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Phytochemical Profile, in vitro Antioxidant Potential and HPTLC Fingerprinting of *Acalypha indica* Linn. Leaves

Research Article

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Abstract

The present study aims to standardize and evaluate bioactive profiling of *Acalypha indica* L. leaves through pharmacognostic parameters, phytochemical screening and High-Performance Thin Layer Chromatography (HPTLC) analysis. Comprehensive standardization was carried out using physicochemical constants, including moisture content, total ash, acid-insoluble ash, and extractive values, by pharmacopoeial guidelines. Macroscopic and Microscopic analysis confirmed key diagnostic features of leaves for identification and quality control profiling. Preliminary phytochemical screening of the ethanolic extract revealed the presence of bioactive constituents such as flavonoids, alkaloids, tannins, glycosides, and phenolic compounds. Antioxidant activity was assessed using in vitro assays including DPPH, ABTS, Superoxide, Hydroxyl radical, Nitric Oxide scavenging, and CUPRAC methods. The extract exhibited significant free radical scavenging potential, particularly in the CUPRAC and Superoxide assays. HPTLC analysis showed the best separation of bands at different retention factors (Rf) when using a solvent system of Toluene: Ethyl acetate: Glacial acetic acid in the ratio of (7:2:1 v/v/v). The quantitative estimation revealed that 10 mg of the ethanolic extract of *Acalypha. indica* L. contained 13.90 µg of kaempferol.

Keywords: HPTLC, Standardization, Antioxidant, *Acalypha indica L.*

Introduction

Acalypha indica L., commonly known as *Indian acalypha* (1). belongs to the Acalypha genus species, which is the fourth largest genus in the Euphorbiaceae family. Many plants from this family are used as medicinal herbs in Asian and African regions (2). *Acalypha indica* L. is a prevalent weed medicinal plant that grows across the plains of India (3). *Acalypha indica* L. thrives in wet, tropical regions and temperate countries across Asia (4). This plant is renowned for its notable medicinal properties, which are widely recognised for their human health benefits. This traditional medicinal plant is well-known among older generations in various countries, particularly in Africa and Asia (5). It is commonly found in regions such as a troublesome weed in gardens, along roadsides, and across the plains of India (6). *Acalypha canescens* Wall and *Acalypha ciliata* Wall are invalid synonyms for the plant, and the accepted name to refer to this plant is *Acalypha indica* L. It has been recognized by local communities for its value, widely used in traditional medicine for various therapeutic purposes (7). Extracts from different parts of the *Acalypha indica* L plant, including the leaves, roots, and stems, are widely utilised in the traditional healing practices across several countries (8). The plant has been reported to aid in the management of conditions such as pneumonia, asthma, scabies, and rheumatism. Additionally, it exhibits diuretic, purgative, and anthelmintic properties (3), (8). Furthermore, *Acalypha indica* L. is also recognised for its potential to lower Blood sugar levels (5). Chemical compounds found in *Acalypha indica* L. include acalyphamide, aurantiamide and its acetate, succinimide, calypholactate, 2-methyl anthraquinone, tri-O-methyl ellagic acid, β-sitosterol and its β-D-glucoside, primarily present in the leaves. The plant also contains a cyanogenic glucoside, acalyphine, two alkaloids—acalyphine and triacetonamine. Additionally, it contains kaempferol, quebrachitol, β-sitosterol acetate, tannins (present in the entire plant), and stigmasterol (found in the roots) (9). More recently, four kaempferol glycosides, mauritianin, clitorin, nicotiflorin, and biorobin, have been isolated from the flowers and leaves of the plant (8). This plant is extensively utilized in the treatment of various metabolic and cellular diseases, including diabetes, cancer (10). The antioxidant activity of plant extracts can be measured in vitro using different assay methods. Polyphenolic compounds, flavonoids, play a crucial role in neutralizing free radicals (11). The current study aims to explore the phytochemical constituents, perform qualitative and quantitative analyses, evaluate the antioxidant activity of crude ethanolic extract, and chromatographic profiling by HPTLC.

