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PHYTONUTRIENTS AND ANTIMICROBIAL ASSAY OF CAT'S TAIL

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Keywords:

Acalypha hispida, Phytochemical screening, Antimicrobial activity, Antioxidant activity

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ABSTRACT: Herbal medicine plays an important role in rural areas, and various locally produced drugs are still being used as household remedies for different ailments. *Acalypha hispida* (Euphorbiaceae) is a plant popularly used in pharmaceutical used and as a commercial product. Hence, the need to explore the potential of this plant, especially in the area of traditional medicine and pharmaceutical industries, arises. The present study was designed to evaluate the phytochemical composition, antimicrobial and radical-scavenging activities of methanol and ethanol extracts of leaf and flower of *A. hispida*. Phytochemical screening showed that phenol, tannin, alkaloid, and flavonoid are present in methanol extract of a leaf. In ethanol extract of leaves, tannin, alkaloid, and flavonoid are present. In the methanol extract of flower, phenol, tannin, and carbohydrate are present, and phenol, tannin, alkaloid, and flavanoid are present in the ethanol extract of the flower. The organisms used for the antibacterial properties assessment were *E. coli*, *Candida albicans*, *B. cereus*, and *Salmonella typhi*. In antimicrobial activity, the desired concentrations were 2.5%, 1.25%, and 0.625% against four bacteria's namely- *E. coli*, *S. albican*, *S. epidermis*, and *S. typhi*. The zone of inhibition was found best in the concentration of 2.5% against *E. coli* in the leaf extract. In the case of a flower zone of inhibition was found best in a concentration of 2.5% against *S. typhi*. Hence, the need to explore the potential of this plant, especially in the area of traditional medicine and pharmaceutical industries, arises.

INTRODUCTION: The study of plants is one of the oldest activities of man essentially as sources of food and medicine for our well being. Herbal medicine plays an important role in rural areas, and various locally produced drugs are still being used as household remedies for different ailments. Medicinal plants are those plants possessing secondary metabolites and are potential sources of curative drugs with a very long list of chemicals and its curative nature [1].

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The increasing use of traditional therapies demands more scientifically sound evidence for the principles behind therapies and for the effectiveness of medicines.

Herbal medicine is still the mainstay of about 75-80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body, and lesser side effects. Also, traditional knowledge is the most affordable and accessible method available for the treatment of various diseases. Forests represent an important resource for local inhabitants who gather and sell medicinal plants as part of their livelihood. Local healers have information and understanding of a wide range of medicinal plants that are useful to cure the common ailments.

In particular, they highlight cure pertinent for skin diseases, stomach disorders, respiratory infections, fever, piles, rheumatism, *etc.* among others. The Indian Himalayan Region (IHR) is one of the richest reservoirs of biological diversity in the world and is considered as a 'storehouse' of the valuable medicinal plant species. The inhabitants of the IHR utilize biodiversity in various forms, *i.e.*, medicine, food, fuel, fodder, timber, making agricultural tools, fiber, religious, and various other purposes.

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by micro-organisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents. Such a fact is cause for concern, because of the number of patients in hospitals who have suppressed immunity and due to new bacterial strains, which are multi-resistant. Consequently, new infections can occur in hospitals resulting in high mortality. The problem of microbial resistance is growing, and the outlook for the use of antimicrobial drugs in the future is still uncertain.

Therefore, actions must be taken to reduce this problem, for example, to control the use of antibiotic, develop research to better understand the genetic mechanisms of resistance, and to continue studies to develop new drugs, either synthetic or natural.

The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient. For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. The use of plant compounds for pharmaceutical purposes has gradually increased in Brazil. According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants. Therefore, such plants should be investigated to better understand their properties, safety and efficiency.

Acalphya hispida common name from the exotic flowers that look like strands of Cat's tail in Assam this plant is known as Bisagos. It is usually grown as a houseplant or seasonal annual for its unusual tassel-like flowers. This evergreen plant can grow to 15 feet tall and 8 feet wide in suitable climates, but in containers will remain much smaller. It thrives in hot, humid summer climates. Cat's tail plant is often grown as an annual, but since it really is a tender perennial, it can be brought indoors to be kept over the winter as a houseplant. It needs as much light and humidity as possible during the winter when water and fertilizer should be reduced. Large, bright green heart-shaped leaves with toothed margins are produced on sparsely branched upright to arching stems. In tropical locations, it will become a large shrub.

