Indian Journal of Natural Products and Resources
Vol. 16(2), June 2025, pp 325-334
DOI: 10.56042/ijnpr.v16i2.13614

Some pharmacopoeial tests for a folklore herb *Cordyline fruticosa* (L.) A. Chev. and monographic standards with antioxidant assay thereof

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Received 10 September 2024; revised received 08 April 2025; accepted 05 May 2025

Cordyline fruticosa (L.) A. Chev. also recognised as a good luck plant, it is commonly used to treat fever, asthma, rheumatic bone pains, smallpox, joint pain, bleeding skin eruptions, and as an abortifacient. In this study, standardisation tests like physicochemical and HPTLC were carried out on leaves of the plant as per Pharmacopoeia. Results of the micro powder study showed fragments of mesophyll parenchymatous tissue embedded with brownish materials, oil globules and bundles of acicular crystals. The mixture of toluene, ethyl acetate, and formic acid (8.5:3.0:0.5v/v) was used as a mobile phase to obtain the HPTLC fingerprint profile. Photo-documentation of methanolic leaf extract, when observed under UV 254 nm, revealed 13 bands, 11 bands under UV 366 nm, and densitometric scanning revealed 13 peaks at 520 nm. By HPLC, 12 peaks were obtained when a methanol and water (60:40) mixture was used as the mobile phase. The antioxidant property of the methanolic leaf extract of *C. fruticosa* was analysed by ABTS and DPPH radical scavenging techniques. The DPPH radical scavenging method showed that the IC_{50} values of *C. fruticosa* and standard ascorbic acid were 70.317±0.51849 and 11.13±1.29179 µg/mL, respectively. The IC_{50} values of *C. fruticosa* and standard trolox were found 47.2348±1.56651 and 37.6146±1.24248 µg/mL, respectively when it was analysed by the ABTS radical scavenging method. The total flavonoid and total phenolic content of the methanol extract of *C. fruticosa* was determined to be 0.7298±0.00162 µg QE/mg and 34.92±0.01808 µg GAE/mg, respectively.

Keywords: Antioxidant, *Cordyline fruticos*a, HPLC, HPTLC, Macroscopy, Microscopy

IPC code; Int. cl. (2021.01)− A61K 36/00, A61K 127/00, A61P 39/00

Introduction

Medicinal plants with important bioactive compounds are a vital source of basic components for modern medicine in both the treatment and prevention of ailments[1]. Traditional medicines have played a remarkable role in providing basic health care in developing countries. More than half of all the new drugs synthesised and medicines discovered till date have been based on active plant phytoconstituents[2,3]. Even many species of ornamental plants are being vastly researched for their significant bioactivities. The most significant example is paclitaxel, derived from *Taxus brevifolia* L. which is widely in clinical use for cancer treatment[4].

However, due to widespread increase of adverse drug reactions compared to benefits of modern medicines, academic and government efforts are enormously being channeled towards traditional therapy[5]. In the case of administration of cisplatin, a medication that cures a variety of cancers, several restrictions exist because of nephrotoxicity caused by cisplatin (CIN), as seen in occurring in between 20 and 35% of cases[6]. Natural antioxidant agents can suppress cisplatin-induced oxidative stress in the kidney[7,8].

Plants belonging to the *Cordyline* genus, are referred to as decorative plants, but have also been utilised as a traditional origin of medications. *Cordyline fruticosa* (L.) A. Chev. (or *C. terminalis* (L.) Kunth) (Asparagaceae) is typically called a good fortune plant, cabbage palm, palm lily, or Ti plant. It is a blooming evergreen plant native to Papua New Guinea and Southeast Asia[9].

Traditionally, it has been applied to treat fever, diarrhoea, asthmarheumatic bone pains, headache,

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smallpox, joint pain, bleeding skin eruptions, as disinfectant for wounds and as an abortifacient[10]. Phytochemical studies of *C. fruticosa*, divulged the presence of multiple biochemically active substances, like as cholestane, tannins, glycosides, steroidal saponins, polyphenols, glucofructan, flavonoid, alkaloids and phytosterols[11-13]. The presence of these compounds has led to a broad spectrum of biological processes such as cytotoxicity, antimicrobial, anti-inflammatory, anti-ulcer, antidiabetic, antidiarrheal, antiproliferative and hypoglycemic activities[14-17]. The leaves of this plant have also been used to treat diarrhoea and dysentery[18]. Studies reported the presence of cytotoxic activities by specific steroidal bioactive components of the plant against HL-60 leukaemia cells, melanoma and colon carcinoma human tumour cell lines[19,20]. *C. fruticosa* plants are significant for their ornamental and medicinal values. So, we were interested in studying its anatomy and powder microscopy, chemical and physical characterisation, antioxidant property, and phenolic and flavonoid compound content.

Materials and Methods

Collection and authentication

The leaves of *C. fruticosa* were obtained from a demonstrative medicinal plant garden of the Central Ayurveda Research Institute (Under CCRAS, New Delhi, Ministry of AYUSH, Government of India), Guwahati, Assam, in June month, 2024. The Department of Pharmacognosy of Siddha Central Research Institute (CCRS, Minister of AYUSH, Government of India), Chennai, Tamil Nadu, verified the authenticity of the collected samples. H/LF-76, the voucher specimens, have been stored in the raw drug museum[21].