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Materials & Methods

Plant collection

Acalypha indica L. leaves were collected locally in Chatgaon, located in the Gadchiroli district of Maharashtra. The leaves were authenticated by Prof. Nitin Dongarwar, botanist, Department of Botany, Rashtrasant Tukadoji Maharaj Nagpur University, Nagpur. The herbarium specimen was deposited in the Department of Botany, Nagpur, with the Herbarium sheet number 10071.

Chemicals and reagents

The reagents were utilized for studies: 2,2-Diphenyl-1-Picrylhydrazyl (DPPH), Gallic acid (GA), Cupric chloride ($CuCl_2$), ABTS, trichloroacetic acid which were purchased from SRL chem Laboratories, Ascorbic acid (AA), DMSO, EDTA, Trio barbituric acid (TBA) were purchased from HiMedia, Hydrogen peroxide (H_2O_2) was purchased from Neurochem Laboratories, Trichloro acetic acid (TCA), ferric chloride and sodium nitroprusside were procured from Fisher Scientific, while Sodium phosphate was procured from Rankem laboratory.

Macroscopic and Microscopic Study of *Acalypha indica* Linn.

The macroscopic study was performed on a dry sample. The Macroscopic study involved colour, odour, surface texture, size, and shape of leaves, and microscopic studies of the leaf powder sample were done using a Leica microscope (DM 2000 LED). For a microscopic study, fresh leaves were collected and soaked in acetone to remove excess chlorophyll for better results. The Transverse section (T.S.) was taken using a razor blade. The section was stained with phloroglucinol and HCl and observed under the Leica microscope using a 10x magnification lens (12), (13).

Physicochemical parameters of crude drug

Determination of Moisture Content

A 2 g sample of *Acalypha indica* L. leaves powder and placed in a crucible. The sample was then dried in an oven at 105°C for 3-5 hrs. Initial drying phase, the powder was removed, allowed to cool in a desiccator, and weighed again. The sample was weighed every hour while the drying process continued at 105°C. This procedure was repeated until a constant weight was obtained. The loss in weight was used to calculate the percentage of moisture content representing the loss on drying (12).

Determination of Total Ash

A 2 g sample of the powder was taken into a silica crucible, which had been previously weighed and kept in a muffle furnace until the sample ignited completely. After cooling, the crucible was weighed again. The total ash content was calculated and expressed as a percentage.

% Total ash value = Wt. of ash /Wt. of drug X 100

Determination of acid-insoluble ash

The total ash obtained was boiled with 25 mL of 2 M HCL for 5 minutes, insoluble residue was collected on ashless filter paper, and the residue was ignited for 10-15 minutes at a temperature not exceeding 450°C, then cooled and reweighed. The acid-insoluble ash was then calculated and expressed as a percentage (12).

Determination of water-soluble ash

The total ash obtained was boiled for 5 minutes with 25 mL of water. The insoluble matters were collected on ashless filter paper and washed with hot water. and residue was ignited for 10-15 minutes at a temperature not exceeding 450 °C, then cooled and reweighed, and water-soluble ash was calculated and expressed as a percentage (12), (13).

Determination of the extractive value of *Acalypha indica* L.

Extractive values are helpful to evaluate the nature of constituents present in the crude drug. Water-soluble extractive and alcohol soluble extractive values were determined as per standard procedures. 5 gm of coarsely powder plant material weighed and transferred into a dry 250 mL conical flask then flask was filled with water and alcohol 30 ml separately flasks were corked and kept for 24 hrs at RT with shaking frequently the mixtures were filtered through filter paper the obtained extracts were concentrated to dryness and the extractive value in percentage was calculated (12).

Extraction of *Acalypha indica* Linn.

The leaves of *Acalypha indica* L. were thoroughly cleaned and shade dried. Once dried, the plant material was ground well into coarse powder using a mortar and pestle. A total of 100g of powdered plant material was initially defatted using petroleum ether and extracted with ethanol by maceration. The extraction process was carried out over seven days. The extraction mixture was filtered using a Whatman filter paper no .01. The solvent was recovered by rotary evaporator and drying the extract.