FIG. 1: *ACALPHYA HISPIDA* (PLANT, LEAF AND FLOWER)

The flowers themselves are very small, mostly just feathery pistils, but are tightly packed along the raceme to form the furry catkin. These catkins that arise from the leaf axils are very long-lasting, gradually fading and browning as they age, but regular dead-heading the spent flowers will encourage more blooms. Flowers are produced at any time of the year when temperatures are warm

enough. This plant is believed by traditional healers to possess medicinal properties that are effective in the management of tuberculosis and other ailments. A flower is used for ulcer treatment. Leaves are used leprosy and gonorrhea. The aim of this study was to analyses the leaf extract and flower extract for the phytochemical composition and to test for its antimicrobial activities and antioxidant activity

Objectives:

1. Plant sample collection, identification, processing, and extraction.
2. Analysis of phytochemical constituents of the selected plant sample.
3. Antimicrobial study of plant sample.
4. Evaluation of the antioxidant activity of the selected sample.

MATERIALS AND METHODS:

Plant Materials: Fresh leaves and flower samples of *Acalypha hispida* were collected. It was identified at the Department of Botany (Gauhati University) ACCESSION NO 18489. The leaves were initially rinsed with distilled water, air-dried in the laboratory, and ground into powder.

FIG. 2: HERBARIUM SHEET OF *ACALYPHA HISPIDA*

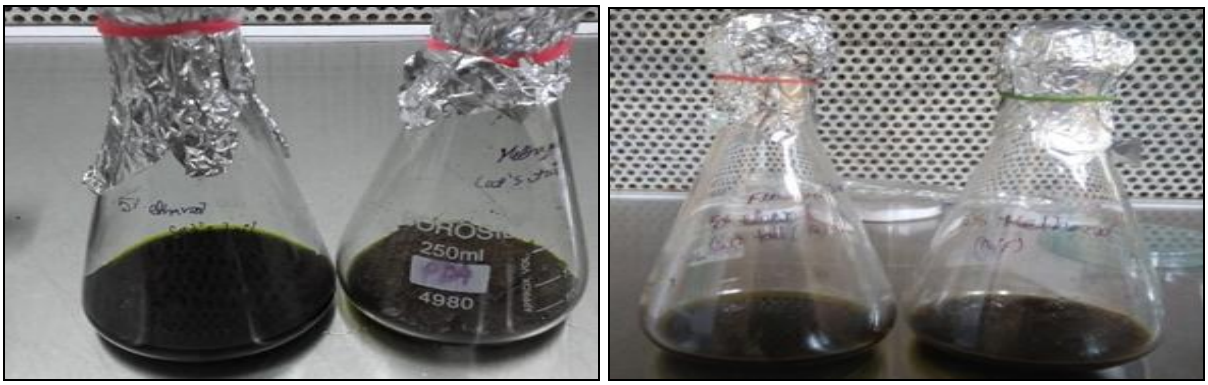


FIG. 3: EXTRACTION (LEAVES AND FLOWER)

Preparation of Plant Extract: The powdered mass of 50 g of *Acalypha hispida* was extracted by methanol and ethanol.

The extract was filtered through filter paper, and the filtrate was concentrated under reduced pressure in a rotary vacuum evaporator. Phytochemical, anti-microbial, and antioxidant tests were carried out on the extract.

Phytochemical Analysis: Phytochemical tests were carried out as per methods described by with some modification [2, 3, 4, 8].

Preparation of Test Sample: Dried extract (5 mg) was dissolved in 5 ml of dilute hydrochloric acid and filtered. The filtrate (test solution) was used to test the presence of alkaloids. For the remaining test, the test solution was prepared by dissolving the extract in DMSO in the concentration of 1 mg/ml.

Tests for Alkaloids:

Hager's Test: To 3 ml of the test solution, few drops of Hager's reagent (saturated aqueous solution of Picric acid) were added to observe the formation of yellow precipitate.

Mayer's Test: To 3 ml of the test solution, few drops of Mayer's reagent (Potassium mercuric iodide solution) were added and observed the formation of a white or cream-colored precipitate.

Wagner's Test: To 3 ml of the test solution, few drops of Wagner's reagent (iodine in potassium iodide) were added and observed for the formation of a red-brown precipitate.