Chemicals and reagents

Analytically superior solvents, including ethyl acetate, formic acid, methanol, and toluene, and HPLC grade solvents, including methanol and water, were purchased from Merck, India. DPPH (1, 1-diphenyl-2-picrylhydrazyl), ABTS (2, 2'-Azinobis (3-ethylbenothiazoline-6-sulfonic acid) diammonium salt), ascorbic acid, potassium peroxy disulphate, phenol reagent, sodium carbonate, aluminium chloride, gallic acid, potassium acetate, and quercetin were purchased from Himedia Laboratories Pvt. Ltd., Mumbai, India. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) was purchased from Sigma Aldrich, Bangalore, India.

Instruments

Zeiss Axiolab 5 trinocular microscope coupled with an Axiocam 208 colour camera was used for TS photomicrography. Powder characters were drawn under 200X magnifications with the help of an Olympus BX43 trinocular microscope with a drawing tube. The parts of CAMAG HPTLC (Switzerland) comprise a twin trough chamber and plate, autosampler ATS4, visualiser, and scanner 4 (Scanner_210441) linked to WINCATS software, a twin trough chamber and plate heater was used for HPTLC analysis. For HPLC analysis, a Shimadzu HPLC (Model: FCV-200AL) with Lab Solution software (Japan) linked to a vacuum degasser (DGU-10B), quaternary pump (LC-20 AP), along with ultraviolet PDA detector (SPD-M20A 230 V) was applied. The antioxidant, total phenol, and total flavonoid quantification were performed using the UV-Vis spectrophotometer (UV-1900i).

Anatomy and powder microscopy

For twelve hand-free hand sections, the dried plant material was dipped in water for 12 hours, and free-hand sections were taken using standard procedures[22]. The section was stained with a mixture of 0.4% Astra Blue and 4% safranin. The powder plant materials were run through sieve 80. The mounting medium is used 75% glycerin, con. H_2SO_4, Jaffrey's maceration fluid, Sudan red IV, chloral hydrate, and iodine solution were used where necessary. The part was photographed on camera with different magnifications. Characters in powder were drawn at 200X magnifications[23].

Preparation of sample extract

Exactly 10 g of powdered raw material was placed in a thimble. Then the thimble was placed in a distillation flask, which was filled with 100 mL methanol. Subsequently, after attaining the level of submersion from the thimble holder, the siphon took up the solvent into the same distillation flask. This flask contained the solutes that had been extracted. This procedure was continuously done till the extraction was over. The extract was vacuum-sealed and dried in a water bath. This dried extract was used for antioxidant, total phenolic, and total flavonoid determination.

Physico-chemical and phytochemical analysis

Pharmacopoeial standard methods[24] were followed in the conduct of each experiment.

HPTLC

A volume of 15 µL of the extract was carefully sprayed onto a TLC plate (measuring 4×10 cm) coated with silica gel ($60F_{254}$), forming a band length of 8 mm and positioned 10 mm from the bottom by the ATS4 Autosampler. Subsequently, the plate was developed in a twin Trough chamber (10×10 cm) previously saturated with the mobile phase toluene: ethyl acetate: formic acid; (8.5:3.0:0.5 v/v). The solvent front reached a height of 85 mm above the base. Following development, the plate was dried on a hot plate, photographed in the CAMAG Visualizer Chamber and scanned under 254 nm (D2 lamp, absorbance mode) and 366 nm (Hg lamp, fluorescence mode). The scanning was performed with 6×0.45 mm slit dimension, at 20 mm/s scanning speed (Scanner_210441, software: WINCATS)[25]. Subsequently, TLC plate was treated with a derivatising reagent (vanillin sulfuric acid) and heated on a TLC plate heater at 105°C until coloured bands were visible. A photograph was taken under white light and then scanned at 520 nm (W lamp, absorption mode).

HPLC

An extract of 10 µL was added to the column for HPLC analysis, and a flow rate of 0.8 mL/min was maintained for 30 minutes throughout the process. A Shimpack GIST (4.6 x 250 mm) C18 analytical column was used as the stationary phase, whereas methanol and water (60:40) mixture comprised the mobile phase. A constant temperature of 40°C was maintained. A PDA detector[26] processed the data at 229 nm.

DPPH radical scavenging activity

A concentration of 40 µg/mL DPPH solution was prepared by dissolving exactly 10 mg analytical grade DPPH reagent in 250 mL methanol. Then, 5 mL of different concentrations (4, 8, 16, 24, 32, and 40 µg/mL) of methanolic extract were combined with 5 mL of prepared DPPH solution. Similarly, 5 mL of the DPPH solution was combined with 5 mL of different concentrations (0.5, 1, 2, 3, 4, and 5 µg/mL) of standard ascorbic acid solutions. The mixtures were given a good shake and were left to settle at ambient temperature in the dark for 30 minutes. Next, the absorbance was determined at 517 nm using spectrophotometer[27]. The formula $[A_c - (A_s - A_o)] \times 100 / A_c$ was employed to figure out the proportion of DPPH radical scavenging in the sample and standard. Here, A_c stands for absorbance of control DPPH solution, A_s for absorbance of sample or standard + DPPH, and A_o for absorbance of sample without DPPH interaction[28].

