Original Article

EVALUATION OF PHYTOCHEMICAL AND IN VITRO ANTIINFLAMMATORY AND ANTIMICROBIAL ACTIVITY OF ACALYPHA INDICA (KUPPAIMENI OR HARITA MANJARI) AGAINST MICROBES CAUSING SKIN DISEASES

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Abstract

Acalypha indica, also known as Indian Copperleaf,(Kuppaimeni) is a tropical medicinal plant found worldwide. The plant has long been used to treat a variety of ailments, including skin infections, inflammation, and fever.

According to recent research, Acalypha indica contains a variety of phytochemicals, which include flavonoids, tannins, and alkaloids, which have anti-inflammatory and antimicrobial properties. Given these properties, the plant could be utilized to treat pathogen-caused skin infections caused by bacteria and fungi. The dried leaves with methanolic extract was subjected to phytochemical analyses, antiinflammatory effect and antimicrobial effects

The antimicrobial assay revealed that the methanolic extract of Acalypha indica leaves was significantly effective against various microorganisms, including bacteria and fungi. The extract was found to have a strong inhibitory effect on the growth of Staphylococcus aureus, Escherichia coli, and Candida albicans. The extract was also found to have a moderate inhibitory effect on the growth of Aspergillus niger and Pseudomonas aeruginosa. To study the anti-inflammatory activity of the extract, the denaturation method was used. The results showed that the extract was able to reduce inflammation in a dose-dependent manner. The extract was found to be more effective in reducing inflammation than the standard drug used in the study. In conclusion, the present study has revealed the phytochemical and antimicrobial properties of Acalypha indica leaves extract in two different solvent extractions like acetone and aqueous extract. The extract was found to contain a variety of phytochemicals, including tannins, saponins, flavonoids, terpenoids, triterpenoids, antroquinone, steroids, polyphenols, glycosides, and coumarins. The extract also showed significant anti-inflammatory and antimicrobial activity. These findings suggest that Acalypha indica leaves extract may have potential therapeutic use in treating inflammatory conditions and microbial infections. Further studies are needed to fully understand the mechanism of action of this extract and to develop new drugs from this plant.

Key words: Skin disease, pathogens, antimicrobial, anti-inflammatory, acalypha, Kuppaimeni Harita manjari

INTRODUCTION:

Acalypha indica, also known as Indian Copperleaf, is a medicinal plant found throughout the world's tropical regions. The plant has traditionally been used to treat a wide range of ailments such as skin infections, inflammation, and fever.

Acalypha indica contains a variety of phytochemicals, including flavonoids, tannins, and alkaloids, which have anti-inflammatory and antimicrobial properties, according to recent research. Because of these properties, the plant could be used to treat skin infections caused by pathogens such as bacteria and fungi[1].

In vitro studies have shown that extracts of Acalypha indica possess significant antimicrobial activity against a range of skin-causing pathogens. The extracts have been found to inhibit the growth of bacteria such as Staphylococcus aureus and Escherichia coli and fungi such as Candida albicans. Additionally, the extracts have been found to have anti-inflammatory properties, which may help to reduce the redness, swelling, and pain associated with skin infections[2].

Despite these promising results, further research is needed to fully understand the mechanisms of action of Acalypha indica and to determine the most effective ways to use the plant for the treatment of skin infections. In this study, we aim to investigate the phytochemical composition of Acalypha indica and to evaluate the in vitro anti-inflammatory and antimicrobial activity of the plant against skin-causing pathogens. By understanding the phytochemical composition and the in vitro activity of Acalypha indica against skin-causing pathogens, we hope to provide valuable information on the potential of the plant as a natural treatment for skin infections and inflammation. This research will also provide a basis for the development of new, more effective and safe skin care products, which will be beneficial for millions of people suffering from skin infections and inflammation worldwide.

AIMS AND OBJECTIVES:

The following are the main objectives of the present study

- To examine the qualitative phytochemicals in Acalypha indica leaves.
- To decide the phytochemicals in Acalypha indica leaves
- To study the histochemical examination in Acalypha indica leaves powder
- To analysis the florescence behaviour of Acalypha indica leaves powder
- To inspect the UV-Visible investigation in Acalypha indica leaves powder.
- To assess the anti-inflammatory of Acalypha indica leaves
- To screen the antimicrobial action of Acalypha indica leaves against skin causing organisms.

MATERIALS AND METHODS:

4.1 Collection of plant materials

The Acalypha indica leaves were collected in Feb. 2021.The separated were washed in a tray and shade dried for 3-5 days.This shade dried leaves after 3 days were milled to obtain fine powder

4.2 Preparation of extract

Take one gram Acalypha indica powder in each extract prepared in 50 ml of different solution (methanol and water) the extract shake it well for 30 minutes by free hand and wait for 24 hours. After extracts were filtered using Whatman filter paper No.1 and filtrate used for further analysis.

