proteins. J Pharm Pharmacol. 1968;20(3):169-173. DOI: https://doi.org/10.1111/j.2042-7158.1968.tb09718.x

[52] Shinde UA, Phadke AS, Nair AM, Mungantiwar AA, Dikshit VJ, Saraf MN. Membrane stabilizing activity – possible mechanism of anti-inflammatory activity.
Fitoterapia. 1999;70(3):251–257. DOI: https://doi.org/10.1016/S0367-326X(99)00030-1