ABTS radical scavenging assay

To prepare 1000 µM ABTS solution 51.462 mg analytical grade ABTS reagent was dissolved in 100 mL distilled water. Then, 5 mL of different concentrations (4, 8, 16, 24, 32, and 40 µg/mL) of sample extract were combined with 5 mL of ABTS solution. Similarly, 5 mL of ABTS solution was mixed with 5 mL of different concentrations (3, 6, 12, 18, 24, and 30 µg/mL) of standard trolox solution. Then the mixtures were vigorously shaken and were allowed to settled for 3 minutes. Next, the absorbance was detected at 734 nm by a spectrophotometer. The same formula $[A_c - (A_s - A_o)] \times 100 / A_c$ was used to calculate the percentage of ABTS radical scavenging in the sample and standard. Here, A_c stands for absorbance of the control ABTS, A_s for absorbance of the sample or standard + ABTS, and A_o for absorbance of the sample without ABTS interaction[29].

Determination of total phenolic content

The total phenolic content of methanolic extracts was determined using the Folin-Ciocalteu reagent., variable concentrations of gallic acid (1, 2, 3, 4, and 5 µg/mL) were used as reference standards to plot the calibration graph. A 2 mL methanolic extract (concentration 500 µg/mL) of sample was combined with 2 mL Folin-Ciocalteu reagent (10% v/v in de-ionised water) and 2 mL sodium carbonate solution (7% w/v in de-ionised water). The mixture was then built up to 10 mL with de-ionised water. After giving the reaction mixture a good shake, it was incubated for 30 minutes. Using a UV-Vis spectrophotometer[30], at 765 nm, the blue colour solution's absorbance was measured. With the help of a standard graph made with gallic acid standard, the total phenolic content of the methanolic extract of the sample was calculated using the linear equation. It was computed to find the total phenolic component materials of the dry extract as µg/mg gallic acid equivalent (GAE)[31].

Determination of total flavonoid content

The methanolic sample solution of concentration 1000 µg/mL was prepared by dissolving 100 mg sample extract in 100 mL methanol. The total flavonoid content of the methanolic extracts was determined using the aluminium chloride

colourimetric method. To draw the calibration graph, quercetin concentrations of 5, 10, 20, 30, and 40 µg/mL were utilised as reference standards. A mixture of 0.2 mL aluminium chloride (10% w/v in de-ionised water) and 0.2 mL potassium acetate (1 M) was combined with 6 mL methanolic extracts. Then, 3.6 mL distilled water was added to it to produce a 10 mL solution. After giving a good shake to the yellow reaction mixture, it was incubated for 30 minutes. Then, using a UV-vis spectrophotometer (UV-1900i), at 415 nm, the blue colour solution's absorbance was measured. A standard graph was created using the quercetin standard, and the linear equation was used to calculate the flavonoid concentration of the methanolic extract. The amount of total flavonoid components in the dry extract, represented as µg/mg quercetin equivalent (QE)[29,31].

Fig. 1 — *Cordyline fruticosa* a) Habit, and b) Dried leaves.

Results

Macroscopy

C. fruticosa plant is found in various habitats, including rain forests, rocky outcrops and coastal cliffs (Fig. 1a). Dried cut pieces of leaf blade are thin, margin scabrous, prominent small. Large thickened parallel veins of the leaf are arranged in alternate mode, midrib U-shaped and more prominent on the lower surface, and surface rough. The broken leaf blade pieces are flat and transversely cut pieces folded; few are recurved, longitudinal fracture smooth, greenish-brown in colour, odour not specific; taste slightly astringent to bitter (Fig. 1b).

Microscopy

Leaf

Diagrammatic transverse section (TS) of the leaf passing through the midrib shows plano-convex wavy outline with mesophyll of parenchymatous ground tissue, embedded with numerous vascular bundles surrounded by mechanical tissue. Linear, isobilateral laminar extensions are found on either side of the midrib, having lateral vascular bundles. Lamina outline is slightly convex on adaxial and abaxial side at the place of lateral vascular bundles (Fig. 2a-b).

Detailed TS of the midrib displays one layer of oval to rectangular upper and lower epidermis covered by thick cuticle followed by wide zone of