4.2.1 Phytochemical screening

Chemical tests were carried out on the extract using standard procedures to identify the constituents as described by Sofowara (1993)[3]

4.2.2 Test for tannins

1ml of extract was dissolved in 5ml of distilled water and heated for few minutes and cooled. The mixture was treated with 1-2 drops of 0.1% ferric chloride (FeCl3) solution and observed for brownish green or a blue black coloration that indicates the presence of tannins.

4.2.3 Test for saponins

1ml of sample and 2ml distilled water was shaken vigorously and add 8 drops of olive oil to obtain stable persistent foam. The formation of emulsion indicates the presence of saponins.

4.2.4 Test for flavonoids

2.5ml of ammonia solution was added to 1 ml of extract in a test tube and few drops of concentrated sulfuric acid was carefully added along the wall of the test tube. A yellow coloration indicates the presence of flavonoids.

4.2.5 Test for steroids

0.5ml sample was added with 1ml of acetic anhydride and 2ml of concentrated sulphuric acid. The formation of violet to blue or green indicates the presence of steroids.

4.2.6 Test for terpenoids

0.5ml of sample was added with 2ml of chloroform and few drops of concentrated sulphuric acid was carefully added along the wall of the test tube to form two layers. An interface with a reddish brown colors layer confirms the presence of terpenoids.

4.2.7 Test for terpenoids

0.5ml of sample was mixed with 1ml of chloroform in a test tube. Then 1ml of acedic anhydride and 2ml Concentrated sulphuric acid was carefully added along the wall of the test tube to form a reddish violet color. It confirms the presence of triterpenoids.

4.2.8 Test for Alkaloids (Mayer's test)

To 1ml of plant extract 5 drops of Mayer's reagent was added. Creamy white precipitate obtained which shows that the alkaloid groups are present.

4.2.9 Test for anthraquinones

1ml leaf extract was mixed with 2ml of concentrated sulfuric acid and 1ml 10% ammonia solution was added. The presence of a rose pink color indicates the anthraquinones.

4.2.10 Test for polyphenol

1ml of sample was added with 4ml of ethanol. The test tubes were kept in boiling water bath for 15 minutes and cool. Then 3 drops of ferric cyanide was added. The appearance of blue green colour indicates the presence of polyphenol.

4.2.11 Test for glycosides

To 1ml of plant extract, 1ml of acetic acid, few drops of 5% ferric chloride, and 1ml of concentrated sulphuric acid was added . The formation of brown ring indicates the presence of cardiac glycosides.

4.2.12 Test for coumarins

2ml of sample was added with 3ml of 10% NaOH. The appearance of yellow color indicates the presence of coumarins.

4.3 Quantitative analysis of phytochemicals

4.3.1 Determination of total phenols by spectrophotometric method

Total phenols estimated by the method of Edeoga et al[4].

Reagent

1. Ether
2. Ammonium hydroxide
3. Amylalcohol

Procedure

Plant powder (250 mg) was boiled with 10 ml of ether for the extraction of the phenolic component for 15 min. 2.5 ml of the extract was pipetted into a 50 ml flask, then 5 ml of distilled water was added. 1 ml of ammonium hydroxide solution and 2.5 ml of concentrated amylalcohol were also added. The samples were made up to mark and left to react for 30 min for colour development. The optical density was measured at 505 nm.

4.3.2 Determination of Flavonoid

Flavonoid determine by the method of Boham[5] and Kocipai-Abyazan (1994)

Reagents

1. 80% aqueous methanol

Procedure

250 mg of the plant sample was extracted repeatedly with 10 ml of 80% aqueous methanol at room temperature. The whole solution was filtered through Whatman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a water bath and weighed to a constant weight.

4.3.3 Estimation of total terpenoid content

Total terpenoid content in the leaf extracts were assessed by standard method

Reagents

1. Methanol
2. Petroleum ether

Procedure

250 mg of plant powder was taken separately and soaked in alcohol (10ml) for 24 hrs. After filtration, the filtrate was extracted with petroleum ether (1:3 ratio) for 2 hours. The dried ether extract was evaporated by complete elimination of petroleum ether under reduced pressure. The dried ether extract was treated as total terpenoid.

4.4 Histochemical tests[6]

A small quantity of dried and finely powdered leaves sample was placed on a grease free microscopic slide and treated with specific chemicals and reagents and waited for 1-2 minutes. A positive test for histochemical was indicated by the appearance of the appropriate colour change after application of the reagent. Using a light microscope to observe and record any colour changes.

