Biochemical, Antioxidant and Antibacterial Analysis of *Andrographis lineata* (Periyanangai) Leaves Extract

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Abstract: Nature has been a source of medicinal agents for thousands of years and since the beginning of mankind. The application of medicinal plants especially in traditional medicine is currently well acknowledged. Medicinal plants are an important source for the therapeutic remedies of various ailments. *Andrographis lineata* is one such plant which is extensively used in herbal medicines due to its vast and time proven medicinal properties. *Andrographis lineata.* Nees belongs to the Acanthaceae family. All parts of this plant are medicinally important in the traditional system of medicine in India and have been used extensively in snake bite and as an antipyretic. In Hilly regions of South India, the leaves extract is a traditional remedy for the treatment of liver disease, infectious disease, diabetes, cancer, fever-causing diseases, loss of appetite, irregular stools and diarrhea. The present study was conducted to evaluate phytochemical screenings of A*ndrographis lineata* leaves. *A. lineata* leaves extract possess a wide range of phytocompounds which has been exposed by the screening with various solvents like Aqueous, Ethanol, Methanol, Acetone and Hexane. The screening showed the presence of various phytochemicals like alkaloids, flavonoids, phenols, tannins, saponins and terpenoids. The quantitative analysis exhibited high amount of Alkaloids, flavonoids and phenols, and biochemical constitutents includes carbohydrate, protein, Irin, Calcium and Vitamin A, C. Thin Layer Chromatography (TLC) method done for identification phytoconstituents. Quantitative analysis indicated high alkaloid concentrations and nutritional components, while antibacterial tests showed concentration- and solvent-dependent efficacy against bacteria like *B. subtilis* and *E. coli*, potentially through mechanisms involving cell membrane disruption or enzyme inhibition. These results provide pharmacological evidence for the plant can be used as medicinal agent in folklore medicine.

I. INTRODUCTION

The "King of Bitters," *Andrographis lineata*, is a popular medicinal herb utilized in several Asian traditional medical systems, such as Ayurveda, Traditional Chinese Medicine (TCM), and Thai herbal medicine (Purohit et al., 2025). Andrographis is known as "King of bitters". *Andrographis lineata* Nees belongs to the family Acanthaceae is an erect herb found widely in Deccan, Carnatic, Yercaud and Nilgiri hills of South India (Gamble, 1956). Reports on the medicinal properties are available only for A.paniculata and much work has not been done on A.alata and A.lineata, The diterpenoid lactones andrographolide, neoandrographolide and deoxyandrographolide, among others dominate this plant's very bitter phytochemical profile, which makes it especially prized. The many biological actions of these substances, such as their anti-inflammatory, antioxidant, hepatoprotective, immunomodulatory, and antibacterial properties, have been well studied (Saravanan et al., 2023).

A. paniculata is reported to contain, andrographolide (C20H30O5), a bitter principle as the main active constituent (Balu et al., 1993) *Andrographis lineata* Nees (Fam. Acanthaceae) is a small plant found in and around Salem district, TamilNadu, India. All parts of this plant are medicinally important in the traditional system of medicine in India and have been used extensively in snake bite and as an antipyretic (Alagesaboopathi and Balu, 1999). It is also used as a blood purifier and also in veterinary medicine. Three flavonoids were isolated from the leaf extract (Harikishore *et al.*, 2003). *Andrographis lineata* commonly known as "Periyanagai" is distributed in south India. The leaves of AL have been reported to be very effective for diabetes, snakebites, ringworms, cancer and inflammation in ancient folklore medicine practiced by the tribal people of Kolli hills Namakkal district, Tamilnadu, India. (Alagesaboopathi, 1993). There are reports on

insecticidal activity, antimicrobial, diuretic and hepatoprotective activity (Sangameswaran.B et al., 2008, Ignacimuthu et al., 2006. chopra et al., 1956). The Present study was undertaken to assess phytochemical analysis, Biochemical profile, and antimicrobial activity of *Andrographislineata*plant extract.

II.TAXONOMY OF *Andrographis lineata*

Scientific classification

Kingdom	:	Plantae
Clade	:	Tracheophytes
Clade	:	Angiosperms
Clade	:	Eudicots
Clade	:	Asterids
Order	:	Lamiales
Family	:	Acanthaceae
Subfamily	:	Acanthoideae
Tribe	:	Andrographideae
Genus	:	Andrographis

Wall. ex Nees

Habit : Undershrub

Habitat : Semi-Evergreen forests and grasslands on hills above 1000m, grasslands

Flower, Fruit : July-October

III. MATERIALS AND METHODS

3.1. COLLECTION OF SAMPLE

Andrographis lineata plant samples were collected from the in the kolli hills, salem district, Tamil Nadu, Authenticated by Department of Botanical Survey of India, Sothern Regional Centre, Coimbatore BSI/SRC/5/23/2022/TECH-200. Leaves were separated from plants and dried at room temperature. The dried samples were grinded properly using a grinder, to obtain the powdered form.


Figure 1 and 2: *Andrograhis lineata* Plant

3.2. PREPARATION OF AQUEOUS EXTRACT

20 gm. of the sample was suspended in 200 ml of distilled water. Extraction was done at 70 ˚C for 30 minutes, followed by filtering of the extracts using Whatman filter paper. After filtration, the Extracts were then further transferred into sterile bottles and refrigerated until use.

3.3. SOXHLET EXTRACTION

3.3.1. Ethanol extract

20g of dried powdered leaves of *Andrographis lineata* was extracted with 200ml of ethanol.

3.3.2. Methanol extract

20g of dried powdered leaves of *Andrographis lineata* was extracted with 200ml of methanol.

3.3.3. Acetone extract

20g of dried powdered leaves of *Andrographis lineata* was extracted with 200ml of Acetone.

3.3.4. Hexane extract

20g of dried powdered leaves of *Andrographis lineata* was extracted with 200ml of Hexane.

3.4.1 PHYTOCHEMICAL SCREENING QUALITATIVE ANALYSIS OF PHYTOCHEMICALS

The plant samples were assessed for the presence of the various photochemical by using the following standard methods

TEST FOR ALKALOIDS

a.Mayer’s test:

To a few ml of plant sample extract, two drops of Mayer’s reagent are added along the Sides of test tube. Appearance of white creamy precipitate indicates the presence of alkaloids.

b.Wagner’s test:

A few drops of Wagner’s reagent are added to few ml of plant extract along the sides of the test tube. A reddish Brown precipitate confirms the test as positive.