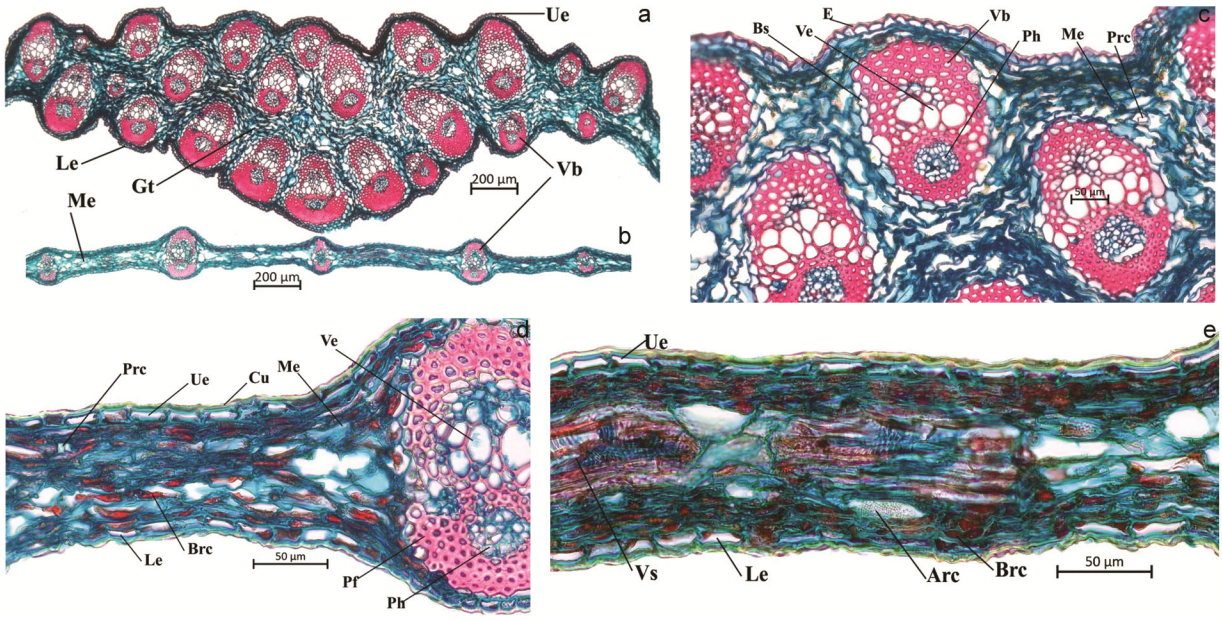


Fig. 2 — TS of a) midrib, b) lamina, c) midrib enlarged view, d) lamina lateral vein enlarged view, and e) lamina enlarged view.
Abbreviation: Arc, acicular crystals of calcium oxalate; Brc, brownish content; Bs, bundle sheath; Cu, cuticle; E, epidermis; Gt, ground tissue; Le, lower epidermis; Me, mesophyll; Pf, pericyclic fibre; Ph, phloem; Prc, prismatic crystals of calcium oxalate; Ue, upper epidermis; Vb, vascular bundle; Ve, vessel; Vs, vascular strand.

thin-walled parenchymatous mesophyll ground tissue embedded with various size vascular bundles, oil globules, brownish content, prismatic crystals of calcium oxalate and raphide bundles of calcium oxalate. Each vascular bundle consists of conjoint, collateral, closed, encircled by thick-walled sclerenchymatous tissue and a layer of thin-walled bundle sheath cells toward the periphery (Fig. 2c).

Lamina

Detailed TS shows single layers of a rectangular size in the upper and lower epidermis. Upper epidermis is embedded with few stomata, and lower epidermis with smaller cells is embedded with more stomata, followed by a wide zone of thin-walled parenchymatous mesophyll tissue embedded with lateral vascular bundles and other cell ergastic substances in the midrib (Fig. 2d-e)

Powder microscopy

Greenish brown colour powder shows fragments of the upper and lower epidermic surface view with paracytic stomata. The density of stomata is lower in the upper and higher in the lower epidermis. A thick and thin-walled fibre tracheid with simple pits and tertiary thickening is present with a surface view of the midrib epidermis. Additionally, thick-walled, sharp ended narrow lumened fibres are present. Fragments of mesophyll parenchyma embedded with brownish content, oil globules and bundles of acicular crystals were also seen. The appearance of vessels with reticulated, scalar form, spiral and pitted thickening; simply pitted tracheid; lamina in sectional view with brownish content; rod-shaped prismatic and raphide bundle of acicular crystals of calcium oxalate; longitudinal cut view of the fibre bundle and vascular elements was observed (Fig. 3).

Physicochemical analysis

Due to microbial load, moisture plays a significant role in drug breakdown and reduces shelf life. It was found that the air-dried sample had a 5.92% loss on drying value, indicating the presence of moisture content. The estimated total ash which represents the drug's whole inorganic content, including both physiological and non-physiological salts was 11.11%. It was found that the acid-insoluble ash which is primarily made up of silica in a plant drug, was 0.778%. The estimated amounts for the water and ethanol soluble extractive values which show how soluble the plant's active ingredients are in water and