Tests for Amino Acids and Proteins:
Ninhydrin Test: To 3 ml of the test solution, 3 drops of 5% ninhydrin solution were added and heated in a boiling water bath for 10 min and observed for formation of purple or bluish colour.

Biuret Test: To 2 ml of the test solution, 2 ml of 10% NaOH and 2 drops of 0.1% $CuSO_4$ solution were added, and observed for the formation of pink colour in the ethanolic layer.

Xanthoproteic Reaction: To 5 ml of the test solution, 1 ml of concentrated HNO_3 was added and boiled. After cooling, excess of 40% NaOH was added and observed the formation of deep orange color.

Tests for Carbohydrate:
Benedict's Test: Equal volume of Benedict's reagent (sodium citrate and sodium carbonate) and test solution were mixed. The mixture was heated in hot water bath for 5 min and observed for the formation of a characteristic colored precipitate (green, yellow, red, depending on the amount of reducing sugar present in the filtrate).

Fehling's Test: One ml of Fehling's solution A and 1 ml of Fehling solution B were mixed and boiled for 1 min.

To this equal volume of sample was added and heated in a hot water bath for 5-10 minutes and observed the formation of a brick-red colored precipitate.

Test for Starch:
Iodine Test: To 3 ml test solution, few drops of dilute iodine solution were added and observed for the formation of blue color.

Tannic Acid Test: Test solution is treated with 20% solution of tannic acid to observe the formation of the precipitate.

Tests for Fixed oils and Fats:
Spot Test: A small amount of the extract was separately pressed between two filter papers and monitored the appearance of oil stains on paper. To 5 drops of the test solution, 1 ml of 1 % copper sulphate solution and 10% NaOH solution was added. A clear blue solution indicates the presence of glycerine.

Tests for Flavonoids: Dilute ammonia solution (5 ml) was added to a small amount of test solution followed by addition of concentrated sulphuric acid and observed the formation of yellow coloration.

Tests for Terpenoids:
Salkowski Tests: To 5 ml of the test solution, 2 ml of chloroform and 3 ml of concentrated sulphuric acid was added carefully to form a layer and observed the formation of reddish-brown color.

Tests for Glycosides: Cardiac Glycosides (Killer Killiani test) To 2 ml of the test solution, 2 ml glacial acetic acid and 1 drop of 5% ferric chloride and concentrated sulphuric acid was added and observed the formation of reddish-brown coloration at the junction of two liquid layers and the upper layer appeared bluish-green in the presence of glycosides.

Anthraquinone Glycosides (Borntrager's Test): To 2 ml of the test solution, 3 ml of chloroform was added, and shaken, chloroform layer was separated out and 10% ammonia solution was added to it and observed the formation of pinkish-red color.

Antimicrobial Activity:
Preparation of Test Extract: The test extracts of desired concentrations (2.5%, 1.25% and 0.625%) were prepared by adding Dimethyl Sulphoxide (DMSO). Test extract so prepared were used for conducting in vitro experiments for antimicrobial efficacy against the pathogens cited above employing agar well diffusion method [5].

Culture Media: Nutrient agar (NA) media and nutrient broth (NB) were used for the culture of the test bacterium.

Preparation of Nutrient Agar (NA) Media: 28 gm of commercially available Nutrient Agar (NA) media was dissolved in 1000 ml of distilled water, stirred it well in a shaker. The media was then heated in an electronic heater till it reaches 100 ºC and becomes sticky, then the media is autoclaved at 15 lb pressure (121 ºC) for 15 min, to avoid contamination. 1.3 gm of nutrient broth (NB) was dissolved in 100 ml distilled water, stirred it well in a shaker, and then the media is autoclaved.

Inoculum: Stock culture was subcultured at regular intervals and used for experimental purposes. Broth

inoculum was prepared by adding one loop full of a pure culture of the test bacterium in a conical flask containing 50 ml of NB and then incubated at 37 ºC for 24 h.

Agar Well Diffusion Method: NA plates were swabbed with a broth culture of the test pathogen. Well of 6mm diameter was made in the inoculated plate (2 wells/plates) with a sterile cork borer. Each well was filed with 150 µl of the test extracts and allowed to diffuse at room temperature v or 2 h. Then the plates were incubated at 37 ºC / 24 h. The antimicrobial activity of the extracts was determined by measuring the diameter of the zone of inhibition around the well [8].