TEST FOR FLAVONOIDS

a.Lead acetate teast :

Extracts were treated with few drops of lead acetate solution. Formation of yellow color precipitate indicates that the presence of flavonoids.

b.Con.H2S04 test:

Extracts were treated with few drops of H2S04. Formation of orange color indicates that the presence of flavonoids.

TEST FOR PHENOLIC COMPOUNDS
Ferric Chloride test:

The extracts were treated with few drops of neutral 5% ferric chloride solution are added. A dark green color indicates the presence of phenolic compound.

TEST FOR TANNINS:

a.Ferric Chloride test:

The extracts were treated with few drops of neutral 5% ferric chloride solutions are added. A dark green color indicates the presence of tannin compound.

b.Lead Acetate Test:

The extract is treated with few drops of 1% lead acetate was added to the tube. The yellow or red color precipitate is indicate the present of tannin.

TEST FOR SAPONIINS

Foam Test:

In a test tube containing 5ml of dioscorea bulbifera tubers extract, a few drops of sodium bicarbonate was added. The mixture was shaken vigorously for 3mins. A honey comb like froth was formed and it showed the presence of saponins.

TEST FOR TERPENOIDS

Salwosik's Test:

5 mg of the extract of the leaves was mixed with 2 ml of chloroform and concentrated H2S04 (3ml) was carefully added to form a layer. An appearance of reddish brown color in the inner face was indicates that the presence of terpenoids.

TEST FOR STEROIDS

Salwosik's Test:

To the extract added few drops of chloroform followed by concentrated sulphuric acid along the sides of the tube. The formation of bluish red to cherry red color change showed positive result.

TEST FOR GLYCOSIDES

Few drop of plant extract added with 2ml glacial acetic acid and then one drop of 5% Fecl3 Formation of brown ring appears indicating the presence of glycosides. Followed by concentrated sulphuric acid were added along the sides of the tubes.

TEST FOR RESINS
1ml of solvent extract was treated with few drops of acetic anhydride solution concentrated sulphuric acid. Resins give color from orange to yellow.

3.4.2 QUANTIITATIVE ANALYSIS OF PHYTOCHEMICAL
ESTIMATION OF ALKALOIDS
Quantitative determination of alkaloid was according to the methodology by Harborne [2]. Exactly 200 cm3 of 10% acetic acid in ethanol was added to each wood powder sample (2.50 g) in a 250 cm3 beaker and allowed to stand for 4 hours. The extract was concentrated on a water bath to one-quarter of the original volume followed by addition of 15 drops of concentrated ammonium hydroxide dropwise to the extract until the precipitation was complete immediately after filtration. After 3 hours of mixture sedimentation, the supernatant was discarded and the precipitates were washed with 20 cm3 of 0.1M of ammonium hydroxide and then filtered using Gem filter 218, the residue was dried in an oven and the percentage of alkaloid is expressed mathematically as

$$\% \text{ Alkaloid} = \frac{\text{Weight of alkaloid}}{\text{Weight of sample}} \times 100.$$

ESTIMATION OF FLAVONOIDS
The flavonoids content was determined by the slightly modified colorimetric method described previously by (Zhishen et al., 1999).

Procedure:
A 0.5ml, aliquot of appropriately (2mg/mL) diluted methanolic extract of Andrographis lineata was mixed with 2ml of distilled water and subsequently with 0.15ml of 5% NaNO2 Solution. After 6 min, 0.15ml of 10% AICI3 Solution was added and allowed to stand for 6 min, and then 2ml of 4% NaOH solution was added to the mixture. Immediately, water was a0ded to bring the final volume to 5 ml, and then the mixture was thoroughly mixed and allowed to stand for another 15min.Absorbance of the mixture was determined at 510nm water blank. The analysis was performed in triplicate and the results were expressed as ruin equivalent.

ESTIMATION OF PHENOLS
The amount of total Phenols in the plant extract was estimated by the method propose by (Mallick., Singh, 1980).

The sample (0.5g) was homogenized in 10X volume of 80% ethanol. The homogenate Centrifuged at 10,000rpm for 20 minutes. The extraction was repeated with 80% ethanol. Supernatants were pooled and evaporated to dryness. The residue was then dissolved in a volume of distilled water. Different aliquots were pipette out and the volume in each made up to 3.0ml with distilled water. Folin-Ciocalteau reagent (0.5ml) was added the tube were placed in a boiling water bath for exactly one minute. The tubes were the Cooled and the absorbance was read at 650mm in a spectrophotometer against a reagent blank, Standard Gallic acid solutions (0.2-Iml) corresponding were also treated as above. The concentration of phenols is expressed as mg/g tissue.

THIN LAYER CHROMATOGRAPHY

Coated the slurry (1:2) over the glass plates at a thickness as 0.25ms 0.25mm and allowed to dry room temperature for 15-30 minutes. Heated the plate in over at 100- 200°C for 1-2 hours to remove the moisture and activate the adsorbent on the plate. The column eluted was applied at 25 cm from one end at the glass plate. Allowed the sample to dry so that spotting can be done repeatedly for more concentrated sample spot. Poured the developing solvent into a tank at depth 1.5cm. After equilibration, remove the cover plate and placed the thin layer (sample applied) vertically in the tank so that it stands in the solvent with the spotted end dipping in the solvent moves upward. Once the solvent reaches the top at the plate, remove it from the tank dried and speared with spraying reagent for the identification at the separated compounds. The sample produce dark blue colour with spraying reagent.

3.5.3 BIOCHEMICAL ANALYSIS

ESTIMATION OF TOTAL CARBOHYDRATE

Total carbohydrate was estimated using the Hedge and Hofreiter method as explained. [Sadasivam and Manickam., 2005]

Prepared the standards by taking 0.2-1.0 ml of the Working standards. And 0.1-0.3ml of the sample were taken in another three different test tubes.1.0 ml of water serves as a blank made up the volume of 1.0 ml in all the tubes with distilled water, then added 4.0 ml of anthrone reagent, heated for eight minutes in a boiling water bath, cooled rapidly, and read the green to dark green color at 630 nm.