Phytochemical screening

The Phytochemical screening was carried out on ethanol extracts of *Acalypha indica* L. leaves. identify the active phytoconstituents, including alkaloids, glycosides, flavonoids, steroids, saponins, etc., present in the ethanolic extract using the following standard phytochemical tests.

Test for alkaloids

Dragendroff's reagent test: 2 mL of extract was heated with 2% H_2SO_4. A small amount of Dragendroff's reagent was added, and orange-red precipitate was observed.

Mayer's test: 1-2 mL of ethanolic extract was taken into a test tube, then 1-2 drops of Mayer's reagent were added. The result was positive; a creamy white precipitate formed.

Wagner's test: 1-2 mL of extract solution was taken, and 1- 2 drops of Wagner's reagent were added. then, a brown precipitate formed.

Test for glycoside

Borntrager test: 1- 2 mL of extract solution was taken, 2-3 mL of Chloroform was added, then shaken, and the chloroform layer was separated. 10% ammonia solution added. The result was positive, a red coloured solution formed.

Test for flavonoids

Shinoda test: 1- 2 mL of extract was taken, and 5 mL of ethanol added, then added few grains of magnesium, turnings, with a few drops of conc. HCL, the sample was positive for flavonoids, red to pink coloured

Test for Phenolic compounds

Gelatin test: The ethanolic extract was dissolved in 5 mL of distilled water, followed by the addition of 1% gelatin solution and 10 % sodium chloride solution, resulting in the formation of a white precipitate.

Test for Steroids

Salkowski's test: The Alcoholic extract solution was taken, and a few drops of concentrated sulphuric acid were added to the sample was positive for steroids when a red colour formed in the lower layer (12), (15).

Test for tannins

Nitric acid test: 2- 3 mL of the ethanolic extract was taken in a test tube, a few drops of dil. Nitric acid was added, then a reddish yellow colour formed (16).

Test for fixed fats and oils

Spot test/stain test: a small quantity of plant extract is pressed between to filter papers, then an oil stain appears on the paper.

Test for saponin

Foam formation test: 2 mL of aq. the solution was taken into a test tube and shaken vigorously. If foam was formed and did not disappear for 5 min. (15)

Quantitative analysis of the ethanolic leaves extract of *Acalypha indica* L.

Total phenolic content

The Folin-Ciocalteu method was used to determine the total phenolic content (TPC) of the ethanolic extract of *Acalypha indica* L. leaves (17). Gallic acid was used as the standard. A standard calibration curve was plotted using gallic acid solution at concentrations (10 µg/ml to 50 µg/ml). A stock solution of extract was prepared by dissolving 10 mg of plant extract in 10 mL of methanol. It was diluted even further to get a solution of 100 µg/ml., 1.5 mL of a 20% sodium carbonate (Na_2CO_3) solution was added to 1 ml of this solution, which was then mixed with 0.5 mL of 2 N Folin-Ciocalteau reagent (FCR) for the experiment. To enable the reaction, this solution was left in the dark for two hours. The absorbances were measured with a UV spectrophotometer (Shimadzu UV-2401PC) at 765 nm (18), (19), (20).

Total flavonoid content

The total flavonoid content of the plant extract, determined using the aluminium chloride colorimetric method. A stock solution of plant extract was prepared by dissolving 10 mg sample in 10 mL of methanol. A 10 mL volumetric flask containing 20% methanolic aluminium trichloride was added with an aliquot of 2 mL of the extract and standard solutions of Rutin (0.5 mg/mL). A few drops of acetic acid were added, and the combination was left to stand for 40 minutes. The absorbance of the resulting solution was determined at 415 nm using a spectrophotometer. A standard curve was prepared using varying concentrations (21).

Total tannin content

10 mg of ethanolic extract was dissolved in 10 mL of methanol as a stock solution. The Folin-Ciocalteu Reagent (FCR) was added to 1 mL of this, further treated with 1.5 mL of a 20% sodium carbonate (Na_2CO_3) solution. To enable the reaction, the resulting mixture was left in the dark condition for 15 minutes. Similarly, the standard calibration curve was prepared using the same process as tannic acid as the reference., The absorbance of the resulting solution was determined at 775 nm using a spectrophotometer (22).