Antioxidant Activity Analysis:

Determination of DPPH Radical Scavenging Activity: The antioxidant potential of any compound can be determined on the basis of the scavenging activity of the 2, 2-diphenyl-1-picrylhydrazyl (DPPH, stable free radical) [6].

Chemicals Used: DPPH, Ascorbic acid, Methanol

Preparation of Standard Solution: L-Ascorbic acid was used as a standard for this assay. A stock solution of the standard was prepared by dissolving 10 mg of Ascorbic acid in 10 ml of methanol. For test solution, 20, 40, 60, 80, and 100 µl of the stock solution was taken in eppendorf tubes separately, and the final volume of 100 µl was adjusted by adding methanol.

Preparation of Test Sample: Stock solution of plant sample in a concentration of 1 mg/ml was prepared by dissolving the extract in methanol. As like the standard, the sample solution of 20, 40, 60, 80 and 100 µl was taken separately in eppendorf tubes. Then the volume was made 100 µl by adding methanol.

Preparation of 0.3 mM DPPH Solution: DPPH (11.82 mg) was dissolved in 100 ml of methanol and was kept protected from light by covering the tubes with aluminum foil. Estimation of DPPH scavenging activity: DPPH scavenging activity was estimated by using the protocol described earlier (Blois, 1958) with some modification. The tubes containing standard and sample were shaken and incubated at dark for 30 min at room temperature. For control, 1 ml of 0.3 mM DPPH solution was added to 2 ml of methanol and allowed to incubate at dark for 30 min at room temperature. After 30 min, absorbance was measured at 517 nm taking methanol as blank using a UV-visible spectro-photometer (SPECTRA max PLUS 384). The percentage of inhibition was calculated by comparing the absorbance values of the control and test samples. All the tests were performed in triplicate. Ascorbic acid was used as the reference compound. The capacity to scavenge the DPPH radical was calculated as the inhibition percentage of free radicals by the sample/standard using the following formula.

$$\% \text{ Inhibition of DPPH scavenging activity} = \{(A_0 - A_t) / A_0\} \times 100$$

Where A_0 is the absorbance of the control reaction, and A_t is the absorbance of test/ standard.

The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} values were calculated by linear regression of plots, where the abscissa represents the concentration of the tested plant extracts, and the ordinate represents the average percent of scavenging capacity. The IC_{50} value is defined as the concentration (µg/ml) of extract that inhibits the formation of DPPH radicals by 50% (Prasad *et al.*, 2009).

RESULTS AND DISCUSSION:

Phytochemical Analysis: The result of methanol extract from the present investigation showed the presence of alkaloids, phenols, flavonoids, tannin in the A. hispida leaf extract **Table 1**. In ethanol extract of the leaves tannin, alkaloid and flavonoid are present **Table 2**. In the methanol extract of flower phenol, tannin, carbohydrates are present **Table 3**. Ethanol extract of lower phenol, tannin, alkaloid and flavenoid are present **Table 4**.

TABLE 1: PHYTOCHEMICAL ANALYSIS OF METHANOL EXTRACT OF *A. HISPIDA* (LEAVES)

Phytochemical	Observation	Result
Flavonoid test	Yellow color formation	Present
Alkaloid test	Formation of yellow ppt	Present
Carbohydrate test	No brick-red coloration	Absent
Amino acid test	No purple or bluish coloration	Absent
Tannin test	Formation of green or black color	Present
Phenol test	Formation of deep blue color	Present

TABLE 2: PHYTOCHEMICAL ANALYSIS OF ETHANOL EXTRACT OF *A. HISPIDA* (LEAVES)

Phytochemical	Observation	Result
Flavonoid test	Yellow color formation	Present
Alkaloid test	Formation of yellow ppt	Present
Carbohydrate test	No brick-red coloration	Absent
Amino acid test	No purple or bluish coloration	Absent
Tannin test	Formation of green or black color	Present
Phenol test	No deep blue coloration	Absent

TABLE 3: PHYTOCHEMICAL ANALYSIS OF METHANOL EXTRACT OF *A. HISPIDA* (FLOWER)

Phytochemical	Observation	Result
Flavonoid test	No yellow color formation	Absent
Alkaloid test	No yellow ppt	Absent
Carbohydrate test	Formation brick-red coloration	Present
Amino acid test	No purple or bluish coloration	Absent
Tannin test	Formation of green or black color	Present
Phenol test	Formation of deep blue color	Present