ESTIMATION OF TOTAL PROTEIN

Total protein content was estimated as depicted in appendix 8 (Lowry et al., 1951)

Extraction of protein from Sample Extraction is usually carried out with buffers used for the enzyme assay. Weigh 500mg of the sample and grind well with a pestle and mortar in 5-10mL of the buffer. Centrifuge and use the supernatant for protein estimation. Pipette out 0.2, 0.4, 0.6, 0.8, and 1.0ml of the working standard into a series of test tubes. Pipette out 0.1 ml and 0.2 ml of the sample extract in two other test tubes. Make up the volume to 1.0 ml in all the test tubes. A tube with 1.0ml of water serves as the blank. Add 5.0 ml of reagent C to each tube including the blank. Mix well and allow to stand for 10mins. Then add 0.5 ml of reagent D, Mix well and incubate at room temperature in the dark for 30min, the blue color is developed. Take the reading at 660nm. Draw a standard graph and calculate the amount of protein in the sample.

ESTIMATION OF IRON

Iron content was estimated by the method of Raghuramula *et al.,* 2005. Taken 1.5 ml of extracted leaves sample and 1.5 ml of prepared ash solution in test tubes, added 1.0 ml of 30% H2SO4 and 1.0 ml of 7% potassium persulphate solution, and 1.5 ml of 40% potassium thiocyanate solution are added. The red color developed was read at 540 nm within 20 minutes. The standard aliquots with concentrations corresponding to 10- 50 µg were treated similarly. The estimation was done in triplicates and the results were expressed in mg/g sample.

ESTIMATION OF CALCIUM:

The estimation of Calcium was done by the method of Raghuramula et al., 2005, Taking 2 ml of extracted resin sample and 2 ml of prepared ash solution in test tubes, adding 2.0 ml of distilled water, and 1.0 ml of 4% ammonium oxalate, mixed well, and allowed to stand overnight. After calcium precipitation, centrifuged and removed the supernatant fluid without disturbing the precipitate. To this 3.0 ml of 2%ammonia was added along the sides of the tube, mixed well, and centrifuged again. The supernatant fluid was poured off. This was repeated until the supernatant gave no precipitate with calcium chloride solution. Added 2.0 ml of 1N H2SO4 and mixed the precipitate well, placed in a boiling water bath for a few minutes. Keeping the mixture at 70–75selcias titrated against 0.01 N KMnO4, to a faint pink color, which persisted for about a minute. Titrated 2.0 ml of 1N H2SO4as blank to the same endpoint. The difference between the titration gives the volume of 0.01N KMnO4

required to titrate the calcium oxalate. The estimation was done in triplicates and the results were expressed in mg/g sample.

ESTIMATION OF VITAMIN A (Carotenoids):

The Vitamin A (Carotenoids) level was determined by the method of Nield and Pearson (1963).

To 1.0 ml of 10% homogenate, 1.0 ml of saponification mixture (2N/KOH in 90% alcohol) was added and heated under gentle reflux for 20 min at 60°C. 25 ml of water was added to the mixture after cooling to room temperature and the solution was transferred to a separating funnel. It was then extracted thrice using 25, 15, and 10 ml of petroleum ether (40- 60°C). The ether extracts were pooled and washed with 50-100 ml of distilled water repeatedly until the wash water was free of alkali.

The petroleum ether extract was then dried by adding anhydrous sodium sulfate. The volume of the 59 extracts was noted as 3.0 ml of petroleum ether phase was transferred to a curette and read at 420 nm against petroleum ether blank without delay to prevent evaporation of the solvent and destruction of Carotenoids by light. Marked this reading as A1. The β-carotene working standards are measured at 450 nm.

The aliquots were evaporated to dryness at 60°C in a water bath. The residue was taken immediately and 2.0 ml TFA reagent was added to it. The mixture was rapidly transferred to a cuvette and the absorbance was measured at 620 nm exactly after the addition of the TFA reagent.

Marked this reading as A2

The vitamin A working standard was read at 620 nm

A1= A2-A1 A1 = Absorbance of carotene at 450 nm 23

A2 = Absorbance at 620 nm due to both carotene and vitamin A

A3 = Absorbance at 620 nm of vitamin A.

$$\text{Sample} = \frac{\text{A3 x µg retinol calibrator / cavetto x 3 x total volume}}{\text{A620 retinol calibrator x 2 x gram}}$$

3 = Vol of petroleum ether from 1.0 ml extract

2 = Aliquot of the petroleum ether used for the assay

1 = 10% extract from the initial sample. The results are expressed as µg/mg protein

ESTIMATION OF VITAMIN C (Ascorbic Acid)

The levels of vitamin-C Ascorbic acid were estimated by the method of Omaye et al., (1979)

0.5 ml of tissue homogenate was mixed thoroughly with 1.5 ml of 6%trichloro acetic acid and centrifuged for 20 minutes at 3,500 x g. To 0.5 ml of the supernatant, 0.5 ml of dinitro phenyl hydrazine reagent was added and mixed well. The tubes were allowed to stand at room temperature for three hours, removed, and placed in ice-cold water. Then, 2.5 ml of 85% sulphuric acid was added to all the tubes and allowed to stand for 30 minutes. The color developed was read at 530 nm. The value of vitamin C was expressed as µg /mg vitamin.

ANTIOXIDANT ACTIVITY

FRAP Assay

Determination of Antioxidant Activity Using the Ferric Reducing/Antioxidant Power (FRAP) Method the FRAP assay was conducted following the method described by. Aliquots of 0.2 mL of methanolic extract (at four different concentrations: 0.1, 0.5, 1, and 2 mg/mL; two replicates per sample and concentration) had 3.8 mL of FRAP reagent added. This reagent was previously prepared by mixing 10 parts of 300 mM sodium acetate buffer solution at pH 3.6, 1 part of 10 mM TPZT, and 1 part of 20 mM FeCl3 hexahydrate (Alfa Aesar, Kandel, Germany). The resulting mix was incubated for 30 min at 37 ◦C. The absorbance increase was measured at 593 nm in a UV-30 spectrophotometer (GIORGIO-BORMAC SRL, Carpi, Italy). The blank was prepared by substituting the same amount of diluted extract with methanol. The results were expressed in milligram equivalents of FeSO4 per milligram of dry weight. The calibration line was established using the following concentrations of FeSO4: 0.0025, 0.005, 0.01, and 0.02 mg/mL.

