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HISTOCHEMICAL, PHYTOCHEMICAL AND ANTIOXIDANT SCREENING FROM AERIAL PART EXTRACTS OF ACALYPHA INDICA LINN. (EUPHORBIACEAE).

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Abstract

The current study was deliberate to investigate the Histochemical, phytochemical screening and antioxidant activities of *Acalypha indica* plant extracts. Histochemical screening showed the presence of Starch, Cellulose, lignins, Tannins, Proteins and polyphenols. Phytochemical screening revealed the presence of Triterpenes, Saponins, Tannins, Phenolic compounds, Flavonoids, Proteins, Carbohydrates, Resins and Glycosides in the extracts of aerial part of *Acalypha indica* and also investigated total antioxidant capacity from Methanol and Aqueous extracts of aerial parts of the plant by Phosphomolybdenum assay and Aqueous extracts showed highest total antioxidant capacity compared to methanol extract. And also assessed the Antioxidant activity of

methanolic and Aqueous extracts by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) Radical Scavenging assay and obtained significant results with IC_{50} value of 22.8µg/µL and 21.3 µg/µL respectively.

Key words: Histochemical, phytochemical, Antioxidants, Phosphomolybdenum, DPPH, IC_{50}

1.Introduction

Without plants, life would not be sustained on earth. People depend upon plants to fulfil their vital human needs such as food, clothing, shelter, and medicine. Many of plants have been used in traditional medicine for many years. Some do seem to work although there may not be sufficient scientific records to ratify their efficiency. The term 'crude drugs of natural or biological origin' is used by pharmacists and pharmacologists to term whole plants or parts of plants which have medicinal stuffs.

Histochemistry is dedicated to study the identification and distribution of chemical compounds within and between biological cells, using stains, indicators and light and electron microscopy. Phytochemistry, one of the early subdivisions of organic chemistry, has been of pronounced significance in the identification of plant substances of medicinal importance.

An antioxidant is a molecule stable enough to donate an electron to a rampaging free radical and neutralize it, thus sinking its capacity to damage. These antioxidants holdup or inhibit cellular damage mainly through their free radical scavenging property. These low-molecular-weight antioxidants can safely interrelate with free radicals and terminate the chain reaction before vital molecules are damaged.

Acalypha indica, commonly known as Indian copperleaf or Indian nettle, belongs to the family Euphorbiaceae, indeed holds a significant place in traditional medicine, particularly in Ayurveda. Its broad spectrum of traditional uses reflects its diverse phytochemical composition, which includes polyphenols, flavonoids, alkaloids, saponins, terpenoids, and tannins. Among its traditional uses, *Acalypha indica* has been employed for treating various ailments including infertility, wound healing, inflammation, bacterial infections, cancer, among others.

The presence of phytoconstituents like polyphenols and flavonoids suggests its potential antioxidant properties, which could contribute to its effectiveness in wound healing and combating oxidative stress-related disorders. Alkaloids and terpenoids found in *Acalypha indica* may play a role in its antimicrobial and anti-inflammatory activities.

Taxonomy:

Kingdom: Plantae

Class : Magnoliopsida

Order: Malpighiales

Family: Euphorbiaceae

Genus: Acalypha

Species: *A. indica*

Binomial name: *Acalypha indica* L.

2. Materials and Methods

Collection of plant material

The plant was collected at the Manasagangotri campus, Mysuru. And removed the roots then washed the aerial parts with running tap water and then with distilled water and shade dried. On complete dry the plant material was make into fine powder using mechanical grinder and powder obtained was stored in air tight containers.

Extraction process

Exactly 5g of powdered plant materials was weighed in an electrical balance and then transferred to 250ml conical flasks and 75ml of Methanol was poured into conical flasks and mouth of the flask was covered with aluminum foil.

Same protocol was followed to get the extracts of Aqueous and Ethyl acetate. And all the three conical flasks were kept in an Orbital Shaker at 100 rpm in room temperature for 24 hours. After this duration each mixture were completely filtered through Whatman No.1 filter paper and these filtrates were used as ‘plant extracts’ and used to carry out further investigations.

Histochemical analysis

For Histochemical analysis the fresh plant was collected and the stem parts were taken, sectioned the plant materials by using the blades. Thin sections were selected for the histochemical analysis according to the following procedures to detect the presence of chemicals secreted by the plants.

Test for Carbohydrates

Callose (Aniline Blue Method): A thin section was taken and placed the sections in 0.05% Aniline blue solution. The excess stain was extracted by treating with Glycerin. If Callose is present stained region still appears blue.

Starch (Potassium-Iodine-Iodide): A thin section was placed in a slide containing potassium-iodine-iodide solution for 15 minutes. The section mounted in starch solution, where the starch stains blue.