Total alkaloid content

A gravimetric analytical method was used to assess the total alkaloid content in the plant material. A 5 g of powdered crude drug was repeatedly extracted with 0.1 N Sulfuric acid (H^2SO^4) in an ultrasonic bath (3x50 ml). The resulting solution was filtered, and the combined acid solution was rinsed four times with 25 mL of chloroform. The chloroform washings were discarded. The acid solution was basified by adding dilute ammonia solution and further extracted with diethyl ether. The combined diethyl ether extracts were washed with distilled water, and the ether was evaporated to dryness in a weighted beaker. The dried extract was then further dried to a constant weight. The total alkaloid content was calculated and expressed as % w/w (23).

Comparative analysis of antioxidant activity using different in vitro assays

DPPH assay

Antioxidant activity is mostly measured using the DPPH radical scavenging test. This technique, which is based on electron transfer, uses an antioxidant to donate electrons to neutralise the stable DPPH radical, which is distinguished by its deep purple. In a 96-well plate, 0.1 mL of a 0.1 mM DPPH solution was mixed with 5 µL of various doses of the test chemical. For every concentration, the reaction was carried out in triplicate, and duplicate blanks were made by mixing 5 µL of the corresponding chemical concentration with 0.2 mL of DMSO/Methanol. For half an hour, the plate was incubated in the dark. Then absorbance was measured at 517 nm against a blank methanol using a UV-Vis

spectrophotometer (Shimadzu UV-2401PC). using a microplate reader (iMark, BioRad). A reaction mixture with 20 µL of deionised water was used as the control. Scavenging activity was expressed as % inhibition relative to the control. The IC_{50} value was calculated using GraphPad Prism 6 software. (24), (25).

%Inhibition = Abs.(control)−Abs. (Sample) /Abs. (control) × 100

Hydroxy Free Radical Scavenging Assay

A reagent mixture was prepared by combining 10 µL of 0.5 M EDTA, 24.14 mg of Deoxyribose, 88 µL of $FeCl_3$ (10 mg/mL), and 28 µL of H_2O_2 (6%), adjusted to a final volume of 33 mL with water. In each well of a 96-well plate, 10 µL of plant extract, 24 µL of phosphate buffer (50 mM, pH 7.4), and 10 µL of ascorbic acid were added to each well, followed by the reagent mixture. The plate was incubated at 37°C for 1 hour. The wells without treatment served as the control, and Gallic Acid was used as the standard. After incubation, 50 µL of 10% TCA and 50 µL of 1% TBA were added to each well. A pink chromogen formed, and absorbance was measured at 540 nm using a microplate reader. IC_{50} was calculated using the Software GraphPad Prism 6. A graph was prepared between X axis (Sample Concentration) Vs. Y axis (% inhibition w.r.t. control (26), (27).

%Inhibition = Abs.(control)−Abs. (Sample) /Abs. (control) × 100

Reactive Nitrogen Oxide Scavenging Assay

A reaction mixture was prepared containing 50 µL of 10 mM sodium nitroprusside, 40 µL of distilled water, and 10 µL of gallic acid. The reaction mixture without treatment was used as the control. The mixture was pre-incubated at room temperature for 15 minutes in the presence of light. After incubation, 100 µL of Griess reagent was added to both the test and control wells. The plate was then incubated for 5-10 minutes at room temperature to allow for chromophore development and stabilization. Absorbance was measured at 540 nm and 660 nm using a microplate reader. The IC_{50} value was calculated using GraphPad Prism 6 software (28).

%Inhibition = Abs. (control)−Abs. (Sample) /Abs. (control) × 100

Super Oxide Anion Radical Scavenging Assay

Different concentrations of the extract (ranging from 1 to 1000 µg/mL) and riboflavin solution (used as the standard, ranging from 1 to 50 µg/mL) were prepared. The reaction mixture was then incubated for 30 minutes in a 96-well plate under light at room temperature. After the incubation, the reaction mixture was added to the previously incubated sample and thoroughly mixed. The wells without treatment were considered the control. Absorbance was then measured using an ELISA plate reader at 560 nm. The IC_{50} value was calculated using GraphPad Prism 6 software (29).