The powder of Acalypha indica leaves powder was treated with specific chemicals and reagents. The treated plant powder further analysed in light microscope. The Acalypha indica leaves powder treated with diluted ammonia and H2SO4 gave yellow colour indicates flavonoids. Plant powder treated with Toludine blue to gave Blue green/Red colour indicates the presence of polyphenol. Plant powder treated with Dinitrophenol hydrazine (few drops) to gave Orange colour indicates the presence of Terpenoids.

4.5 Determination of Fluorescence behavior of plant powder[7]

Fluorescence analysis of leaves powder of Acalypha indica leaves has been carried out in daylight and under UV light. Florescence analysis of leaf powder of Acalypha indica leaves was carried out by the treatment of different chemical reagents such as AlCl3, H2SO4, HCl, NH3, CH3OH, HNO3 and NaOH..The powders were observed in normal daylight and under short (245 nm) and long UV light (365 nm).

4.5.1 In Vitro Anti-inflammatory activity

Inhibition of egg albumin denaturation

In vitro anti-inflammatory activity was carried out by the method of Sangita Chandra et al[8]. (2012)

Reagent

1. Egg albumin
2. Phosphate Buffer (pH 6.4)
3. Diclofenac sodium as standard

Procedure

The reaction mixture (5 mL) consisted of 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate buffered saline (PBS, pH 6.4) and 2 mL of varying concentrations of Acalypha indica leaves extracts (100, 200, 300, 400 and 500 µg/ mL respectively). Similar volume of double-distilled water served as control. Then the mixtures were incubated at (37± 2 °C) in a incubator for 15 min and then heated at 70'c for 5 min. After cooling, their absorbance was measured at 660 nm by using vehicle as blank. Diclofenac sodium at the final concentrations (100- 500µg/ mL) of were used as reference drug and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ inhibition} = 100 \times (Vt / Vc - 1)$$

Where, Vt = absorbance of test sample, Vc = absorbance of control.

The extracts concentration for 50% inhibition (IC50) was determined by plotting percentage inhibition with respect to control against treatment concentration.

4.5.2 Inhibition of Bovine serum albumin denaturation:

In vitro anti-inflammatory activity was carried out by the method of Sangita Chandra[8] et al. (2012)

Reagent

1. Bovine albumin
2. Phosphate Buffer (pH 6.4)
3. Diclofenac sodium as standard

Procedure

The reaction mixture (5 mL) consisted of 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate buffered saline (PBS, pH 6.4) and 2 mL of varying concentrations of Acalypha indica leaves extracts (100, 200, 300, 400 and 500 µg/ mL respectively). Similar volume of double-distilled water served as control. Then the mixtures were incubated at (37± 2 °C) in a incubator for 15 min and then heated at 70°C for 5 min. After cooling, their absorbance was measured at 660 nm by using vehicle as blank. Diclofenac sodium at the final concentrations (100- 500µg/ mL) of were used as reference drug and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ inhibition} = 100 \times (Vt / Vc - 1)$$

Where, Vt = absorbance of test sample, Vc = absorbance of control.

The extracts concentration for 50% inhibition (IC50) was determined by plotting percentage inhibition with respect to control against treatment concentration.

4.6 UV-Visible analysis

The extracts were examined under visible UV-Visible spectrum. The sample is dissolved in same solvent. The extracts were scanned in the wavelength ranging from 340-800 nm using Systronic Spectrophotometer. These solutions were scanned in turn at intervals of 10 nm and the characteristic peaks were detected. The peak value of the UV-Visible was recorded.

4.7 Determination of antimicrobial activity

The antimicrobial activity was performed by disc diffusion method [9]

4.7.1 Preparation of Media

Nutrient Agar (NA-Himedia) Media for Bacteria

Composition of Media

Animal's tissue: 5.00 g

Sodium chloride: 5.00g

Beef extract: 1.50g

Yeast extract: 1.50g

Agar: 15.0g

Preparation of medium:

Suspend 28.0 grams of nutrient agar in 1000 ml distilled water. Heat to boiling and dissolve the medium completely. Sterilize by autoclaving at 15 Ibs pressure (121˚C) for 15 minutes. Mix well and pour into sterile Petri plates.

4.7.2 Preparation of Media

Potato Dextrose Agar (PDA-Himedia) Media for Fungi

Composition of Media

Potatoes infusion from: 200.00g

Dextrose: 20.00g

Agar: 15.00g

Preparation of medium

Suspend 39.0 grams of PDA in 1000 ml distilled water. Heat to boiling and dissolve the medium completely. Sterilize by autoclaving at 15 Ibs pressure (121˚C) for 15 minutes. Mix well before dispensing in specific work, when pH 3.5 is required; acidify the medium with sterile 10% tartaric acid. The amount of acid required for 100 ml of sterile cooled medium is approximately 1 ml.