Cellulose (Potassium-Iodine-Iodide & Sulphuric Acid Method): Sections were placed in Potassium-Iodine-Iodide solution for 15 minutes and observe under microscope and further confirmed by adding a few drops of Sulphuric acid on the sides of cover clip, then observed. Because of the presence of cellulose in the walls are swollen and take bright blue colour.

Test for Lignin

Lignin's (Schiff's Method): Sections were placed in Schiff's reagent for 15 minutes and washed, dehydrated, cleared and mounted, then observed under microscope. Because of the presence of Lignin it stains Pink or Purplish red.

Test for Tannins

Tannins (Ferric Chloride Method): Sections were placed in 10% Formalin and then placed it in 2% Ferric chloride solution and then observed under microscope. Appearance of blue or blue green precipitate indicates the presence of Tannins

Test for proteins

Proteins (Mercuric Bromophenol Blue Method): Sections were placed in mercuric Bromophenol Blue Dye solution for 15 minutes and observed under microscope for any colour changes. Because of the presence of Proteins, cells stains blue colour.

Biuret test for Proteins: Sections were immersed in Biuret's reagent for about 2-5 minutes and observed under the microscope. If sections takes violet higher the proteins, or red, less protein molecules are present.

Test for Polyphenols

Phenolics (Nitro-reduction Method): Sectioned material was placed in equal volume of 10% Sodium nitrate, 10% Urea and 10% Acetic acid. 3-44 minutes later, transferred it into another clean slide and treated the section with 2N Sodium hydroxide and observed for any colour changes. If phenol is present it gives cherry red colour.

Phytochemical analysis

Test for Triterpenes:

Salkowski's test:

Test solution was treated with few drops of Con.H_2SO_4, shaken and allowed to stand. Appearance of golden yellow colour indicates the presence of triterpenes.

Liebermann- Burchard's test:

To the test solution drops of Acetic anhydride was added and few drops of Conc. H_2SO_4 were added along the sides of the test tube. Appearance of red ring at the junction of the two liquids indicates the presence triterpenes.

Test for Saponins:

Foam test:

Crude extract was mixed with distilled water in the test tube and it was shaken vigorously. The formation the stable foam was taken as an indication for the presence of Saponins.

Test for Alkaloids:

Extracts were dissolved individually in the dilute HCl and filtrated. The filtrates were subjected to the following tests to determine the presence or absence of alkaloids.

Mayer's test: Filtrates were treated with Mayer's reagent (Potassium mercuric iodide). Formation of a cream or yellow coloured precipitate indicates the presence of alkaloids.

Wagner's test: Filtrates were treated with Wagner's reagent (Iodine in potassium iodide). Formation of brown/reddish precipitate indicates the presence of alkaloids.

Dragendorff's test: Filtrates were treated with Dragendorff's reagent (solution of Potassium bismuth -iodide). Formation of red precipitate indicates the presence of alkaloids.

Test for Tannins:

Ferric chloride test: To the test solution few drops of neutral $FeCl_3$ solution was added. The formation of a bluish green colouration indicated the presence of tannins.

Gelatin test: To the test solution, 1% solution of Gelatin containing Sodium chloride solution was added. Presence of tannins was indicated by the formation of white precipitate.

Test for Phenolic compounds:

Ferric chloride test: The extract was treated with 1 ml of neutral 5% ferric chloride solution. A dark green colour indicates the presence of phenolic compounds.

Test for Flavonoids:

Shinoda test: Crude extract was mixed with few fragments of Magnesium ribbon. Then concentrated HCl was added drop wise along the side of the test tube. Pink scarlet colour appeared after few minutes which indicated the presence of flavonoids.

Lead acetate test: 10% Lead acetate solution was added to the test solution. The formation of a yellow precipitate revealed the presence of flavonoids.

Test for proteins:

Biuret test: To the test solution 2 ml of 10% NaOH was added and mixed well, then 2 ml of 0.1% Copper sulphate solution was added. The formation of purplish violet or pink colour indicates the presence of proteins.

Ninhydrin test: Crude extract was boiled with 2 ml of 0.2% solution of ninhydrin, violet colour appeared suggesting the presence of amino acids and proteins.

Test for carbohydrates:

Molisch's test: Crude extract was mixed with 2ml of Molisch's reagent and the mixture was shaken properly. After that, 2ml of concentrated H_2SO_4 was poured carefully along the side of test tube. Appearance of a violet ring at the interphase indicated the presence of carbohydrates.

Fehling's test: Equal volume of Fehling's A and Fehling's B reagent were mixed together and 2ml of it was added to crude extract and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicates the presence of reducing sugars.

Benedict's test: Crude extract when mixed with 2ml of Benedict's reagent and boiled, a reddish brown precipitate formed which indicates the presence of the carbohydrates.

Test for Resins:

Turbidity test: Crude extract when mixed with 2ml of distilled water, shaken well and allowed to stand. Appearance of turbidity indicated the presence of resins.