%Inhibition = Abs. (control)−Abs. (Sample) /Abs. (control) × 100

ABTS Radical Scavenging Assay

ABTS radicals were generated by mixing a 2.45 mM APS solution with a 7 mM ABTS solution, which was then diluted 100-fold to prepare the ABTS free radical reagent. To evaluate antioxidant activity, 10 µL of different concentrations of ascorbic acid (standard) and the test samples were added to 200 µL of the ABTS free radical reagent in a 96-well plate. The plate was incubated at room temperature for 10 minutes in the dark. Control wells, containing no treatment, were included for comparison. After incubation, the absorbance of the decolorized solution was measured at 750 nm using a microplate reader. The results were expressed relative to the negative control (30), (31), (32).

%Inhibition = Abs. (control)−Abs. (Sample) /Abs. (control) × 100

CUPRAC Assay

10µl of different concentrations of the sample were added to defined wells of a 96-well plate. Then, 200µl of reagent mixture was added. Reaction mixture in triplicate form and blank in duplicate form were prepared, containing 200µl Methanol and 10µl of compound of different concentrations for sample and standard (Trolox – Ottokemi - Cat no–T7723) and incubated for 30 minutes in the dark. The wells without treatment were considered as controls. At the end of the incubation, absorbance of the decolorization was measured at 490 nm (33).

%Inhibition = Abs.(control)−Abs. (Sample) /Abs. (control) × 100

HPTLC Fingerprint Analysis

A CAMAG HPTLC system equipped with a Linomat 5 applicator fitted with a 100 µL syringe, a CAMAG TLC scanner, and visionCATS software was used.

Sample Preparation and Application

0.1 mg/mL of standard (Kaempferol) solution was prepared in HPTLC grade methanol and 10 mg/mL ethanolic crude extract was prepared in methanol of chromatographic grade and filtered through Whatman filter paper no .01 prepared extract and standard were applied on a TLC aluminium sheet, silica gel 60 F 254 (Merck). Standard reference solution kaempferol (1 to 8 µL) and sample ethanolic extract (2 to 14 µL) were applied sequentially on a 200 ×100 mm plate, each with a band length of 8.0 mm, using a CAMAG Linomat 5 sample applicator fitted with a 100 µL syringe, set at a dosage speed of 150 nL/s.

Sample system development

For the separation of phytoconstituents in the extract, various solvent systems were tried. Optimal resolution and the maximum number of spots were obtained in the solvent system Toluene: Ethyl acetate: Glacial acetic acid in the ratio 7:2:1 (14).

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Development of Chromatogram

The TLC plate was developed in a twin trough glass chamber of 20 ×10 cm and saturated the mobile phase system for 20 min at RT up to a distance of 70 mm. The standard and sample-loaded plates were developed in an automated development chamber. The plate was then allowed to dry at room temperature for 10 min. the Rf values and colour of the bands were noted.

Scanning and detection of spots

The dried plate was visualised using under UV cabinet at 254nm and 366 nm. All spots are UV visible in the extract, and spectrum scanning of the developed plate was performed on a CAMAG TLC scanner 4 using the deuterium and tungsten lamp at 190-450 nm wavelength and a spectrum speed of 20 nm/s. The separation of per track was used, and Rf values were noted.