TABLE 4: PHYTOCHEMICAL ANALYSIS OF ETHANOL EXTRACT OF *A. HISPIDA* (FLOWER)

Phytochemical	Observation	Result
Flavonoid test	Yellow color formation	Present
Alkaloid test	Formation of yellow ppt	Present
Carbohydrate test	No brick-red coloration	Absent
Amino acid test	No purple or bluish coloration	Absent
Tannin test	Formation of green or black color	Present
Phenol test	Formation of deep blue color	Present

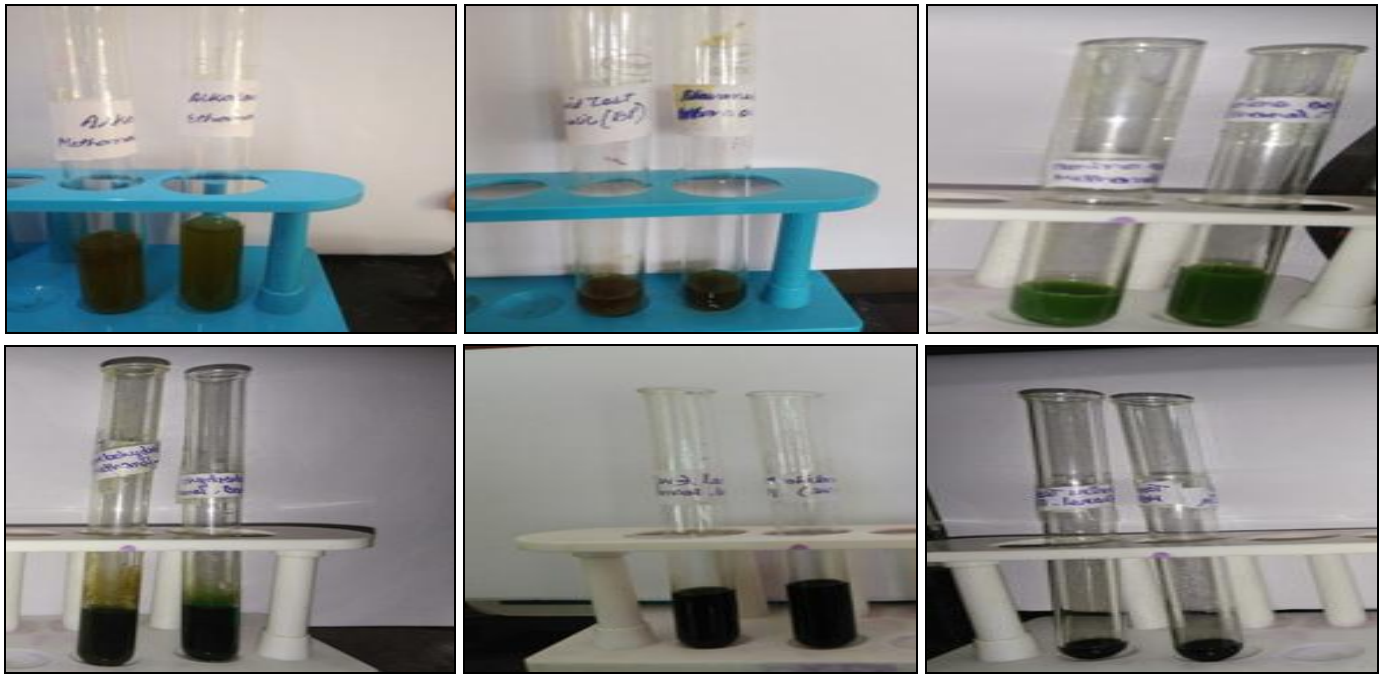


FIG. 4: PHYTOCHEMICAL ACTIVITY OF *A. HISPIDA* LEAVES (ETHANOL, METHANOL EXTRACT)

Antioxidant Activity:

DPPH Radical Scavenging: The molecule of 1, 1-diphenyl-2-picryl-hydrazyl is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecules do not dimerize, as would be the case with most other free radicals. The delocalization gives rise to deep violet color, characterized by an absorption band at 520 nm. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of the violet color, although there would be a residual pale yellow color from the picryl group still present [7].