Acetic anhydride test: To the test sample 5ml of acetic anhydride was added, boiled and cooled to room temperature. Then 0.5ml of concentrated H_2SO_4 was added. Appearance of bright purplish- red colour changes to violet which indicated the presence of resins.

Test for glycosides:

Keller- Kiliani's test: Crude extract was mixed with 2ml of Glacial acetic acid containing 1-2 drops of 2% solution of $FeCl_3$. The mixture was then poured into another test tube containing 2ml of concentrated H_2SO_4. A reddish brown ring at the interphase of two liquid layers and appearance of bluish green colour in the upper layer indicated the presence of glycosides.

Assessment of Total Antioxidant Capacity

Total antioxidant capacity of the plant extract like Methanol and Aqueous extracts of *A. indica* were determined by Phosphomolybdenum assay. Stock solution the plant extracts were prepared by dissolving 10 mg of plant extract in their respective solvent.

Different concentrations like 100, 200, 300, 400 and 500 µg/ml were prepared from the six extracts. To obtain these concentrations, to 100µL of the plant stock solution 400µL of Methanol was added. Likewise, other concentrations of different plant extracts were also made and duplicates were maintained. To all the test tubes which are of plant sample 3 ml of Phosphomolybdenum reagent (0.6 M sulfuric acid, 28mM sodium phosphate and 4mM Ammonium molybdate) was added. 'Blank' was prepared by adding 500µL of Solvent and 3ml of reagent in the test tube. All the test tubes were incubated in a boiling water bath at 95 ℃ for 90 minutes. After 90 minutes the absorbance of the solution was read at 695nm using UV- Spectrophotometer.

Evaluation of Antioxidant activity by DPPH method

The free radical scavenging activity or the antioxidant activity of all the extracts was evaluated by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) according to Brand- Williams *et al* (1995) with slight modification. Here DPPH free radical scavenging activity of the plant extract like Methanol and Aqueous extracts of *A. indica* were determined. The stock solution of radical was prepared by dissolving 3.94mg of DPPH in 50 ml of Methanol, was wrapped with Aluminum foil and kept it in a refrigerator until further use.

Different concentrations like 10, 20, 30, 40 and 50 µg/µL were prepared from the six extracts. To obtain these concentrations, to 10µL of the plant stock solution 90 µL of Methanol was added. To all the wells which are of plant sample test, 100 µL of DPPH was added with the help of Micropipette.

'Control' was prepared with 100 µL of Methanol and 100 µL of DPPH solution. 'Blank' was prepared by 200 µL of Methanol in the well plate. The mixture were shaken vigorously and allowed to stand at room temperature for 30 minutes. Then the optical density of the solution in the micro wells were read at 517nm using the Microplate reader UV- spectrophotometer.

After that, OD for 'samples' were obtained by sample test (ST) - sample blank (SB). All the tests were performed in triplicates and the results were averaged. The values obtained are subjected to graph and calculations from which the percentage of scavenging activity or antioxidant activity percentage and IC_{50} values were determined.

Here, the Percentage of Scavenging activity was determines using the following formula,

$$\% \text{ of Scavenging activity} = \frac{\text{Abs. of control} - \text{Abs. of sample}}{\text{Abs. of control}} \times 100$$

From the % of antioxidant values a graph was plotted. On the graph, a trend line was added without (0, 0) intercepts. An equation of straight line from % of antioxidant values was obtained as, y = 12.75x + 13.55 for methanol plant extract. By this equation 'IC_{50}' value was determined by substituting '50' in the place of 'y'.

3. Results and discussion

Figure 1: Habit of *A. indica* L.

Table 1: Results of Qualilative Histochemical analysis of *A. indica*

Sl.No	Tests	Results
1	Callose	_
2	Starch	+
3	Cellulose	+
4	Lignins	+
5	Tannins	+
6	Proteins	+
7	Polyphenols	+

Here (+) = Present, (-) = Absent

Histochemical analysis of the stem sections of *A. indica* revealed the presence of Starch, Cellulose, Lignin, Tannin, protein and Polyphenols. The test showed negative result for callose.