4.8 Microorganisms

The microbial strains employed in the biological assays were Gram – positive bacteria: Staphylococcus aureus (MTCC 3160), Streptococcus pyogenes (MTCC 442), Staphylococcus epidermidis, (MTCC 435) and Gram – negative bacteria: Escherichia coli, (MTCC 732), Pseudomonas aeruginosa (MTCC 358). The fungus of Candida albicans (MTCC 183) and Candida tropicalis (MTCC 184), Trichophyton rubrum (MTCC 7859), Trichophyton tonsurans (MTCC 8475). Obtained from Microbial type culture collection (MTCC) at the institute of Microbial Technology (IMTECH), Chandigarh, India.

4.8.1 Preparation of 24 hours pure culture

A loop full of each of the microorganisms was suspended in about 10ml of physiological saline in a Roux bottle. Each of these was streaked on to the appropriate culture slants and was incubated at 37ºC for 24 hours except for fungal which was incubated at 25ºC for 48 hours. After completion of incubation period, when growth was observed the tubes were kept into 2-8˚C until use.

4.8.2 Preparation of samples solutions for the experiment

The sample were weighed (10mg/10ml) and dissolved in methanol to prepare appropriate dilution to get required concentrations of about 50µl (50µg), 100µl (100µg) and 150µl (150µg). Standard solution as Chloramphenicol for bacteria (25mg/ml distilled water), Fluconazole (25mg/ml distilled water) for fungi. They were kept under refrigerated condition unless they were used for the experiment.

4.8.3 Preparation of dried filter paper discs

Whattman filter paper (No:1) was used to prepare discs approximately 6 mm in diameter, which are placed in hot air for sterilization. After sterilization, the discs were loaded with 50µl, 100µl and 150µl of samples and again kept under refrigeration for 24 hrs. Standard solution as Chloramphenicol and Fluconazole (25mg/ml distilled water- 30µl) used to compare the test solution. They were kept under refrigerated condition unless they were used for the experiment.

4.9 Antimicrobial assay

Antibiogram was done by disc diffusion method using samples. Petri plates were prepared by pouring 30 ml of Nutrient agar (NA) medium for bacteria and Potato Dextrose agar (PDA) for fungal. The bent glass rod is sterilized and used to spread the microbe-containing liquid uniformly on the plates using 24 hours culture of respective bacteria. Briefly, inoculums containing Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes, Staphylococcus epidermidis, Pseudomonas aeruginosa bacteria specie were spread on Nutrient agar plates and Candida albicans, Trichophyton rubrum, Trichophyton tonsurans, Candida tropicalis fungus strains were spread on potato dextrose agar. Using sterile forceps, the sterile filter papers (6 mm diameter) containing the crude samples (50µl, 100µl and 150µl) were laid down on the surface of inoculated agar plate. The plates were incubated at 37 ºC for ± 2˚C for 24 h for bacterial strains and at room temperature (30±1) for 24-48 hr. for yeasts strains. Each sample was tested in triplicate. Each sample was tested in triplicate. The antimicrobial capability of test compounds was resolved based on mean distance across of zone of restraint around the plate in millimeters.The zones of inhibition of the tested microorganisms were measured in a millimeter scale.

4.10 Statistical analysis

Tests were carried out in triplicate for 3 separate experiments. The result was graphically determined by a linear regression method using Ms- Windows based graphpad Instat (version 3) software. Results were expressed as graphically and mean ± standard deviation.

RESULTS AND DISCUSSION:

5.1 Qualitative and quantitative analysis

In the present study was carried out on the *Acalypha indica* leaves revealed the presence of medicinally active constituents. The phytochemical characters of the *Acalypha indica* leaves investigated and summarized in Table-1 and figure 1 and 2. The phytochemical screening *Acalypha indica* leaves showed that the presence of tannin, saponin, flavonoids, terpenoids, triterpeniods, antroquinone, steroids, polyphenol, glycosides and coumarins while alkaloids was absent in both extract. Such analyses with different herbs were done by authors [10.11]

Table.1: Qualitative analysis of Phytochemicals in *Acalypha indica* leaves extract

S. No	Phytochemicals	Aqueous extract	Methanol extract
1	Tannin	+	+
2	Saponin	++	++
3	Flavonoids	+	++
4	Steroids	+	++
5	Terpenoids	+	++
6	Triterpeniods	+	+
7	Alkaloids	-	-
8	Antroquinone	+	++
9	Polyphenol	++	++
10	Glycosides	+	++
11	Coumarins	++	++

(+) Presence, (++) High concentrations and (–) Absences

Figure 1: Qualitative analysis of Phytochemicals in *Acalypha indica* leaves aqueous extract