Two different solvent extracts, namely ethanol and methanol extract derived from A. hispida leaves, were tested for DPPH radical scavenging activity. The results were depicted in **Fig**. **6** and **7**.

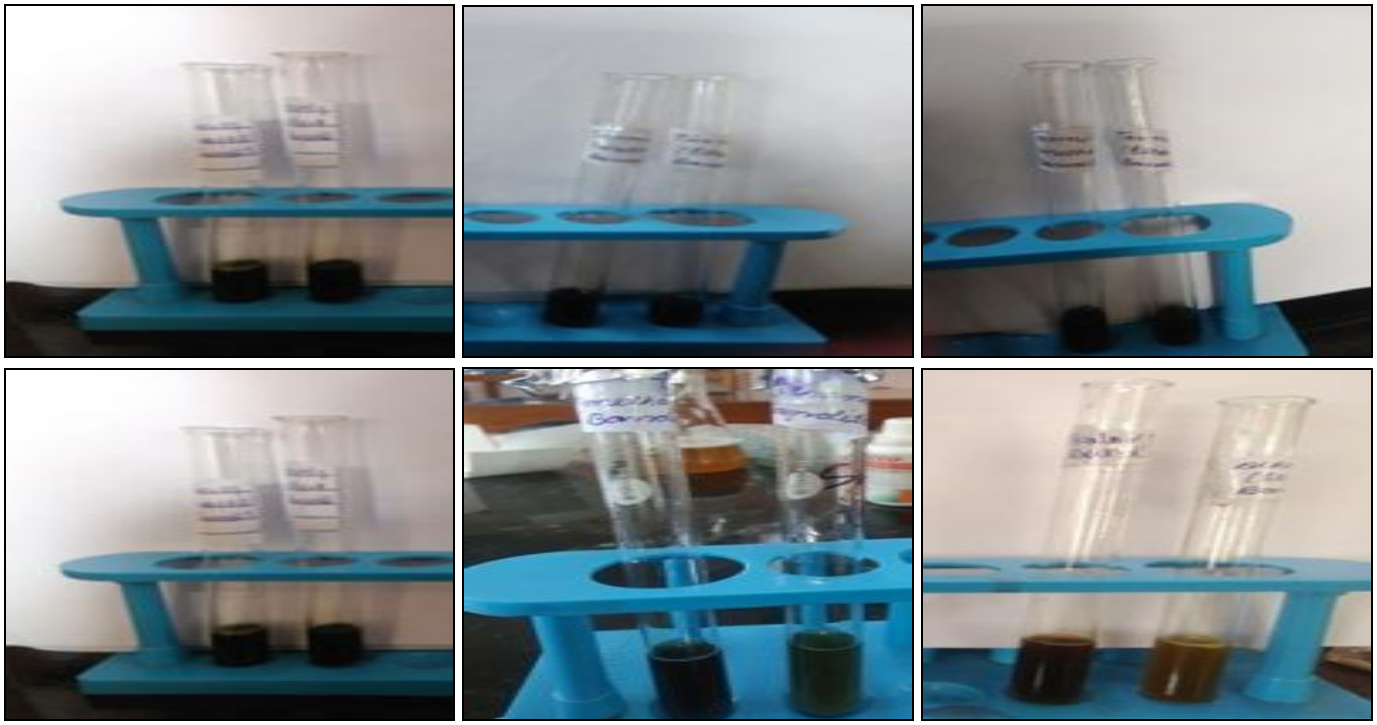


FIG. 5: PHYTOCHEMICAL ACTIVITY OF *A. HISPIDA* FLOWER (ETHANOL, METHANOL EXTRACT)

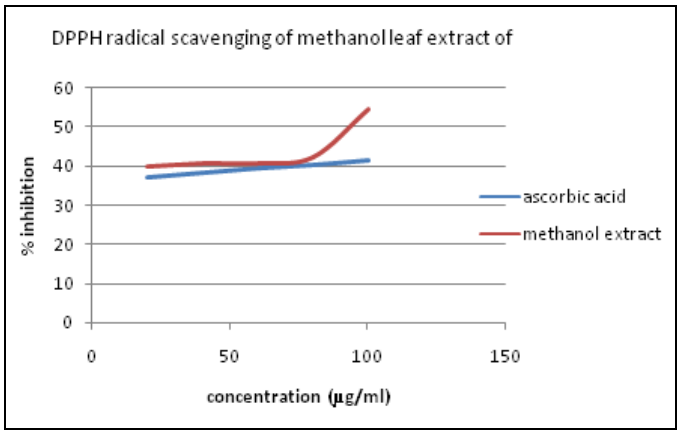


FIG. 6: ANTIOXIDANT ACTIVITY OF METHANOL EXTRACT (LEAVES)