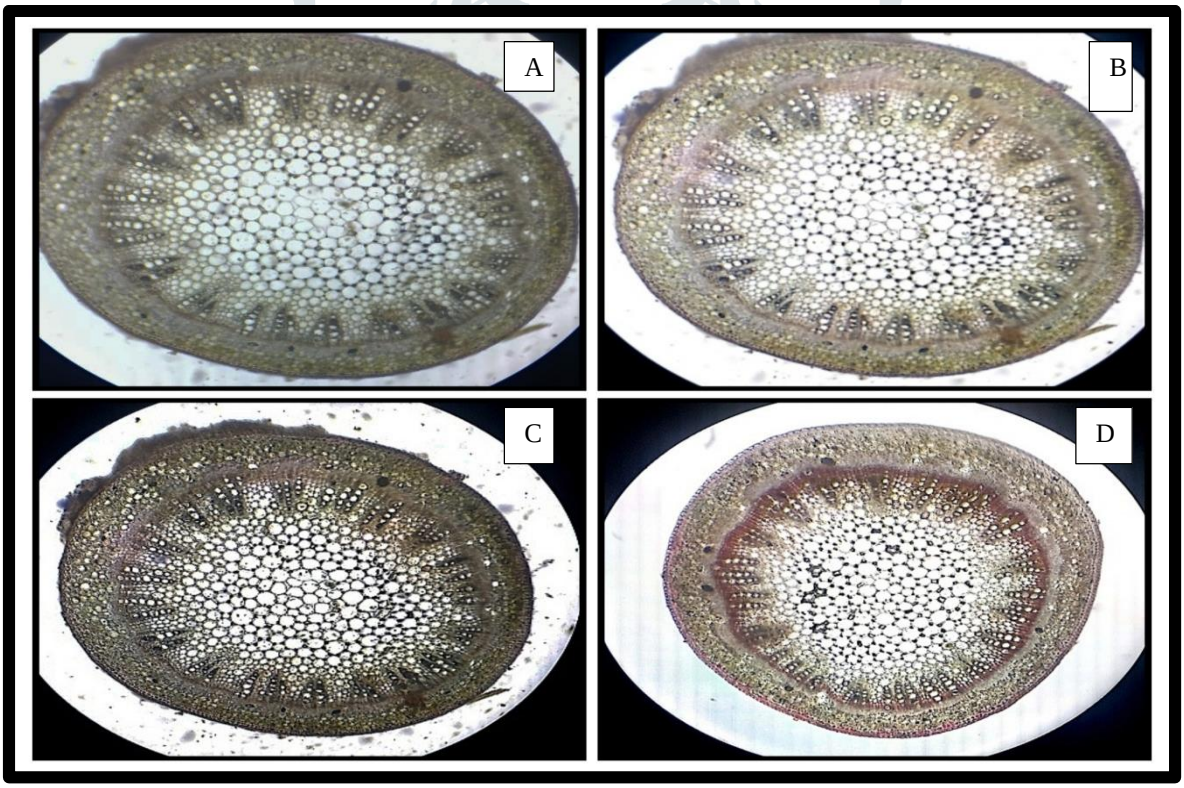

Figure 2: Histochemical analysis: Stained sections of *A. indica* stem- A) Control, B) Starch, C) Cellulose, D) Lignin

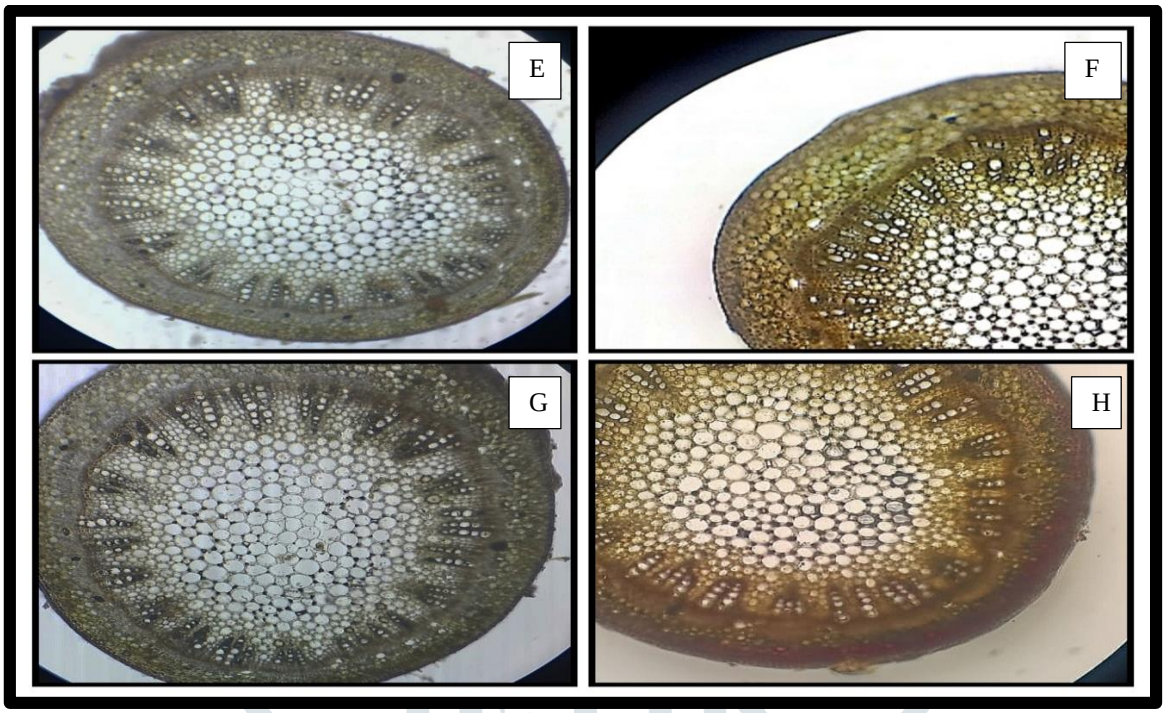

Figure 3: Histochemical analysis: Stained sections of *A. indica* stem- E) Control, F) Tannin, G) Protein, H) Polyphenol