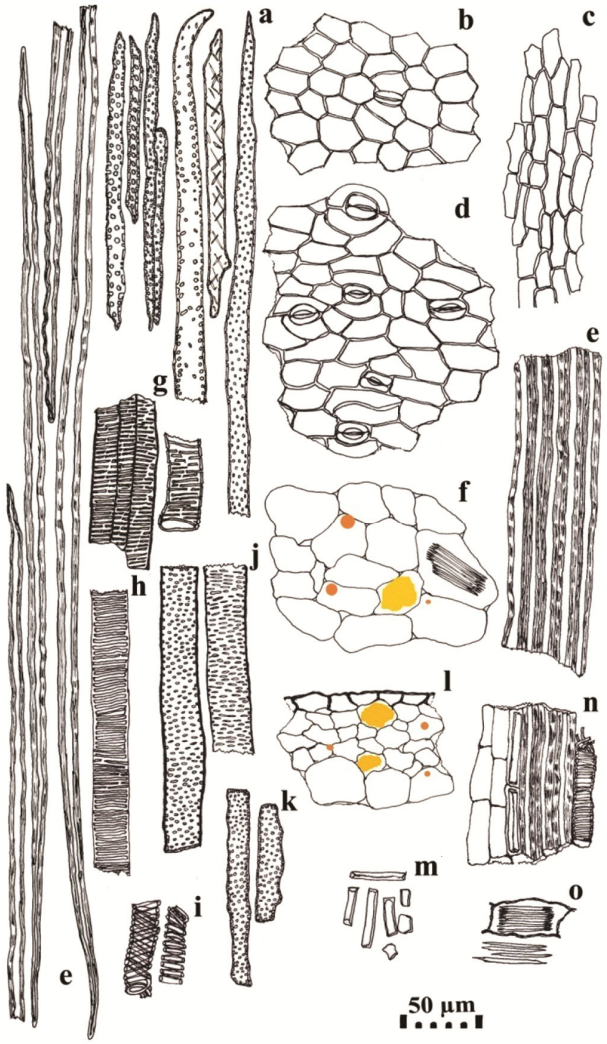


Fig. 3 — Powder microscopy of *C. fruticosa* leaf. a) thick and thin walled fibre-tracheids, b) upper epidermis in surface view with paracytic stomata, c) midrib epidermis in surface view, d) fragment of lower epidermis in surface view with paracytic stomata, e) thick walled, sharp end narrow lumen fibres, f) fragment of mesophyll parenchymatous tissue embedded with brownish content, oil globules and bundle of acicular crystals, g) fragment of vessels with reticulated thickening, h) scalar form vessels, i) spiral vessels, j) pitted vessels, k) tracheids, l) fragment of lamina in sectional view with brownish content, m) rod shaped prismatic crystals of calcium oxalate, n) fragment of fibre associated with vessels and parenchymatous cells with rod shaped crystals, o) raphide bundles of calcium oxalate.

ethanol respectively, were 8.64 and 28.29%. The drug's pH was found to be 6.68 indicating that the plant is acidic (Table 1).

Phytochemical analysis

The components of plant extracts were evaluated, and their predominance was determined through phytochemical screening which also looked for

Table 1 — Physico-chemical results of *C. fruticosa* leaf

Parameters	Results
Loss on drying (%)	5.92
Total Ash (%)	11.19
Acid insoluble Ash (%)	0.778
Alcohol soluble extractive (%)	28.29
Water soluble extractive (%)	8.64
pH (10% suspension)	6.68±0.02

Table 2 — Phytochemical results of methanol extract of *C. fruticosa* leaf

Tests	Results	Tests	Results
Acids	-	Protein	-
Alkaloid	+	Quinone	+
Anthraquinone	-	Reducing sugar	+
Cardiac glycosides	+	Saponins	+
Coumarin	+	Steroids	-
Flavonoids	+	Tannins	-
Glycosides	+	Triterpenoids	-
Phenol	+	Quinone	+
Alkaloid	+		

'+' represents presence and '-' represents absence

bioactive components that could be used in the formulation of medicinal drugs. The methanol extracts of *C. fruticosa* are subjected to a qualitative phytochemical examination in this current investigation, as shown in Table 2. Extracts were found to contain alkaloids, cardiac glycosides, cumarin, quinone, reducing sugar, saponins, phenols, and flavonoids. These phytochemicals may be the cause of *C. fruticosa'*s medicinal potential. One of the primary and most abundant substances that plants make is alkaloids, which are metabolic byproducts derived from amino acids and possess potent antibacterial, anti-HIV, and antiparasitic properties. It is well known that flavonoids contain antioxidant properties that prevent tumour development, growth, and spread. Numerous biochemical actions including antioxidant, antimutagenic, anti-carcinogenic, and gene-expression-modifying properties are possessed by phenols[32].

HPTLC

The methanol extract of the plant sample was analysed using thin-layer chromatography, photodocumentation showed 13 green bands (Fig. 4a) under short UV light with R_f values 0.07, 0.14, 0.19, 0.22, 0.27, 0.30, 0.33, 0.46, 0.47, 0.61, 0.69, 0.76, and 0.83. When it was exposed to long UV light, 11 bands (Fig. 4b) were visible with R_f values 0.16(red), 0.23(red), 0.27(red), 0.31(red), 0.37(fluorescent green), 0.44(red), 0.49(red), 0.56(fluorescent green), 0.61(red) & amp; 0.68(red). The postderivatized plate under white light exhibited 13 bands (Fig. 4c) with R_f values 0.01(black), 0.04(violet), 0.16(violet), 0.24(violet), 0.27(yellow), 0.33(violet), 0.38(violet), 0.42(pink), 0.48(pink),0.54(purple), 0.62(purple), 0.72(pink); 0.84(pink) (Table 3).