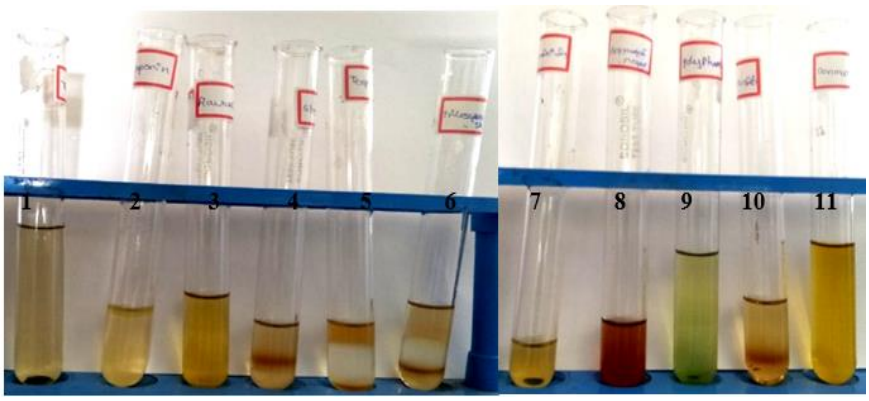
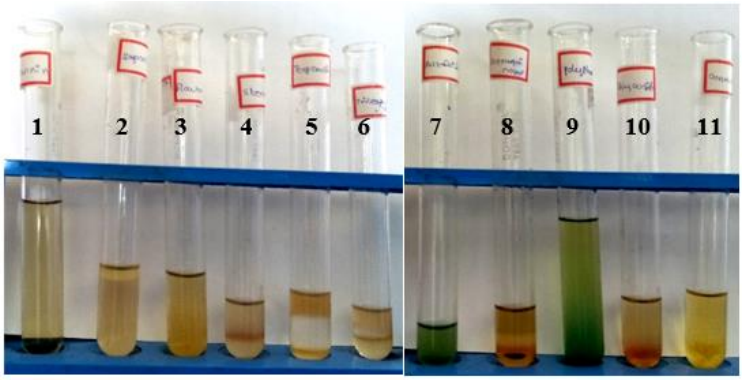


Figure 2: Qualitative analysis of Phytochemicals in *Acalypha indica* leaves methanol extract



(1. Tannin, 2. Saponin, 3. Flavonoids, 4. Steroids, 5. Terpenoids, 6. Triterpenoids, 7. Alkaloids, 8. Antroquinone, 9. Polyphenol,10. Glycoside and 11. Coumarins)

Kumar *et al*.,[12] (2013) investigated the The phytochemical screening showed that the methanol and aqueous extracts contained alkaloid, the carbohydrates and the phenolic compounds were present in all of the solvent extract except petroleum ether extract. The chloroform, ethyl acetate and the aqueous extract contained glycosides whereas the saponins present in methanol and aqueous extract. The ethyl acetate extract contain only the flavonoids.

5.2 Quantitative analysis

Quantitative analysis revealed that the *Acalypha indica* leaves powder has significant amount of polyphenol (25.00mg/gm), flavonoids (51.42mg/gm), and Terpenoids (10.00mg/gm) were presented (Table 2). The above phytoconstituents were tested as per the standard methods.Similar results were found with a study by Jagatheeswari et al [13]

Table.2: Quantitative analysis of Phytochemicals in *Acalypha indica* leaves powder

Phytochemicals	**Results (mg/gm)**
Poly phenol	25.00
Flavonoids	51.42
Terpenoids	10.00

5.3 Histochemical analysis of *Acalypha indica* leaves powder

Histochemistry is the part of histology managing the recognizable proof of synthetic segments of cells and tissues; it is an amazing asset to identify low amount of substances present in organic tissues. Histochemical strategies have been utilized to identify the structure and improvement, and to contemplate time course of statement and dissemination of major phytocompounds

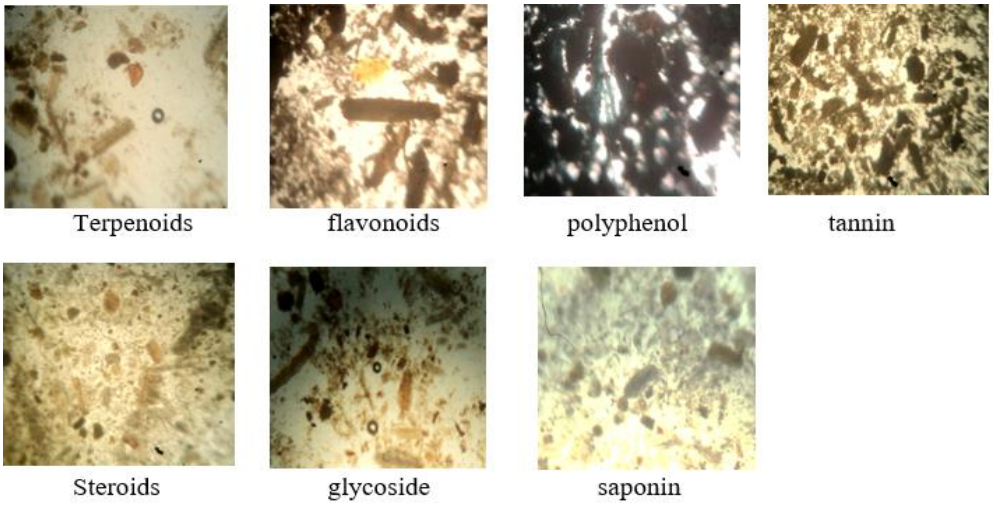
In the present study, *Acalypha indica* leaves powder were treated with specific chemicals and reagents. The *Acalypha indica* leaves powder treated with diluted ammonia and H_2SO_4 gave yellow colour negundotes Flavonoids, treated with $FeCl_3$ reagent gave dark blue to black colour. Tannin, treated with Few drops toluidine blue reagent gave Blue green / red colour negundotes Polyphenol. (Table 3**)**. This results further confirmed the presence of phytochemicals.Similar results were found with vinitha et al[14]