Results And Discussion

Macroscopic and Microscopic Study

The leaves of *Acalypha indica* L. are ovate to rhomboid in shape, generally measuring 4 to 5 cm in length and 3-4 cm in width. the upper surface is dark green, while the lower surface is pale green. Leaf margins are serrate to crenate, and the petiole is notably long, from 1 to 8 cm, as observed in macroscopic studies fig.1 and 2. The transverse section (TS) of *Acalypha indica* L. reveals a differentiated structure comprising both upper and lower epidermal layers. Beneath the epidermis, parenchyma cells are present, consisting of loosely arranged, thin-walled cells. Vascular bundles are visible, exhibiting a reddish coloration, and non-glandular types of trichomes are observed in Fig. 3 on the epidermal surface under the Leica microscope (DM 2000 LED). Microscopic evaluation of the powdered material revealed several diagnostic features. Xylem vessels (A) appeared as thick-walled, elongated structures. Parenchyma cells (B) were noted to be thin-walled and polygonal in shape. Volatile oil globules (C) were observed as spherical droplets. Starch grains (D) were observed when iodine solution was applied. Under polarized light, needle-shaped and crystal-shaped calcium oxalate crystals (E) became distinctly visible with 60% H_2SO_4, as shown in Fig. 4.

Table 1: Macroscopic parameters

Macroscopic parameters	Observation
Shape	Ovate to rhomboid
Size	Length – 4 to 5 cm, Width- 3 to 4 cm
Colour	Upper dark green and lower pale green
Margin	Serrate-crenate
Petiole	Very long (1 to 8 cm)

Physicochemical study of crude drug

The percentage of moisture content, total ash, acid-insoluble ash, and water-soluble was carried out and the results are shown in Table 2. The physicochemical standardization parameters results showed that moisture content, total ash, acid insoluble ash, and water-soluble ash values are 6.50±0.06, 14±0.03, 5.6±0.05, and 3.4±0.04 % w/w, respectively. The alcohol soluble and water-soluble extractive values are determined. the alcohol soluble extractive value indicates the presence of polar phytoconstituents like flavonoids, glycosides, phenols, etc., and the water-soluble extractive value indicates the presence of sugar, amino acids. The alcohol and water-soluble extractive values of *Acalypha indica* L. were 12 ±0.02 % and 17.5 ±0.08 %, respectively.

Figure 1: Upper surface

Figure 2: Lower surface

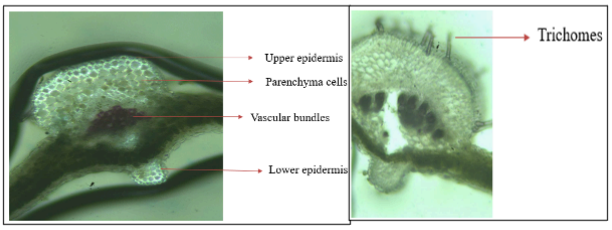

Figure 3: T.S. of *Acalypha indica* L

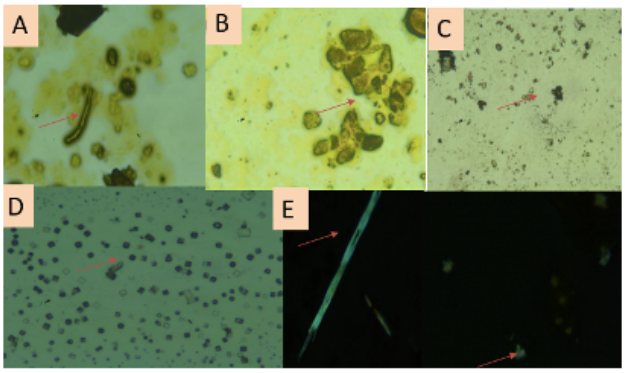

Fig. 4: Powder microscopy of leaves of *Acalypha indica* L

Table 2: Physicochemical Standardization Parameters

Sr. no	Physicochemical parameters	% yield (%w/w)
1	Moisture content	6.50 ± 0.06
2	Total ash value	14.5±0.03
3	Acid insoluble ash	5.6±0.05
4	Water-soluble ash	3.4±0.04
5	Water-soluble Extractive value	17.5 ±0.08
6	Alcohol-soluble extractive value	12 ±0.02

*Values were the means ±SEM

Phytochemical screening

Phytochemical screening (Table 3) revealed that the leaf extract of *Acalypha indica* L. is rich in secondary metabolites, particularly flavonoids, alkaloids, glycosides, tannins, and phenolic constituents present and saponins and fixed fats and oils were absent in the ethanolic extract.