Table 2: Phytochemical analysis of extracts of aerial parts of *A. indica*

Phytochemical Test					
Sl.No	Phytochemicals	Tests	*A. indica* aerial part extract		
			MeOH	E.A.	Aqua
1	Triterpenes	1. Salkowski's test	+	+	+
		2. Liebermann-Burchard's test	+	-	-
2	Saponins	1. Foam test	-	-	+
3	Alkaloids	1. Mayer's test	+	-	-
		2. Wagner's test	+	-	-
		3. Dragendorff's test	+	+	+
4	Phenolics	1. Ferric chloride test	-	-	+
5	Tannins	1. Gelatin test	+	-	-
6	Flavonoids	1. Shinoda test	-	-	+
		2. Lead acetate test	+	-	+
7	Proteins	1. Biuret test	+	-	+
		2. Ninhydrin test	-	-	+
8	Carbohydrates	1. Molisch's test	+	+	-
		2. Fehling's test	+	+	+
		3. Benedict's test	+	-	+
9	Resins	1. Turbidity test	-	-	-

		2. Acetic anhydride test	+	+	-
10	Glycosides	1. Keller-Killiani's test	-	-	+

MeOH= Methanol, E. A. = Ethyl acetate, Aqua= Aqueous extract.

(+) = Present, (-) = Absent

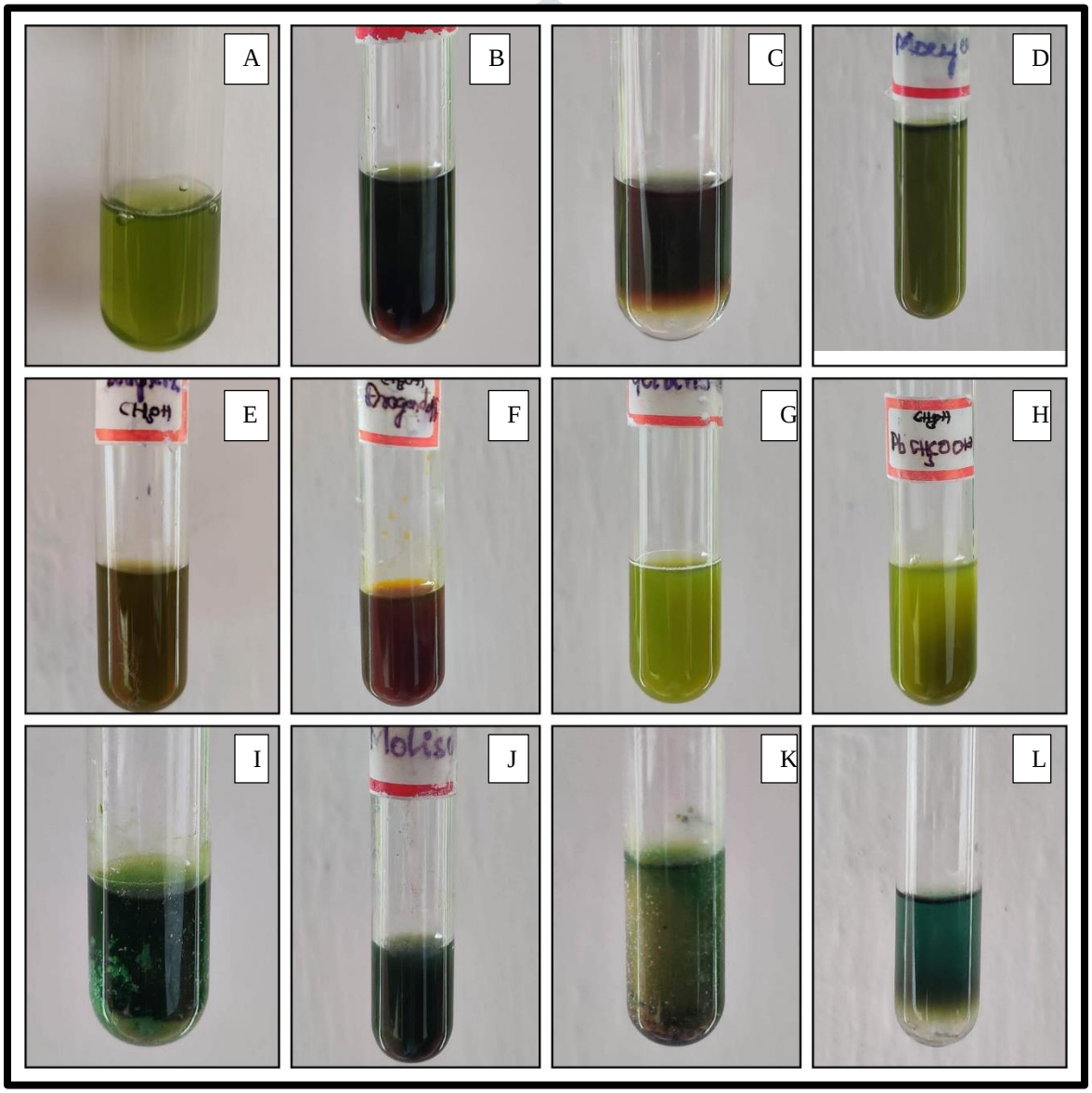

Figure 4: Methanol extract of *A. indica* aerial parts: A) Control, B) Salkowski's test, C) Libermann- Burchard's test, D) Mayer's test, E) Wagner's test, F) Dragendorff's test, G) Gelatin test, H) Lead acetate test, I) Biuret test, J) Molisch's test, K) Fehling's test, L) Acetic anhydride test.

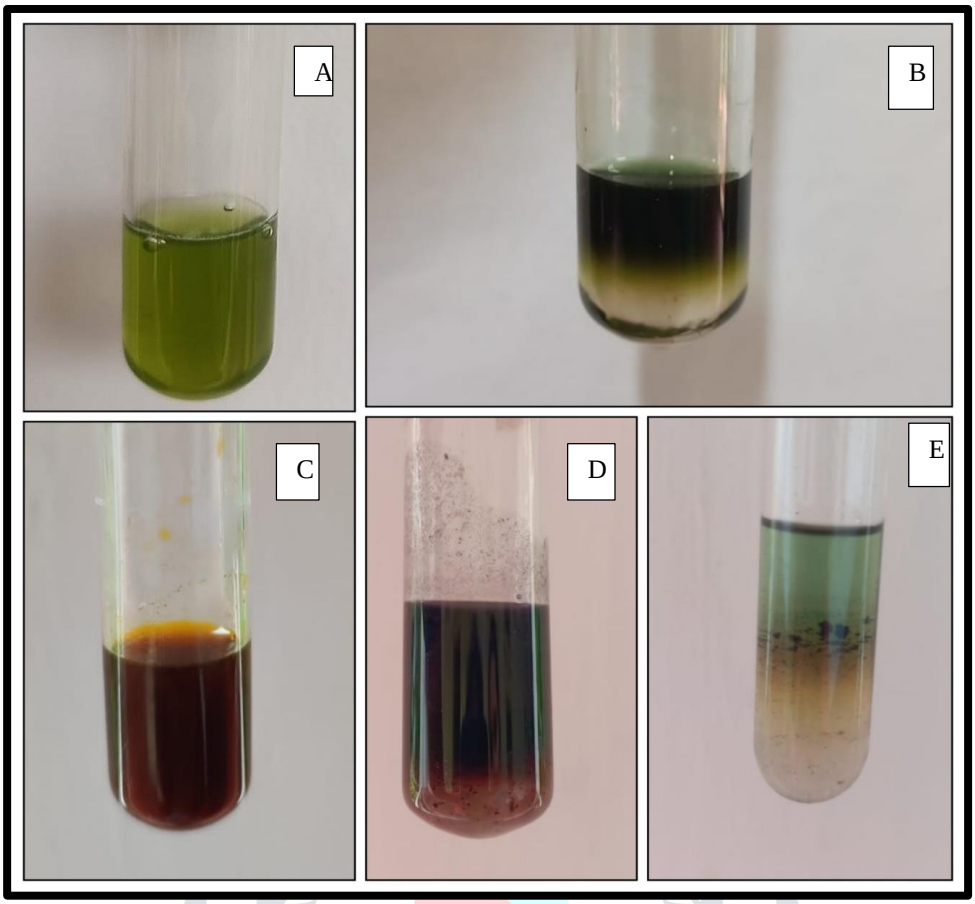

Figure 5: Ethyl acetate extract of *A. indica* aerial part: A) Control, B) Salkowski's test, C) Dragendorff's test, D) Molisch's test, E) Acetic anhydride test