Calculation

$$\text{Scavenging effect (\%)} = \frac{\text{Absorbance of test} - \text{Absorbance of control}}{\text{Absorbanceofcontrol}} \times 100$$

ABTS ASSAY

the ABTS solution from the refrigerator was taken and allow it to come to room temperature. Prepare Trolox standards as follows: Briefly spin down the contents of the 1.5 mM Trolox standard tube after thawing. Pipette 80 micro litre of Assay Buffer into the 1.5 mM Trolox standard tube provided and mix

well by vortexing. This produces a diluted stock Trolox standard of 300 µM. Pipette 50 µL of assay buffer into 6 tubes (not provided). Using the newly diluted stock Trolox solution, prepare a dilution series as depicted below. Mix each new dilution thoroughly before proceeding to the next. The 1mg/mL stock dilution serves as the highest standard, and the assay buffer serves as the zero standard.

3. Prepare the Working Solution. Briefly spin down the contents of the stock solution tube.

4. Add 20 µL,40 µL,60 µL,80 µL,100 µL of samples or Trolox standards to individual wells of the assay plate provided, add 10 µL of assay buffer to individual wells as a negative control.

5. Add 20 µL,40 µL,60 µL,80 µL,100 µL of the SAMPLE working solution to each of the wells.

6. To begin the assay, add 100 µL of the ABTS solution per well and place on plate shaker at room temperature. Allow the reaction to proceed for 5 minutes. To stop the reaction, add 50 µL of Stop Solution per well.

7. Read absorbance using plate reader at a wavelength of 745nm.

Calculation

$$\text{Scavenging effect (\%)} = \frac{\text{Absorbance of test} - \text{Absorbance of control}}{\text{Absorbanceofcontrol}} \times 100$$

ANTIBACTERIAL ACTIVITY

Phytochemical constituents	Aqueous	Ethanol	Methanol	Acetone	Hexane
Alkaloids	+	++	+++	-	+
Flavonoids	+	++	+++	+	+
Phenols	+	++	++	-	+
Tannins	-	++	++	+	+
Steroids	++	-	+	-	-
Saponins	++	++	+++	-	+
Glycosides	++	+	-	++	-
Terpenoids	++	+++	-	-	-

The antibacterial activity was evaluated using *E.coli and Bacillus*. The bacterial strains were provided from the microbiology laboratory, Periyar University. Bacterial strains were maintained by subculture on nutrient agar favorable to their growth for 24 h in the dark at 37°C. The antibacterial assay was done by using agar diffusion and pour plate method with standard antibiotics drug. The solvent of aqueous, ethanol, methanol, hexane, acetone were used against *E.coli* and *Bacillus subtilis* bacterial strains.

The Muller Hinton nutrient agar was poured on petriplates and allowed to solidify under laminar airflow chamber. About 1 ml of each bacterial inoculum was spread on the agar surface using sterile glass spreader or cotton swab. Then a well of 0.5 cm was made in the agar medium using a sterile cork borer. About 100µl of each sample of crude extract was transferred into separate wells and plates were incubated at 37° C for 24 hrs. A well with respective solvent served as negative control and 50µl of standard antibiotics tetracycline at the concentration of 10 mg/ ml served as positive control. The development of inhibition zone around the sample loaded well was recorded.

IV.RESULT AND DISCUSSION

4.1. QUALITATIVE ANALYSIS OF *Andrographis lineata*

The result of the qualitative analysis carried out *Andrographis lineata leaves* extract revealed the presence of wide range of phyto constituent (flavonoids, phenols, tannin and alkaloids) It shown in Table 1. Among the distribution of the phytochemicals that table no.1 figure clearly significance the abundant of flavonoids and alkaloids. The qualitative screening the phyto compounds of *Andrographis lineata leaves varied* among the solvents (ethanol, methanol, acetone,hexane, aqueous). Among the various solvent used for extraction, the methanolic fraction exhibited significant present for flavonoids.

4.2. THIN LAYER CHROMATOGRAPHY (TLC)

The plant extract of *Andrographis lineata* the subjected to thin layer chromatography (TLC) exihibited destinct spot for the present of secondary metabolites namely flavonoids, Phenols and Alkaloids.

Table 1: Qualitative Phytochemical analysis of *Andrographis lineata*

(++) indicates highly presence, (+)indicates presence, (-) indicate absence

An analysis of phytochemical constituents extracted using different solvents found that solvent polarity significantly affects which compounds are extracted, with highly polar solvents like methanol being most effective for a broad range of compounds. Aqueous and ethanol extracts were found to be more effective for specific compounds like terpenoids and glycosides, respectively

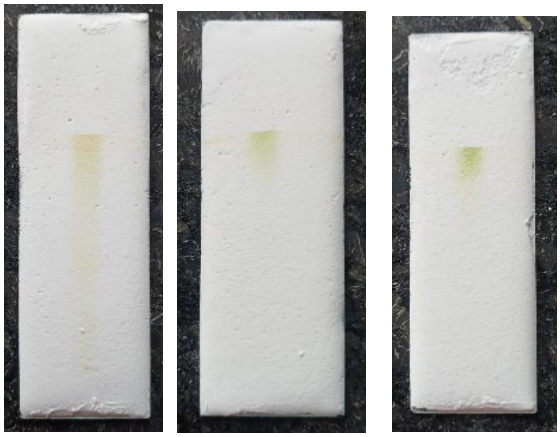


Figure 3: Plate 1. Flavonoids Plate 2. Alkaloids Plate 3. Phenols

The result of the thin layer chromatography spot further confirmed the presence of valuable secondary compound present Andrographis lineata.