Further scanning of *C. fruticosa* showed 13 peaks (Fig. 4a) with R_f 0.07(area 0.86%), 0.14(3.33%), 0.19(1.13%), 0.22(2.06%), 0.27(6.96%), 0.30(2.41%), 0.33(8.22%), 0.46(3.97%), 0.47(3.78%), 0.61(27.81%), 0.68(21.18%), 0.76(12.68%) and 0.83(5.63%) under 254 nm. Scanning under long UV revealed 11 peaks (Fig. 4b) with R_f0.01(area 6.40%), 0.16(1.15%), 0.23(2.74%), 0.27(3.54%), 0.31(0.83%), 0.37(4.41%), 0.44(0.66%), 0.49(3.97%), 0.56(36.17%), 0.61(18.71%), and 0.68(21.41%). White light scanning of post derivatised plate under 520 nm displayed 13 peaks (Fig. 4c) with R_f 0.01(area 1.86%), 0.04(0.45%), 0.16(1.92%), 0.24(3.74%), 0.27(5.47%), 0.33(9.48%), 0.38(1.84%), 0.42(2.14%), 0.48(8.47%), 0.54(8.86%), 0.62(34.16%), 0.72(4.92%), and 0.84(16.69%).

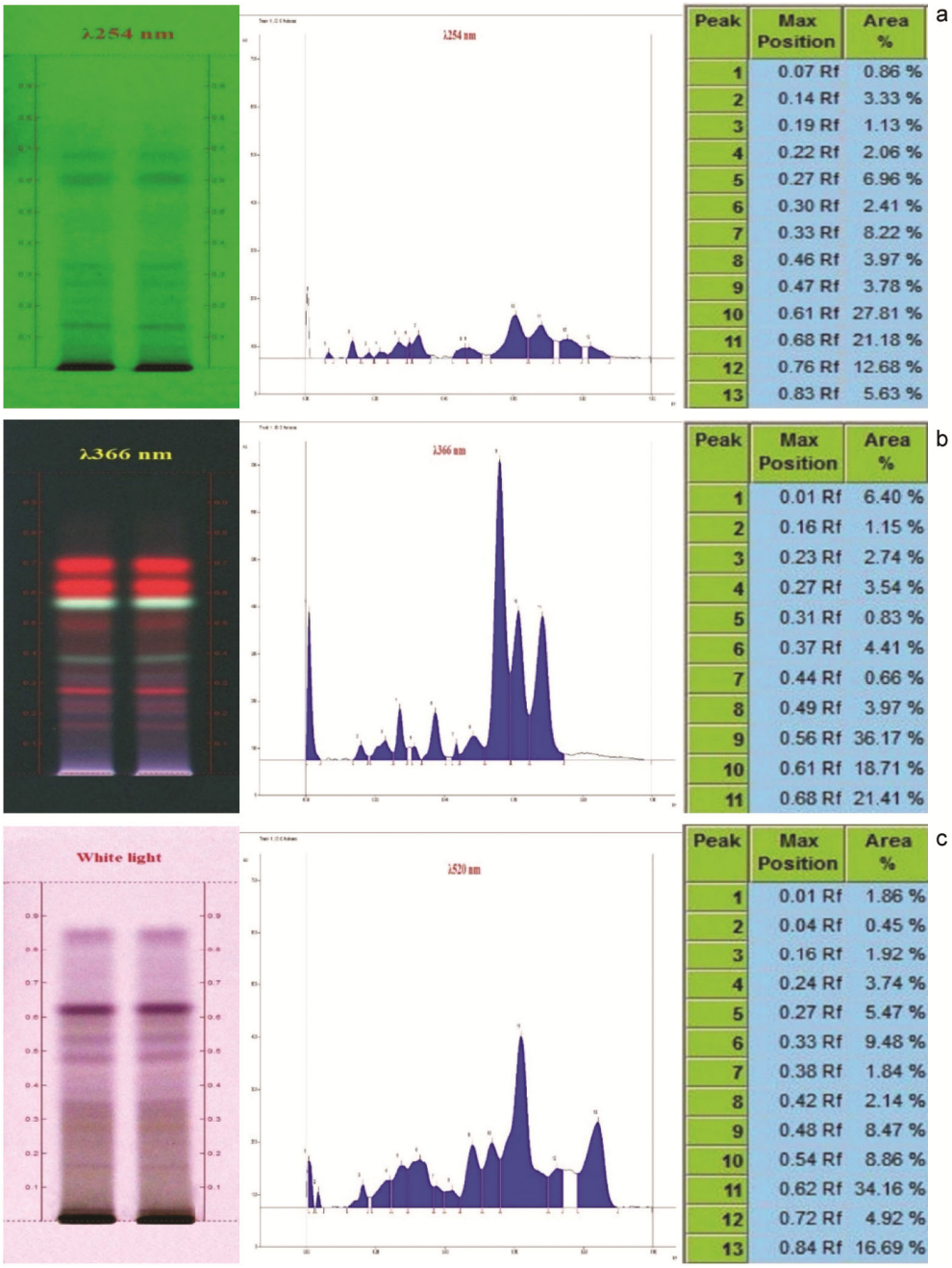
HPLC

HPLC analysis resulted in 12 peaks with retention time (RT) 1.241(0.365%), 1.496(1.191%), 2.875 (50.335%), 3.308(13.375%), 3.953(18.627%), 4.681 (5.878%), 8.273(2.503%), 15.532(2.535%), 17.619 (2.217%), 20.711(1.097%), 26.304(0.912%) and 28.033 (0.963%) (Table 4).

Peak with RT 2.875 (50.335%) was found to be the major, followed by peaks 3.953 (18.627%) and 3.308 (13.375%) in HPLC analysis in Fig. 5.