Table 3: Phytochemical screening of leaves extract of *Acalypha indica* L

Sr.no.	Phytochemical tests	Observation	Result
1	Alkaloids Dragendroff's test Mayer's test Wagner's test	A reddish-brown precipitated White precipitated Brown precipitated	+ ve + ve + ve
2	Glycosides Borntrager test	Red colour solution formed	+ ve
3	Tannins Ferric chloride test	Greenish blue colour formed	+ ve
4	Phenolic compound Gelatin test	White precipitated	+ ve
5	Flavonoids Shinoda test	Red to pink precipitated	+ ve
6	Steroids Salkowski's test	Red colour formed at a lower layer	+ ve
7	Saponin Foam test	No foam formed	- ve
8	Fixed Fats and oils Spot test	No oil spot observed	- ve

Key: +ve = Positive test, - ve = Negative

Quantitative estimation

Total phenolic content was expressed as microgram of gallic acid equivalent per mg of crude extract (µg GAE/mg), Total flavonoids content was expressed as microgram of rutin equivalent per mg of extract (µg RT/mg), and total tannin content was expressed as mg of tannic acid equivalent per mg of crude extract. (µg TA/mg). Total phenolic, flavonoids, and tannins content were recorded as 220.9 ± 0.83, 68.50 ± 0.33, and 21.65 ± 0.41, respectively, in Table 4. The alkaloid content was determined to be 0.4 % w/w of the total yield.

Table 4: Quantitative estimation

Sr.no	Quantitative parameters	Result(µg/mg)
1	Total phenolic content (µg GAE/mg)	220.9 ± 0.83
2	Total flavonoid content (µg RT/mg)	68.50 ± 0.33
3	Total tannin content (µg TA/mg)	21.65 ± 0.41

*Values were the means ± SEM

Comparative analysis of antioxidant activity

The in vitro antioxidant activities of ethanolic extracts of *Acalypha indica* L. were evaluated using various antioxidant assays, as shown in Table 5.

The antioxidant activity was evaluated by using different in vitro assays. The DPPH assay showed modest activity with an IC_{50} of 415 ± 0.05 µg/ml. The assays for Superoxide Scavenging and Hydroxyl Radical showed stronger antioxidant activity, with IC_{50} values of 13.07 ± 0.53 µg/ml and 16 ± 0.16 µg/ml, respectively. The CUPRAC assay also showed significant potency (IC_{50} = 9.29 ± 0.9 µg/ml). The IC_{50} values for the Nitric Oxide and ABTS tests were 510.9 ± 0.20 µg/ml and 28.28 ± 0.06 µg/ml, respectively.

Table 5: Comparative analysis of antioxidant activity using different in vitro assays

Sr. no	Antioxidant assay	Name of Standard	IC_{50} value
1	DPPH radical quenching capacity	Ascorbic acid	415 ± 0.05
2	Hydroxy Free Radical Scavenging Assay	Gallic acid	16±0.16
3	Reactive Nitrogen Oxide Scavenging Methods	Gallic acid	510.9 ± 0.20
4	Super Oxide Anion Radical Scavenging Assay	Gallic acid	13.07 ± 0.53
5	ABTS Radical Scavenging Assay	Ascorbic acid	28.28 ± 0.060
6	CUPRAC	Trolox	9.292± 0.09

*Values were the means ± SEM

HPTLC analysis of the ethanolic extract of *Acalypha indica* L.

Fingerprint profiling and separation of bioactive phytoconstituents of the ethanol extract of *Acalypha indica* L. (Leaves) were carried out using HPTLC, with kaempferol used as a standard reference compound. The best separation of the bands was achieved when using the solvent combination of Toluene: Ethyl acetate: Glacial acetic acid in the ratio (7:2:1,v/v/v). The plate was visualised under UV at 254 nm and 366 nm. The plate scanned at 254 nm for the standard reference compound shows that kaempferol has an Rf value of 0.410. The quantitative estimation revealed that 10 mg of the ethanolic extract of *Acalypha. indica* L. contained 13.90 µg of kaempferol.