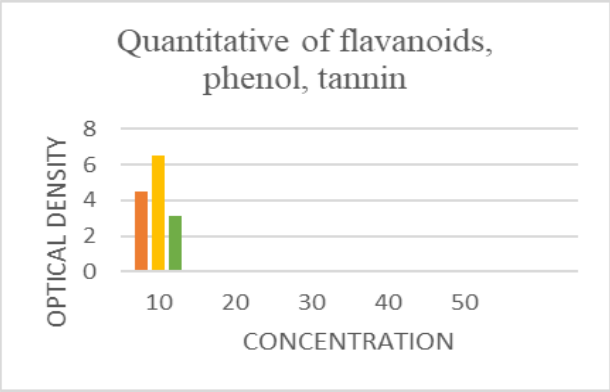
4.3 QUANTITATIVE ANALYSIS OF *Andrographis lineata*

The results show that alkaloids are the most abundant phytochemical in the sample, with a concentration of 6.5 mg/100gm. This is significantly higher than both flavonoids and phenols.

Table 2. Quantitative analysis of *Andrographis lineata*

S.No	Phytochemical analysis	Mg/100gm
1	Flavanoids	4.5±1.8
2	Alkaloids	6.5±2.4
3	Phenols	3.1±0.95

Figure 4. Estimation of flavonoids, Alkaloids, Phenols



1.Flavonoids 2. Alkaloids 3.Phenols

Among the phytochemical from the ethanol extract of the *Andrographis lineata shows alkaloids* predominant value (6.5 ± 2.4). Followed by Flavanoid (4.5±1.8) and followed by Phenols (3.1±0.95).

4.4 BIOCHEMICAL ASSESSMENT

The primary constitution in the globe provided by the plants of the source of food and nutrients. The prime phytochemical constitutional namely carbohydrates, proteins and vitamins. which collectively exhibit the biological significance in the life existence of living system in the globe.

Carbohydrates (polysaccharides)

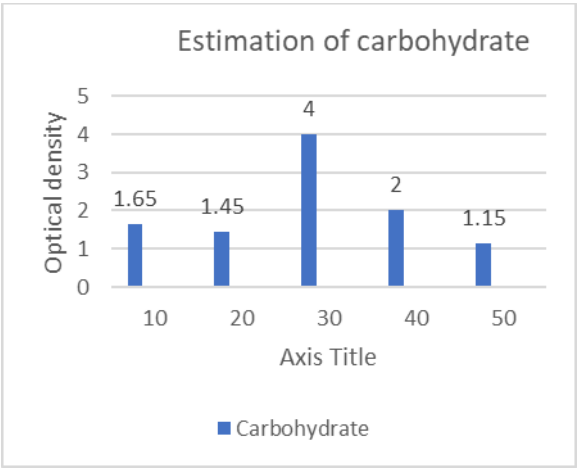
The results show a clear correlation between the solvent's polarity and its effectiveness in extracting carbohydrates. Methanol is the most effective solvent for carbohydrate extraction, yielding the highest concentration of 4 mg/dl. This is expected, as carbohydrates are polar molecules and dissolve readily in highly polar solvents like methanol.

Table 3. Estimation of carbohydrate of *Andrographis lineata* of leaves

S.no	Extract	Carbohydrate mg/dl
1	Aqueous	1.65±0.66
2	Ethanol	1.45±0.58
3	Methanol	4±1.6
4	Acetone	2±0.8
5	Hexane	1.15±0.46

The result from the table (4±1.6) shows that is various in the biochemical composition. Reveals the present of high content of carbohydrates(polysaccharides) followed by protein and cholesterol respectively.

Figure 5. Estimation of carbohydrate of *Andrographis lineata* of leaves



Protein

The most effective solvents for protein extraction were Ethanol (9.7±4.369.7 plus or minus 4.369.7±4.36mg/dl) and Aqueous (8.9±4.08.9 plus or minus 4.08.9±4.0 mg/dl). This is consistent with

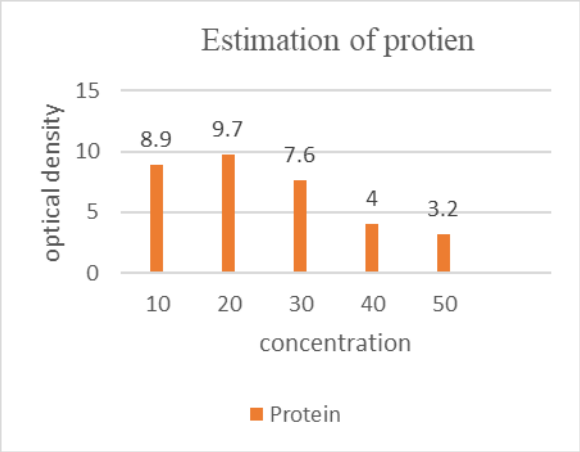
common knowledge that proteins are generally soluble in polar solvents, and hydro-alcoholic mixtures like aqueous and ethanol are often used for this purpose.

Table 4. Estimation of protein of Andrographis lineata

S.no	Extract	Protein mg/dl
1	Aqueous	8.9±4.0
2	Ethanol	9.7±4.36
3	Methanol	7.6±3.41
4	Acetone	4±1.79
5	Hexane	3.2±1.43

The presents quantified amount of protein in *Andrographis lineata* clearly exhibits from the table values (9.7±4.36) in the chloroform fraction which distinctly provides the nutritive potential of *Andrographis lineata.*

Figure 6. Estimation of protein of Andrographis lineata



Iron

The most effective solvents for extracting iron are the highly polar ones: Aqueous (33.2±15.933.2 plus or minus 15.9 33.2±15.9 mg/dl) and Ethanol (30.1±14.430.1 plus or minus 14.4 30.1±14.4mg/dl). The high concentration of iron extracted by these solvents indicates that iron compounds in the source material are highly soluble in polar solvents. This is a common finding, as metal ions and their salts often dissolve readily in water and other polar media.