Antioxidant property

Plant components like flavonoids and phenolic compounds have a significant role in antioxidant activity. So, determining these chemicals in plants are crucial[33]. The antioxidant property of this plant leaf was determined by DPPH radical scavenging power and ABTS radical scavenging power methods. DPPH radical scavenging power of methanolic leaf extracts of *C. fruticosa* was increasing with increased concentration of sample solution and standard ascorbic acid solution which was shown in Fig. 6a. The maximum inhibition of *C. fruticosa* was 31.12% at the concentration 40 µg/mL and the maximum inhibition of standard ascorbic acid was 23.38% at the concentration 5 µg/mL. The IC_{50} values of the sample solution and the standard



Peak	Max Position	Area %
1	0.07 Rf	0.86 %
2	0.14 Rf	3.33 %
3	0.19 Rf	1.13 %
4	0.22 Rf	2.06 %
5	0.27 Rf	6.96 %
6	0.30 Rf	2.41 %
7	0.33 Rf	8.22 %
8	0.46 Rf	3.97 %
9	0.47 Rf	3.78 %
10	0.61 Rf	27.81 %
11	0.68 Rf	21.18 %
12	0.76 Rf	12.68 %
13	0.83 Rf	5.63 %

Peak	Max Position	Area %
1	0.01 Rf	6.40 %
2	0.16 Rf	1.15 %
3	0.23 Rf	2.74 %
4	0.27 Rf	3.54 %
5	0.31 Rf	0.83 %
6	0.37 Rf	4.41 %
7	0.44 Rf	0.66 %
8	0.49 Rf	3.97 %
9	0.56 Rf	36.17 %
10	0.61 Rf	18.71 %
11	0.68 Rf	21.41 %

Peak	Max Position	Area %
1	0.01 Rf	1.86 %
2	0.04 Rf	0.45 %
3	0.16 Rf	1.92 %
4	0.24 Rf	3.74 %
5	0.27 Rf	5.47 %
6	0.33 Rf	9.48 %
7	0.38 Rf	1.84 %
8	0.42 Rf	2.14 %
9	0.48 Rf	8.47 %
10	0.54 Rf	8.86 %
11	0.62 Rf	34.16 %
12	0.72 Rf	4.92 %
13	0.84 Rf	16.69 %

Fig. 4 (a-c) — HPTLC Chromatograms, fingerprint profiles and peak tables of leaf extract of *C. fruticosa*.

solution were also calculated. The IC_{50} values of the sample solution were calculated by using the equation y = 0.6301x + 5.69288 and the value was found to be 70.317±0.51849 µg/mL. In the same way, the IC_{50} value of the standard solution was calculated by using the equation, y = 4.30986x + 2.02452 and the value was found to be 11.13±1.29179 µg/mL. The IC_{50} suggests that the DPPH radical scavenging power of the sample solution is much less than that of the standard ascorbic acid solution.

The ABTS radical scavenging power of methanolic leaf extracts of *C. fruticosa* also increased with increased concentration of the sample solution and the standard trolox solution, as shown in Fig. 6b.

The maximum inhibition of *C. fruticosa* was 42.5% at the concentration 40 µg/mL and the maximum inhibition of standard trolox was 39.89% at the concentration 30 µg/mL. The IC_{50} value of the sample solution was 47.2348±1.56651 µg/mL, and it was calculated by using the equation y = 0.83916x +

Table 3 — R_f values and colour of spots

Under UV 254 nm		Under UV 366 nm		Under White light post Derivatisation	
R_f	Colour	R_f	Colour	R_f	Colour
--	--	--	--	0.03	Violet
0.06	Green	--	--	--	--
0.14	Green	0.15	Red	0.16	Violet
--	--	0.21	Red	--	--
0.23	Green	0.23	Red	0.24	Violet
0.27	Green	0.28	Red	0.28	Yellow
0.33	Green	--	--	0.32	Violet
--	--	--	--	0.35	Violet
--	--	0.38	F green	--	--
0.50	Green	0.49	Red	0.48	Violet
--	--	0.56	Green	0.54	Violet
0.61	Green	0.62	Red	0.62	Violet
0.69	Green	0.69	Red	--	--
--	--	--	--	0.73	Pink
--	--	--	--	0.84	Violet

F for Fluorescent

Table 4 — HPLC peak table of *C. fruticosa*

Peak	Retention Time	Area	Concentration (%)
1	1.241	8218	0.365
2	1.496	26816	1.191
3	2.875	1133314	50.335
4	3.308	301155	13.375
5	3.953	419402	18.627
6	4.681	132350	5.878
7	8.273	56362	2.503
8	15.532	57087	2.535
9	17.619	49916	2.217
10	20.711	24707	1.097
11	26.304	20538	0.912
12	28.033	21682	0.963