Table.3: Histochemical analysis of *Acalypha indica* leaves powder

S. No	**Phytochemicals**	**Results**
1	Terpenoids	++
2	Flavonoids	++
3	Polyphenol	++
4	Terpenoids	++
5	Steroids	+
6	Glycoside	+
7	Saponin	++

Note: (+) Presence; (++) present with high intensity of the colour

Figure 3: Histochemical analysis of Acalypha indica leaves powder



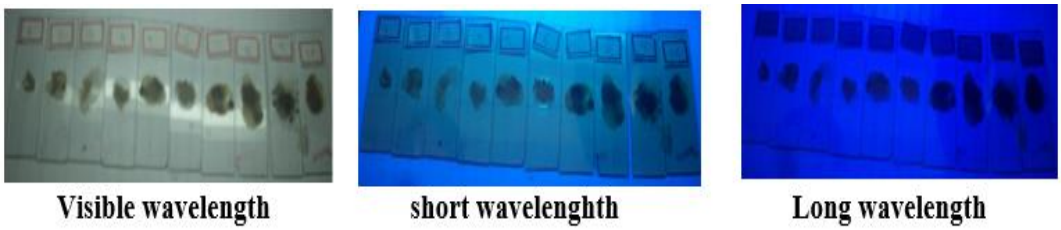
5.4 Fluorescence behavior of *Acalypha indica* leaves powder

Fluorescence analysis of entire leaves of *Acalypha indica* has been carried out in daylight and under UV light. Fluorescence analysis of leaf powder of *Acalypha indica* leaves was carried out by the treatment of different chemical reagents such as $AlCl_3$, H_2SO_4, HCl, NH_3, HNO_3, CH_3OH and NaOH. The powders were observed in normal daylight and under short (245 nm) and long UV light (365 nm) and the results were presented in Table 4 and figure 4.

Table 4: Fluorescence behavior of leaf powder of *Acalypha indica* on treatment with different chemical reagents

S. No	Test	Visible Light	Short UV	Long UV
1	Plant powder	Light brown	Sandal	Black
2	Plant powder treated with water	Light sandal dark brown	Sandal light brown	Dark brown
3	Plant powder treated with Hexane	Light sandal	Light sandal	Dark brown
4	Plant powder treated with Chloroform	Light sandal	Light sandal	Black
5	Plant powder treated with Methanol	Dark brown	Light brown	Black
6	Plant powder treated with Acetone	Light brown	Sandal	Black
7	Plant powder treated with 1N NaOH (water)	Dark brown	Yellowish green	Dark green black
8	Plant powder treated with 1N HCl	Dark brown	Dark brown	Black
9	Plant powder treated with sulphuric acid with an equal amount of water	Black	Dark brown	Black
10	Plant powder treated with Nitric acid dilute with an equal amount of water	Yellowish brown	Dark yellow	Dark brown

Figure 4: Fluorescence behavior of leaf powder of *Acalypha indica* on treatment with different chemical reagents



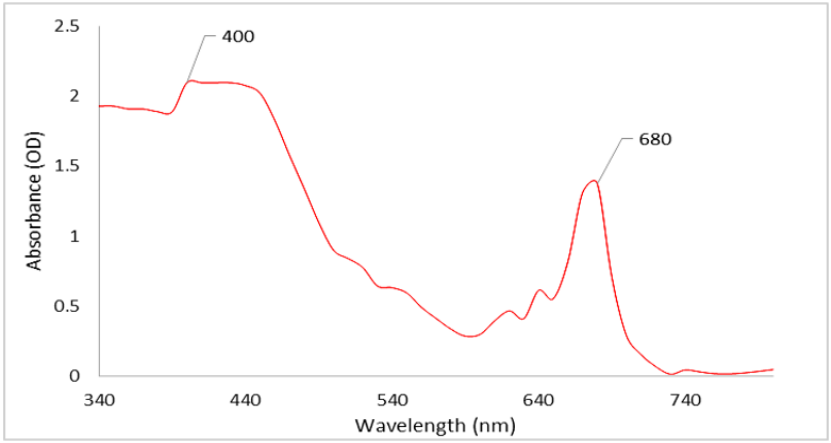
Fluorescence is the phenemenon shown by different substance constituents present in the plant. A few constituents show fluorescence in the noticeable reach in daylight. The uv light delivers fluorescence in numerous items, which don't noticeably fluorescence in sunlight. Fluorescence investigation is one of the pharmacognostic methodology helpful in the Identification of real examples and perceiving debasements