Table 5. Estimation of Iron of *Andrographis lineata*

S.no	Extract	Iron mg/dl
1	Aqueous	33.2±15.9
2	Ethanol	30.1±14.4
3	Methanol	21.3±10.1
4	Acetone	17.5±8.3
5	Hexane	9.8±4.6

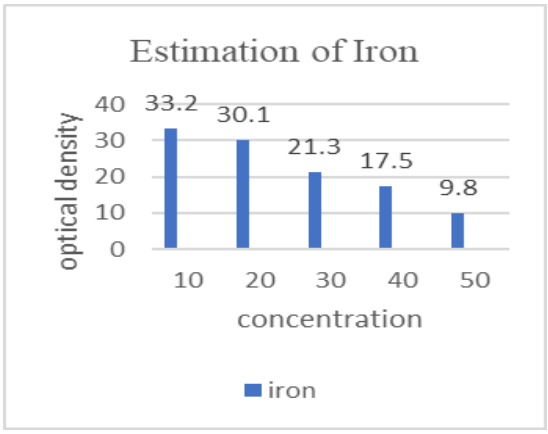


Figure 9. Estimation of Iron of *Andrographis lineata*

Calcium

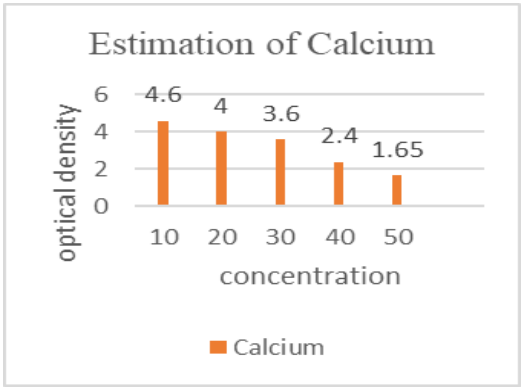
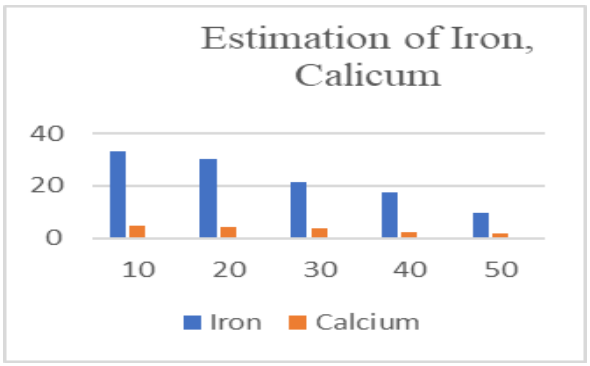


Figure 11. Estimation of iron,calcium



ESTIMATION OF VITAMINS OF *Andrographis lineata*

Vitamins

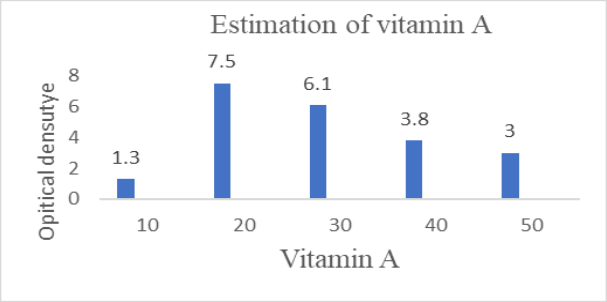
The most effective solvents for extracting Vitamin A were Ethanol (7.5±2.817.5 plus or minus 2.81 7.5±2.8 mg/dl) and Methanol (6.1±2.286.1 plus or minus 2.28 6.1±2.28 mg/dl). These are polar organic solvents and their high efficiency is consistent with the fact that Vitamin A, while fat-soluble, has a

hydroxyl group that makes it moderately soluble in alcohols.

Table 7. Estimation of vitamin A

S.no	Extract	Vitamin A
1	Aqueous	1.3±0.41
2	Ethanol	7.5±2.81
3	Methanol	6.1±2.28
4	Acetone	3.8±1.42
5	Hexane	3±1.18

Figure 12. Estimation of vitamin A



Vitamin C:

An analysis of the provided data indicates that aqueous is the most effective solvent for extracting vitamin C, yielding the highest concentration of 7.2 ± 3.23 mg/dl. The data suggests a correlation between solvent polarity and extraction efficiency, with polar solvents like water, ethanol, and methanol showing higher yields compared to the non-polar solvent hexane.

Table 9. Estimation of vitamin C

S.no	Extract	Vitamin C
1	Aqueous	7.2±3.23
2	Ethanol	6.3±2.8
3	Methanol	4.3±1.93
4	Acetone	3.5±1.57
5	Hexane	2.4±1.07

Figure 13. Estimation of vitamin C

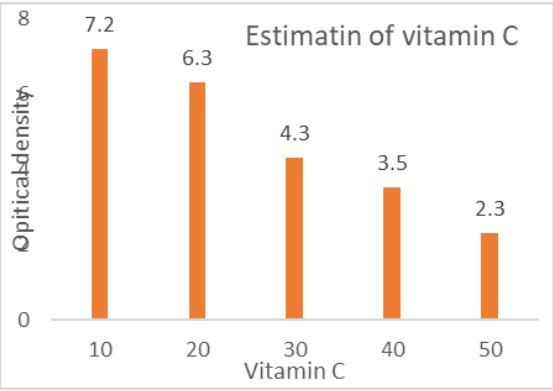
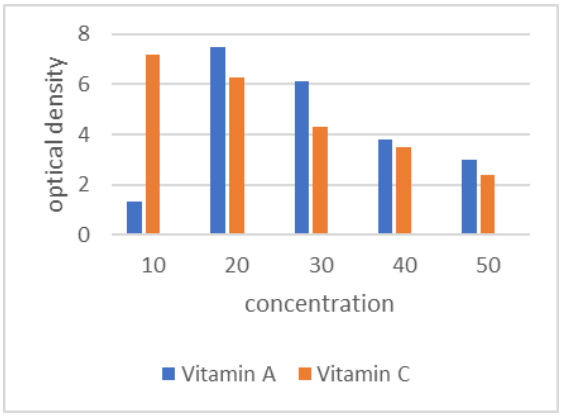
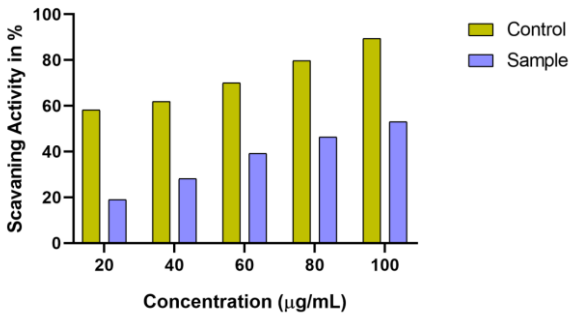


Figure 14. Estimation of vitamin A, vitamin C

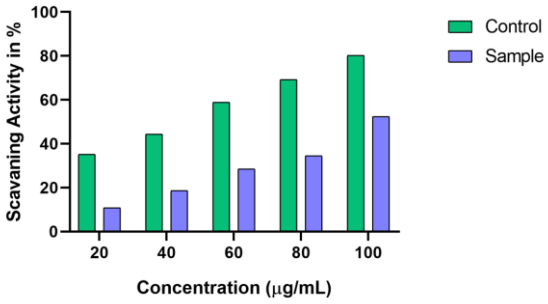


that *Andrographis lineata* plant have high antioxidant properties.