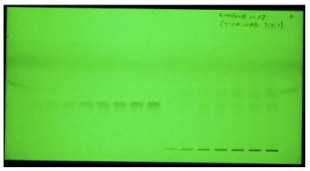

Fig.5 : Scanned image of kaempferol and sample at 254 nm

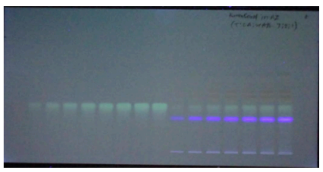

Fig.6: Scanned image of kaempferol and sample at 366 nm

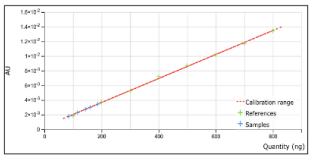

Fig. 7: Area calibration for standard kaempferol

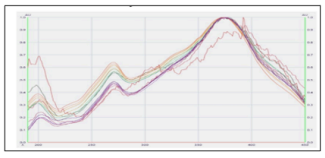

Fig.8: Spectrum scan of 190-450

Discussion

The standardization of *Acalypha indica* L. powdered crude drug by different physicochemical parameters was evaluated, including moisture content, total ash value, acid insoluble value, and extractive values evaluated to assess the purity and stability of the crude drug. The results of the phytochemical screening show that the ethanolic extract contains alkaloids, flavonoids, polyphenolic compounds, tannins, saponins, phenols, steroids, and glycosides. The saponins and fixed fats, and oils were absent. Macroscopic evaluation of the leaves further supports the identification of the plant. The leaves were observed to be ovate to rhomboid in shape with serrate to crenate margins, and

the petiole was generally 1 to 8 cm in length. In microscopic evaluation, Vascular bundles are visible, exhibiting a reddish coloration, and non-glandular types of trichomes are present. Total phenolic, flavonoids, and tannins content were recorded as 220.9 ± 0.83 µg/mg, 68.50 ± 0.33 µg/mg, and 21.65 ± 0.41 µg/mg, respectively. The DPPH assay showed modest activity with an IC_{50} of 415 ± 0.05 µg/ml. The assays for Superoxide Scavenging and Hydroxyl Radical showed stronger antioxidant activity, with IC_{50} values of 13.07 ± 0.53 µg/ml and 16 ± 0.16 µg/ml, respectively. Optimal separation was achieved using a mobile phase solvent system comprising Toluene: Ethyl acetate: Glacial acetic acid (7:2:1, v/v/v) in HPTLC analysis. Quantitative analysis further confirmed the presence of kaempferol in the ethanolic extract, with 13.90 µg in 10 mg of crude extract. These findings support the use of HPTLC as a reliable method for standardization and quality assessment of flavonoid content in *Acalypha indica* L.

Conclusion

In the present study, Phytochemical screening revealed that the leaf extract of *Acalypha indica* L. is rich in secondary metabolites, particularly flavonoids, Alkaloids, glycosides, tannins, and phenolic compounds. The presence of these compounds is consistent with the traditional medicinal uses of *Acalypha indica* and supports its potential antioxidant and therapeutic properties. Comparative analysis of antioxidant activity using different in vitro assays findings imply that the extract has strong antioxidant qualities, especially against cupric, superoxide, and hydroxyl radicals. In the HPTLC profiling analysis, kaempferol was quantified. Rf value of kaempferol found to be 0.410, when the mobile phase solvent system comprising Toluene: Ethyl acetate: Glacial acetic acid (7:2:1, v/v/v), 13.90 µg of kaempferol was found in 10 mg of leaves extract of *Acalypha indica* L.

Abbreviations

- **DPPH:** 2,2-diphenyl-1-picrylhydrazyl
- **ABTS:**2, 2′-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid
- **IC 50**: 50% Inhibitory concentration
- **TPC**: Total polyphenol content
- **TFC**: Total flavonoid content
- **GAE:** Gallic acid equivalent
- **RE:** Rutin equivalent
- **TA**: Tannic acid equivalent
- **HPTLC**: High-performance thin-layer chromatography
- **FCR**: Folin-Ciocalteu Reagent
- **Rf:** Retention factor
- **RT:** Room temperature

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