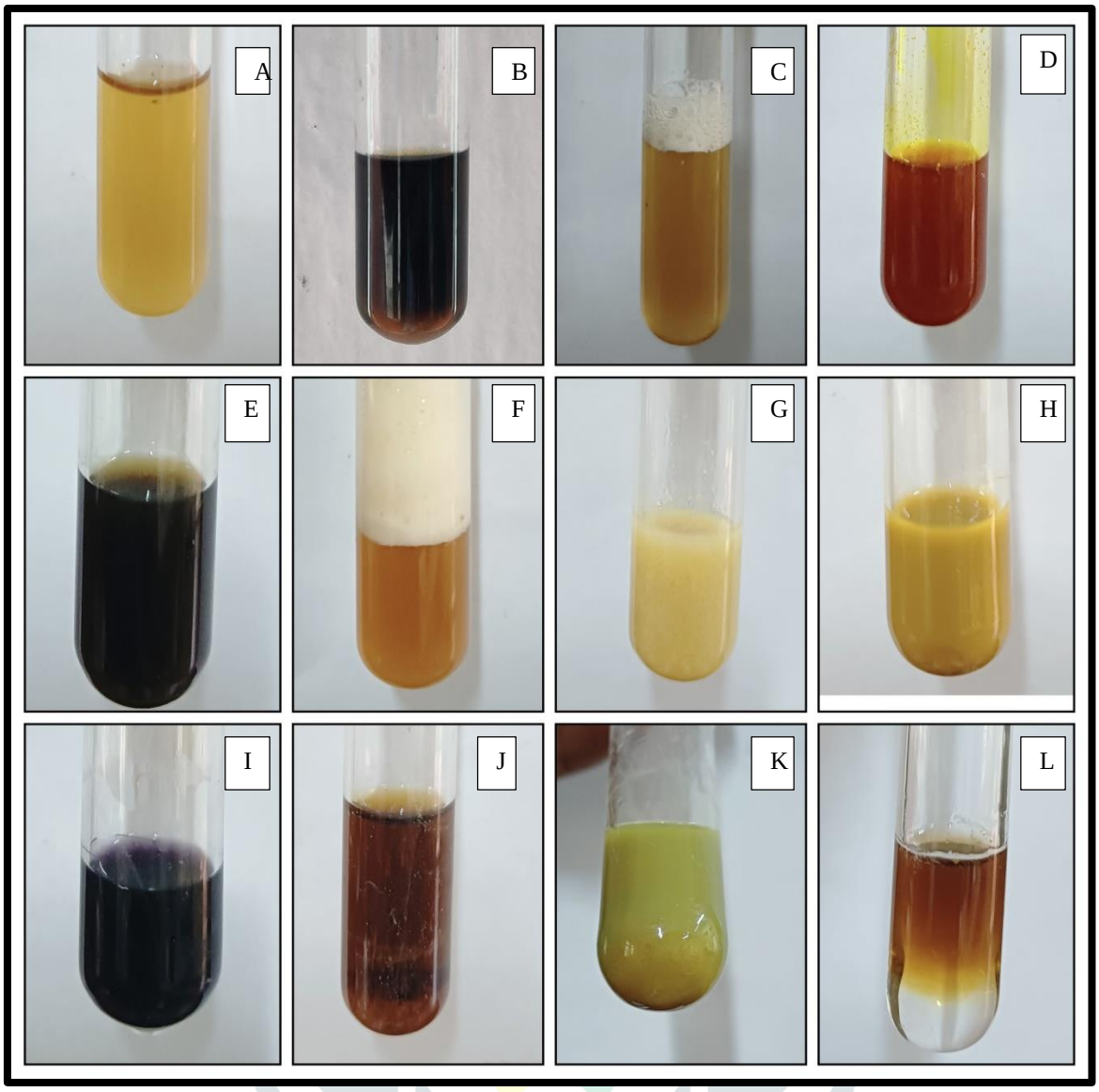

Figure 6: Aqueous extract of *A. indica* aerial plarts: A) Control, B) Salkowski's test, C) Foam test, D) Dragendorff's test, E) FeCl3 test, F) Shinoda test, G) Lead acetate test, H) Biuret test, I) Ninhydrin test, J) Fehling's test, K) Benedicts test,L) Keller- Kiliani's test.

Table 3: Total antioxidant capacity (Abs.695nm)

Sl.No.	Conc. µg/mL	Absorbance at 695 nm	
		Methanol extract	Aqueous extract
1	100	0.074	0.184
2	200	0.138	0.268
3	300	0.257	0.395
4	400	0.329	0.437
5	500	0.401	0.564

SB- Sample blank T_1: Trial-1, T_2: Trial-2, T_3: Trial-3

ST- Sample test OD- Optical density

Here, in case of total antioxidant activity by phosphomolybdenum assay, the Aqueous extract shows more absorbance when compared to Methanol extract. It means that Aqueous plant extracts shows highest total antioxidant capacity compared to methanol extract.