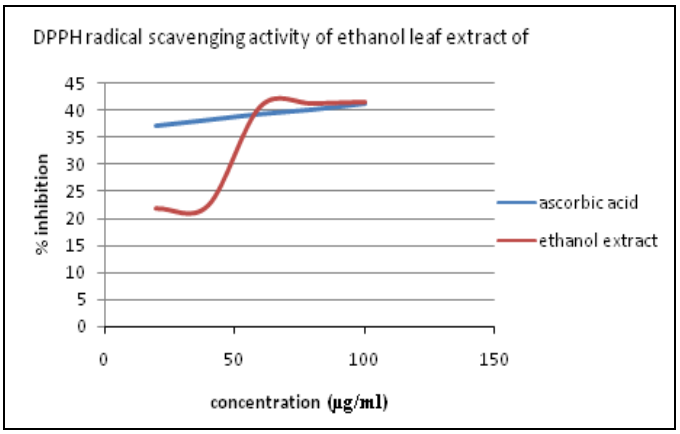
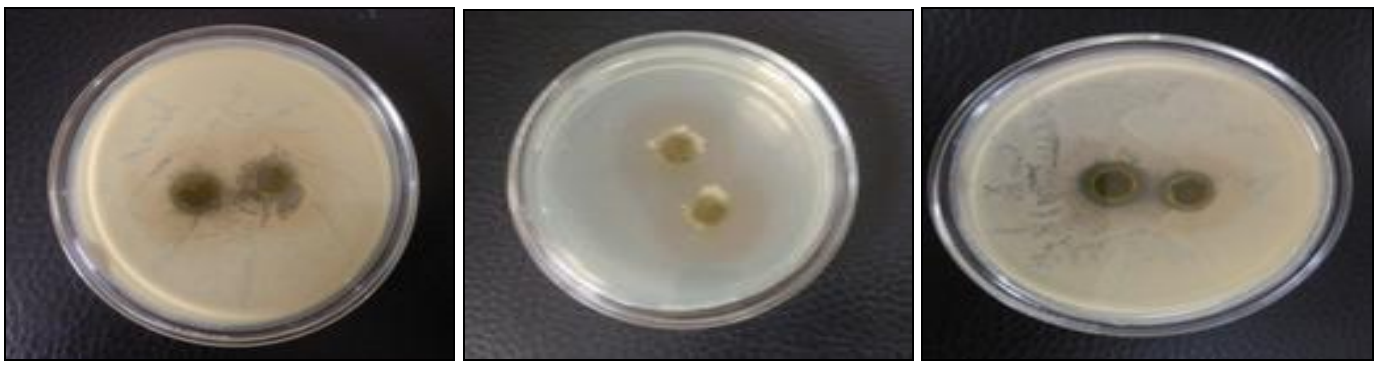


FIG. 7: ANTIOXIDANT ACTIVITY OF ETHANOL EXTRACT (LEAVES)

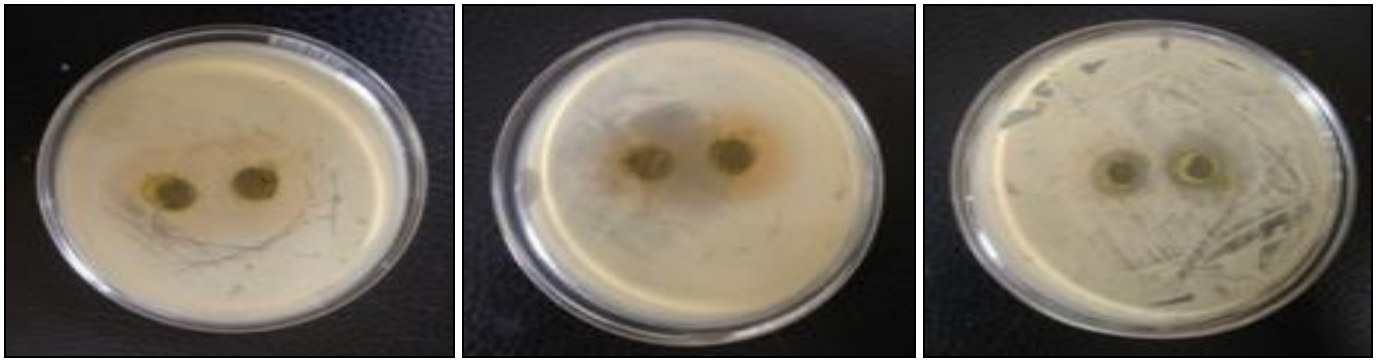
The methanol extract exhibited the highest antioxidant activity as compared to ethanol extracts as well as standard. The extract showed free radical scavenging activity in a dose-dependent manner.