ANTIOXIDANT ACTIVITY OF *Andrographis Lineata*



FRAP ASSAY



ABTS ASSAY

ANTIBACTERIAL ACTIVITY FOR *Andrographis Lineata*

These extracts showed positive inhibitory effect against bacterial strains *(E.coli and B.subtilis*) and it is depends on the concentration (25µ, 50µ, 75µ, 100µ) It zone inhibition represent the effectiveness of *Andrographis lineata* extract against the selected bacterial strains.

Antibacterial activity aqueous extract by cup diffusion method

S. no	Name of the microorganisms	25 µl (mm)	50 µl (mm)	75 µl (mm)	100 µl (mm)
1	*Bacillus*	2.89±0.53	3.60±0.65	4.88±0.72	5.92±86
2	*E.Coli*	1.60±0.19	2.01±0.75	3.80±0.72	4.90±0.71

Antibacterial activity ethanol extract by cup diffusion method

S. no	Name of the microorganisms	25 µl (mm)	50 µl (mm)	75 µl (mm)	100 µl (mm)
1	*Bacillus*	2.09±0.38	3.19±0.45	4.14±0.73	6.42±0.71
2	*E.Coli*	1.67±0.21	3.10±0.81	4.87±0.75	5.63±0.78

Antibacterial activity methanol extract by cup diffusion method

S. no	Name of the microorganisms	25 µl (mm)	50 µl (mm)	75 µl (mm)	100 µl (mm)
1	*Bacillus*	2.00±0.23	3.08±0.41	3.70±0.58	5.12±0.60
2	*E.Coli*	1.44±0.16	2.02±0.73	3.72±0.69	4.50±0.62

Antibacterial activity acetone extract by cup diffusion method

S. no	Name of the microorganisms	25 µl (mm)	50 µl (mm)	75 µl (mm)	100 µl (mm)
1	*Bacillus*	1.80±0.19	2.89±0.38	3.65±0.48	4.98±0.58
2	*E.Coli*	1.41±0.12	2.45±0.61	3.68±0.67	4.44±0.74

Antibacterial activity hexane extract by cup diffusion method

S.no	Name of the microorganisms	25 µl (mm)	50 µl (mm)	75 µl (mm)	100 µl (mm)
1	*Bacillus*	1.77±0.16	2.81±0.33	3.60±0.42	4.80±0.53
2	*E.Coli*	1.38±0.10	2.33±0.58	3.64±0.62	4.41±0.71

Plate:E.coli *Plate B.subtilis*

Antibacterial activity of Andrographis lineata Aqueous extract against (E.coli and Bacillus subtilis) by cup diffusion method

Plate:E.coli *Plate B.subtilis*

Antibacterial activity of *Andrographis lineata* Ethanol extract against (*E.coli* and *Bacillus subtilis*) by cup diffusion method

Plate:E.coli *Plate B.subtilis*

Antibacterial activity om *Andrographis lineata* Methanol extract against (*E.coli* and *Bacillus subtilis*) by cup diffusion method

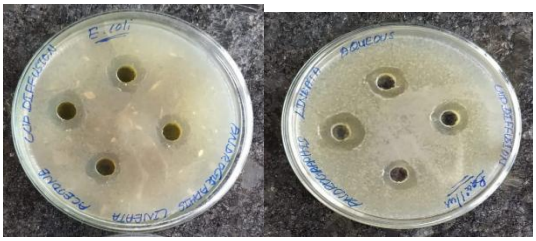


Plate:E.coli *Plate B.subtilis*

Antibacterial activity om *Andrographis lineata* Acetone extract against (*E.coli* and *Bacillus subtilis*) by cup diffusion method

Plate:E.coli *Plate B.subtilis*

Antibacterial activity om *Andrographis lineata* Hexane extract against (*E.coli* and *Bacillus subtilis*) by cup diffusion method

Antibacterial activity of different extracts (Aqueous, Ethanol, Methanol, Acetone, and Hexane) of *Andrographis lineata* by pour plate method.

S.no	Name of the microorganisms	Bacterial growth on the control plate	Bacterial growth on test plate with different extracts
1	*Bacillus*	+	-
2	*E.coli*	+	-

Table showing the growth of bacterial strain on control and test plate. (+) indicates the growth and (-) indicates the absence of growth.

Antibacterial screening *(E.coli* and *Bacillus subtilis*) by pour plate method- Control Plates

Plate:E.coli *Plate B.subtilis*

Antibacterial activity on *Andrographis lineata leaves* Aqueous extract against *(E.coli* and *Bacillus subtilis*) by pour plate method

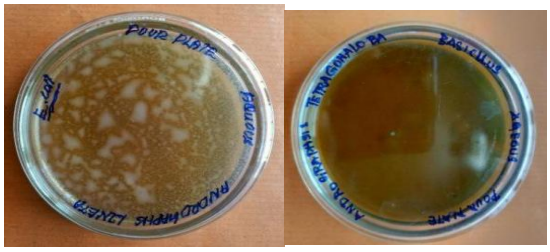


Plate:E.coli *Plate B.subtilis*

Antibacterial screening of *Andrographis lineata* leaves Ethanol extract *(E.coli* and *Bacillus subtilis*) by pour plate method

Plate:E.coli *Plate B.subtilis*

Antibacterial activity on *Andrographis lineata leaves* Methanol extract against *(E.coli* and *Bacillus subtilis*) by pour plate method