Table 12: Percentage RSA and IC_{50} values of Methanol plant extract of *A. indica*

Sl. No	Concentration µg/µL	Control	Sample OD (ST-SB)	% Antioxidant activity	IC_{50} Value
1	10	0.7763	0.550	29.15	**22.8 µg/µL**
2	20		0.430	44.60	
3	30		0.310	60.06	
4	40		0.180	76.81	
5	50		0.100	87.11	

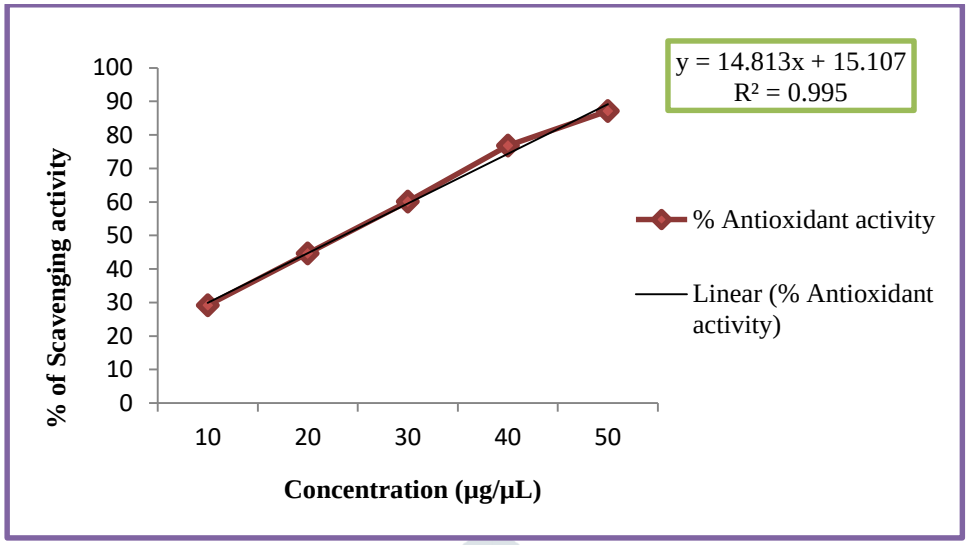

Figure 7: Graph of Antioxidant assay in Methanol extract of *A. indica*

Table 18: Percentage RSA and IC_{50} values of Aqueous plant extract of *A. indica*

Sl. No	Concentration µg/µL	Control	Sample OD (ST-SB)	% Antioxidant activity	IC_{50} Value
1	10	0.7763	0.520	33.01	**21.3 µg/µL**
2	20		0.400	48.47	
3	30		0.280	63.93	
4	40		0.150	80.67	
5	50		0.070	90.04	

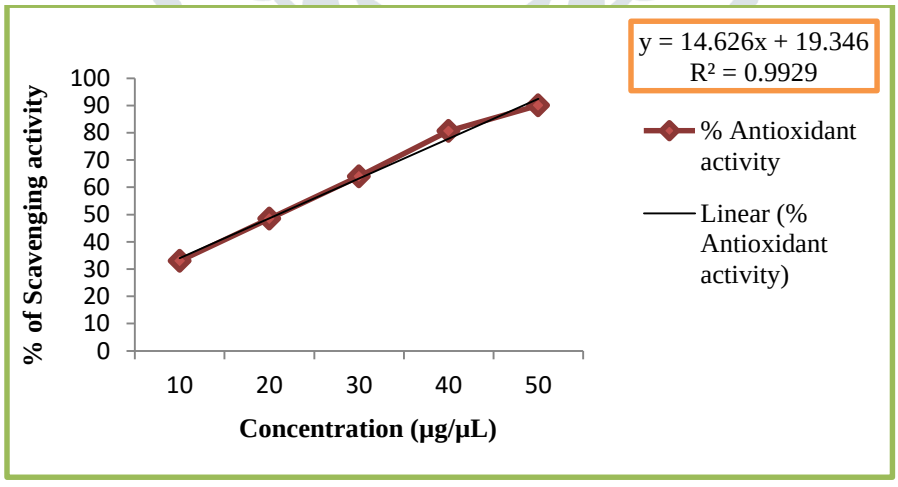

Figure 8: Graph of Antioxidant assay in aqueous extract of *A. indica*

Here, in case of DPPH antioxidant assay, the aqueous plant extract gave the higher IC_{50} values of **21.3 µg/µL,** when compared to the Methanol extracts value.

4. Summery and Conclusion

Plants are the treasure house of numerous medicines which are playing an important role in curing many diseases thus saving many lives since ancient time. The selected plant A. indica for present investigations is ethnopharmacologically important. Qualitative Histochemical analysis showed the presence of highest starch, Tannin, Lignin, Protein and Polyphenols. On screening of plant extract to various qualitative phytochemical tests reveals the presence of Triterpens, Saponins, Alkaloids, Phenolics, Flavonoids, Proteins, Carbohydrates and Glycosides. Total Antioxidant Capacity by Posphomolybdenum method showed the highest absorbance in Aqueous extract than in methanol extract and on assessing antioxidant activity of plant extract by DPPH assay reveals highest activity in aqueous extract with IC_{50} value of 21.3 µg/µL. It is thereby apparent and promising; to state the obtained research findings of histochemistry, phytochemistry and antioxidant activity for the selected plant species clearly reveals that, the plant is potential and challenging enough to be worked out further.

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