In the fluorescence investigation, the plant parts or unrefined medications might be inspected accordingly or in their powdered structure or in arrangement or as concentrates. Although, in a large portion of the cases the real substances answerable for the fluorescence properties has not been distinguished, the benefits of simplicity and quickness of the interaction makes it an important scientific instrument in the Identification of plant tests and crude drugs. Henceforth, a few medications are frequently surveyed subjectively and it is a significant boundary of pharmacognostical assessment. We have clearly delineated the fluorescence behaviour[15] even there are a few studies

5.5 Ultraviolet/visible (UV/VIS) spectroscopy

UV-Visible spectrophotometry technique is simple, rapid, moderately specific and applicable to small quantities of compounds. UV-visible spectroscopy can be performed for qualitative analysis and for identification of certain classes of compounds in both pure and biological mixtures. Preferentially, UV-visible spectroscopy can be used for quantitative analysis because aromatic molecules are powerful chromophores in the UV range[16]

Figure 6: UV-Visible spectrum analysis of Acalypha indica leaves



Natural compounds can be determined by using UV-visible spectroscopy. Phenolic compounds including anthocyanins, tannins, polymer dyes, and phenols form complexes with iron that have been detected by the

ultraviolet/visible (UV-Vis) spectroscopy. The UV-VIS profile (Figure 5) of the *Acalypha indica* leaves extract was studied at a wavelength range of 340 to 800 nm. Three major bands were recorded at 400 and 680nm. The result confirms the occurrence of peaks at 340-800 nm reveals that the absorption bands are due to the presence of flavonoids, phenol and its derivatives . The result of UV-VIS spectroscopic analysis confirms the presence of phenolic compounds in the extract of *Acalypha indica* leaves.

5.5.1 In vitro Anti-inflammatory activity of Acalypha indica leaves extract

Recent studies[13] have investigated the anti-inflammatory properties of the leaves of this plant. In vitro studies have shown that the leaves of A. indica possess potent anti-inflammatory activity, which is likely due to the presence of various phytochemicals such as flavonoids, tannins, and phenolic compounds. These compounds are known to inhibit the production of pro-inflammatory mediators, such as nitric oxide and prostaglandins, which contribute to inflammation.

The anti-inflammatory activity of A. indica leaves may be useful in the treatment of conditions such as rheumatoid arthritis, asthma, and other inflammatory disorders. Further research is needed to confirm these findings and to determine the most effective dosage and administration of A. indica for therapeutic use.

Present study was alcoholic *Acalypha indica* leaves extract confirm the anti-inflammatory activity in Egg albumin activity of highly in 93.17 for 500µg/ml, and Bovine Serum albumin activity of highly in 94.39 for 500µg/ml (Table 5 and 6, fig 6, 7).

Table.5: *In vitro* anti- inflammatory activity of *Acalypha indica* leaves

(Egg albumin)

Concentrations (µg/ml)	***Acalypha indica* leaves**	**Standard (Diclofenac sodium)**
100	17.95 ±1.25	28.00±1.58
200	33.03 ±2.31	43.80±3.25
300	56.29±3.94	62.47±4.56
400	80.07±5.60	83.66±5.32
500	93.17±6.45	95.51±6.52
IC_{50}	273.33	228.56

Values are expressed as Mean ± SD for triplicates

Figure 6: *In vitro* anti- inflammatory activity of *Acalypha indica* leaves

(Egg albumin)