Antimicrobial Activity: Methanol extract derived from *A. hispida* leaves and flowers were tested for antimicrobial activity. The results were depicted in **Fig. 8** and **9**.



E. COLI

S. TYPHI



B. CEREUS

C. ALBICANS

FIG. 8: ZONE OF INHIBITION OF *A. HISPIDA* LEAF EXTRACT

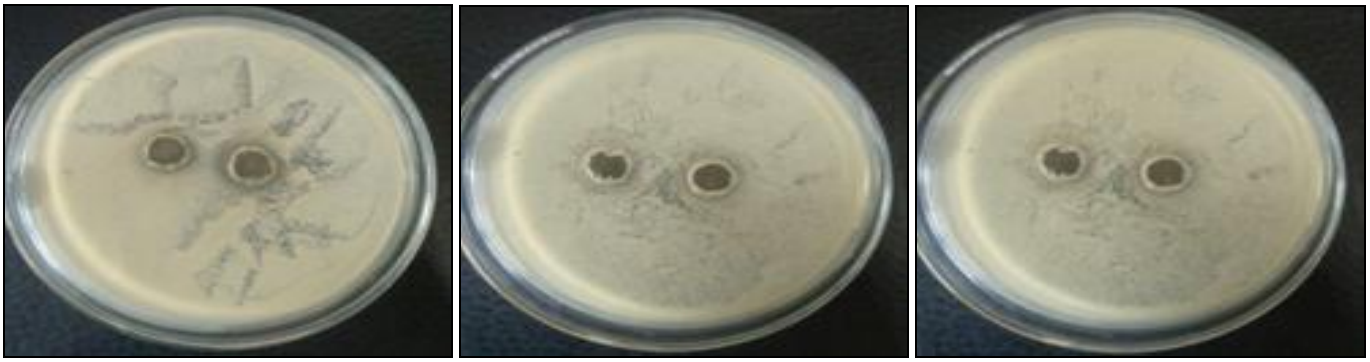
TABLE 5: ANTIMICROBIAL ACTIVITY OF *A. HISPIDA* LEAF EXTRACT

Concentration / Strain	2.5%	1.25%	0.625%
E. coli	35 mm	30 mm	27 mm
S. typhi	26 mm	28 mm	29 mm
C. albican	30 mm	28 mm	25 mm
B. cereus	33 mm	31 mm	27 mm

TABLE 6: ANTIMICROBIAL ACTIVITY OF *A. HISPIDA* FLOWER EXTRACT

Concentration/ Strain	2.5%	1.25%	0.625%
E. coli	10 mm	0 mm	2 mm
S. typhi	12 mm	0 mm	0 mm
C. albican	11 mm	0 mm	0 mm
B. cereus	10 mm	4 mm	3 mm

E. COLI



S. TYPHI

B. CEREUS

C. ALBICANS

FIG. 9: ZONE OF INHIBITION OF *A. HISPIDA* FLOWER EXTRACT

In the antimicrobial activity, the desired concentrations were 2.5%, 1.25% and 0.625% against four bacteria's namely- *E. coli*, *S. albican*, *S. epidermis* and *S. typhi*.

The zone of inhibition was found best in the concentration of 2.5% against *E. coli* in the leaf extract. In the case of a flower zone of inhibition was found best in a concentration of 2.5% against *S. typhi*.

CONCLUSION: In conclusion, the plant studied here can be seen as a potential source of useful drugs. Also, this work revealed that this extract has activity against micro-organism in a dose-dependent manner. However, further studies can be done on this plant to isolate, identify, characterize, and elucidate the structure of the bioactive compounds.

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CONFLICTS OF INTEREST: The author declares no conflict of interest.

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