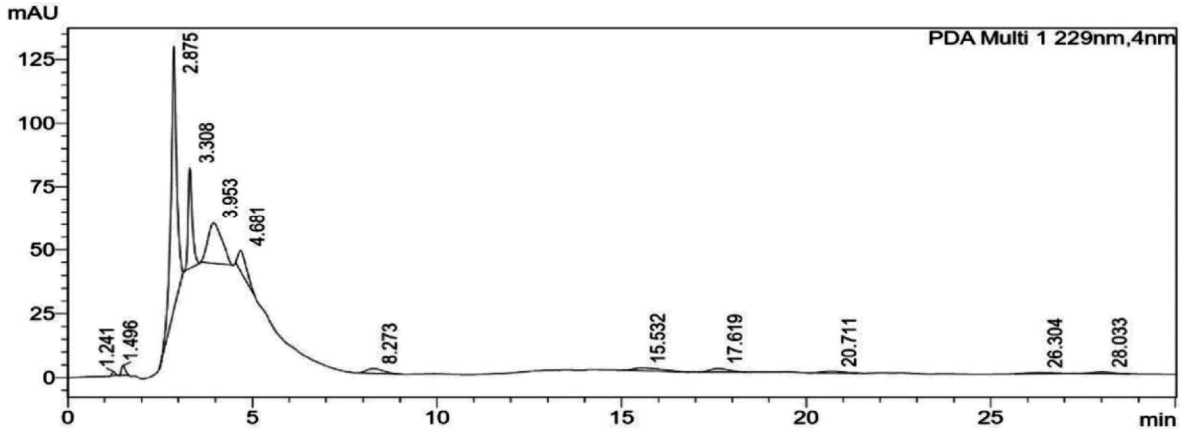


Fig. 5 — HPLC chromatogram of methanolic leaf extracts of *C. fruticosa*.

10.3624. The IC_{50} value of the standard solution was 37.6146±1.24248 µg/mL, and it was calculated by using the equation, y =1.2243x + 3.94833. These IC_{50} values suggest that the ABTS radical-scavenging activity of the sample solution is almost equal to the standard trolox solution.

The total phenolic and flavonoid content in *C. fruticosa* leaves was also determined. The total

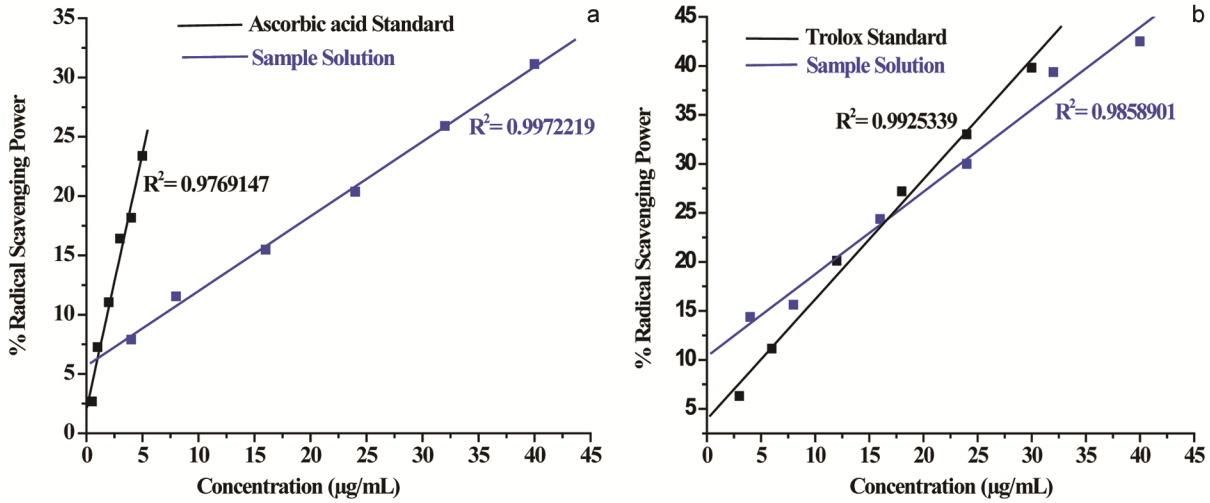


Fig. 6 — Antioxidant activity of methanolic leaf extracts of *C. fruticosa* by a) DPPH method, and b) ABTS method.

phenolic content in the methanol extract of *C. fruticosa* was found to be 34.92±0.01808 µg GAE/mg of dry extract, and the total flavonoid content of *C. fruticosa* was found to be 0.7298± 0.00162 µg QE /mg of dry extract.

Conclusion

The current research is the initial account of the detailed macroscopic, powder microscopic, HPTLC, HPLC, physicochemical, antioxidant, total phenolic and total flavonoid content of *C. fruticosa*. The high alcohol extractive value of *C. fruticosa* leaf indicates that a sufficient amount of phytochemical compound might be present when it is dissolved in alcohol. The phytocompound is a major contributor to its medicinal importance. HPLC and HPTLC fingerprint profiles of methanolic leaf extract also support the presence of different phytocompounds. The DPPH and ABTS radical scavenging activity of the methanolic extract of *C. fruticosa* indicates that the plant leaf has a significant antioxidant property. The phenolic and flavonoid content also supports its nutritional values and antioxidant properties. Further studies must be carried out to evaluate its medicinal applications by performing detailed phytochemical and pharmacological studies. These details, as well as macroscopic, powder microscopic, HPTLC, HPLC, and physicochemical data will help to characterise the leaf of *C. fruticosa* for further research.

Conflict of interest

The authors declare that they have no conflict of interest.

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