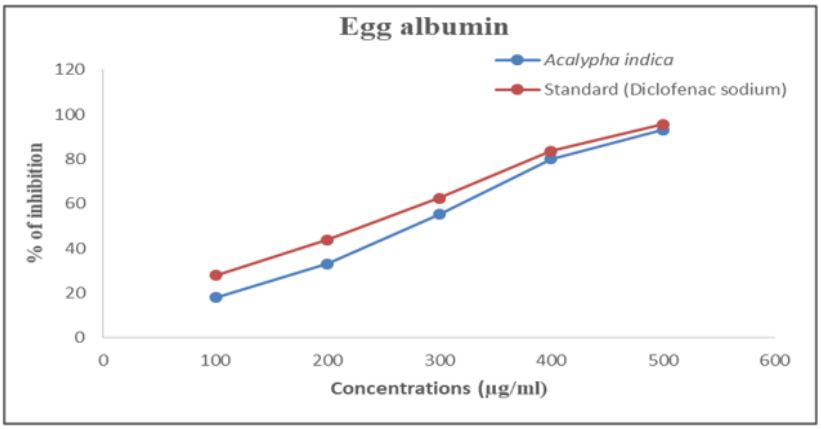


Table.6: *In vitro* anti-inflammatory activity of *Acalypha indica* leaves

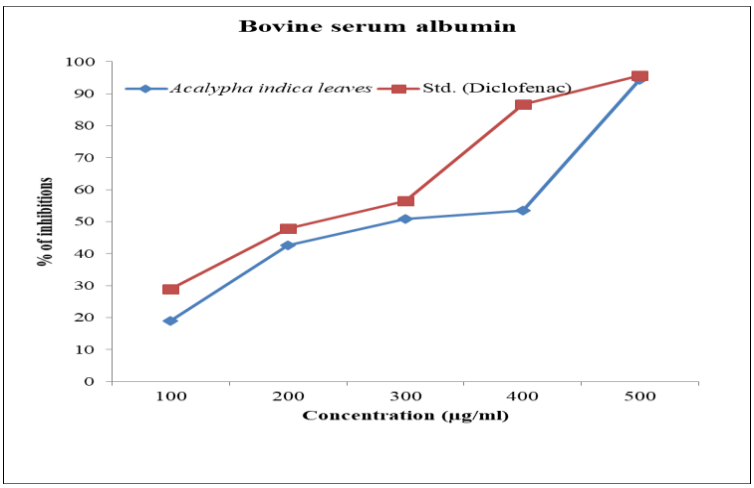
(Bovine Serum albumin)

Concentrations (µg/ml)	***Acalypha indica* leaves**	**Standard (Diclofenac sodium)**
100	18.96±1.32	28.87±2.02
200	42.67±2.98	47.84±3.34
300	50.86±3.56	56.46±3.95
400	53.49±3.74	86.63±6.06
500	94.39±6.60	95.68±6.69
IC_{50}	288.38	224.59

Values are expressed as Mean ± SD for triplicates

Figure 7: *In vitro* anti-inflammatory activity of *Acalypha indica* leaves

(Bovine Serum albumin)



5.6 Antimicrobial activity

The plant's leaves and roots have been found to inhibit the growth of bacteria such as E. coli and S. aureus and fungi such as Aspergillus niger and Candida albicans. The antimicrobial activity is thought to be due to the presence of compounds such as flavonoids, tannins and alkaloids. Further research is needed to determine the most effective dosage and administration of A. indica for therapeutic use.[17,18]

Table 7: Evaluation of antimicrobial activity of *Acalypha indica* leaves against skin causing microbes

Microorganisms	**Concentrations (µl/ml)**			**Std.**
	50	**100**	**150**	**(30µl/ml)**
Bacteria				
Escherichia coli (mm)	8.50	10.50	11.50	13.00
Staphylococcus aureus (mm)	6.00	10.50	12.00	12.50
Streptococcus pyogenes (mm)	8.00	9.50	11.00	17.50
Staphylococcus epidermidis (mm)	8.50	15.00	16.00	17.00
Pseudomonas aeruginosa (mm)	8.50	10.50	11.00	14.50
Fungal				
Candida albicans (mm)	12.00	11.00	14.50	17.50
Candida tropicalis (mm)	11.00	12.50	13.50	14.50
Trichophyton rubrum (mm)	11.00	13.50	14.50	16.00
Trichophyton tonsurans (mm)	9.50	10.00	12.00	15.00

Elrofaei *et al*., (2018)[19] petroleum ether, methanol and chloroform extracts of five plants were evaluated to detect antibacterial activity against five standards bacterial strain viz *Bacillus subtitles* (NCTC 8236), *Klebsiella pneumonia* (ATCC 53657), *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) using well-diffusion agar diffusion method. The petroleum ether and chloroform extracts were inactive compared to methanol extracts. The maximum antibacterial activity against the test organisms was found in methanol extract.

CONCLUSION:

In summary, the study found that Acalypha indica leaves contain a variety of phytochemicals including tannins, saponins, flavonoids, terpenoids, triterpenoids, antroquinone, steroids, polyphenols, glycosides, and coumarins. The leaves also showed significant anti-inflammatory and antimicrobial activity, specifically against microbes causing skin disease. This study is the first to provide scientific evidence for the medicinal properties of Acalypha indica leaves, supporting their traditional use in India.

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CONFLICT OF INTEREST – NIL

MRS -concept and design, RG and SPS – data collectiona nd manuscript SB – communication and supervision

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