Plate:E.coli *Plate B.subtilis*

Antibacterial activity on Andrographis lineata leaves *Acetone extract against* (E.coli *and* Bacillus subtilis*) by pour plate method*

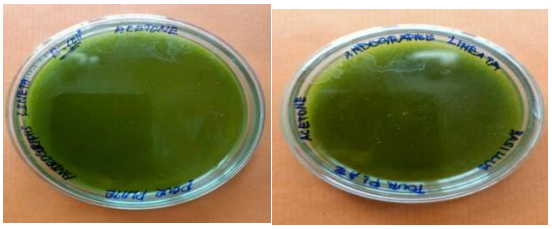


Plate:E.coli *Plate B.subtilis*

Antibacterial activity on *Andrographis lineata leaves* Hexane extract against *(E.coli* and *Bacillus subtilis*) by pour plate method

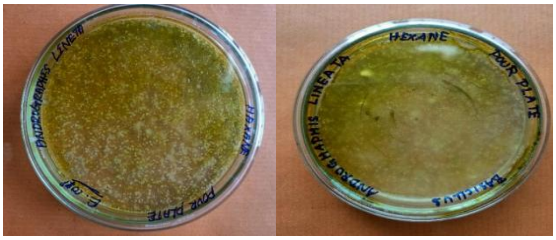


Plate:E.coli *Plate B.subtilis*

DISCUSSION

The phytochemical analysis on *Andrographis lineata* leaves extraction were carried out using the various extracts (ethanol, methanol, acetone, hexane, aqueous) of dry leaves powder.The

Qualitative screening for phytochemicals revealed the presence of phyto constituents like Alkaloids, Flavonoids, phenols and Tannins. Thin layer chromatography showed the presence of phenol at high concentration than flavonoids and Tannins respectively.The phytochemical quantification showed significant amount of alkaloids (6.5 ± 2.4).The amount of total carbohydrates (4 ± 1.6) of Andrographis lineata leaves possess a good store of carbohydrates. The protein content of methanolic extracts *Andrographis lineata* possess notable amount of protein (9.7 ± 4.3). The iron, (33.2 ±15.9) levels present in *Andrographis lineata* upholds good store. Andrographis lineata upholds good store of antioxidant vitamin A and vitamin C.

The *A.lineata* demonstrated strong antibacterial and antioxidant properties. Although somewhat less efficient than ascorbic acid, *A.lineata* antioxidant activity demonstrated a respectable FRAP assay and ABTS assay effectiveness, indicating their potential as robust free radical neutralizers. Antibacterial characteristics of *A.lineata* revealed improved activity against. five species. These findings highlight the potential of *A.lineata* an environmentally friendly alternative for antimicrobial therapeutics, The study highlighted the prospect of green-produced *A.lineata* in applications in medicine.

CONCLUSION

Qualitative analysis of *Andrographis lineata* leaf extracts using various solvents confirmed the presence of phytochemicals including flavonoids, phenols, tannins, alkaloids, steroids, saponins, glycosides, and terpenoids. Methanol was the most effective solvent for extracting a broad spectrum of compounds, Thin-layer chromatography (TLC) validated these findings by separating and identifying distinct spots for flavonoids, phenols, and alkaloids in the leaf extract. The leaves are rich in alkaloids, carbohydrates, proteins, iron, vitamin A, and vitamin C, suggesting potential nutritional and medicinal value. Andrographis lineata leaf extracts show antibacterial activity against *Bacillus subtilis* and *Escherichia coli*, with effectiveness depending on the solvent and concentration. Ethanol extracts at higher concentrations demonstrated potent activity against both bacterial strains, while acetone and hexane extracts were less effective. The study demostrates the plant's traditional medicinal use and potential for developing new therapeutic agents.

Future research on *Andrographis lineata* leaf extracts should prioritize isolating specific bioactive compounds and investigating their mechanisms of action and potential applications. Key areas include employing advanced analytical techniques, exploring synergistic effects with antibiotics, conducting in-vivo studies, and assessing antioxidant and other biological activities.

REFERENCE

[1] Alagesaboopathi C, Balu S. 1999. *Andrographis wallich* ex and*Andrographis lineata* Nees is used as an herbal drug in the treatment of snake bite. *Indian J Econ Taxon Bot* 23: 29–32.

[2] Alagesa Boopathi. 2000. Andrographis SPP.: A Source of Bitter Compounds for Medicinal Use. Anc Sci Life. 2000 Jan-Jun; 19(3-4): 164–168

[3] Balu.S, Alagesaboopathi and Elango.V. 1993. Antipyretic activities of some species of Andrographis wall. Ancient Science of Life. Vol No.XII . 399-402.

[4] Balu S, Alagesaboopathi C. 1993. Anti-inflammatory activities of some species of Andrographis Wall. Acanthaceae. Anc. Sci. Life 13: 180–184

[5] Balu S, Alagesaboopathi C. 1995. Antivenom activities of some species of Andrographis Wall. Anc. Sci. Life 14: 187–190

[6] Gamble J.S., 1956. Flora of the Presidency of Madras. Botanical Survey of India, Calcutta, Vol. 2, p. 1052.

[7] Hari Kishore P, Vijaya Bhaskar Reddy N, Kesava Reddy N. 2003. Flavonoids isolated from *Andrographis lineata. J Phytochem* 63: 457–461.

[8] J. Purohit, H. Solanki, A. Mishra, B. Shelke, A. Navale, R. Wakchaure, Translating Tradition into Therapy: Evidence-based Medicinal Plants for the Modulation of Osteoarthritis Biomarkers. Journal of natural remedies, -2547-2591

[9] Saravanan, V. Parthasarathy, P.S Kumar Synthesis and characterization of bioactive silver nanoparticles: evaluation of their anticancer potential against MCF7 cell line and antibacterial activity against foodborne pathogens J. Sol-Gel Sci. Technol. (2023), 10.1007/s10971-023-06243-2

[10] Sangameswaran*, T. Chandraganth Reddy and B. Jayakar. 2008. Hepatoprotective Effect of Leaf Extracts of *Andrographis lineata* Nees on Liver Damage Caused by Carbon Tetrachloride in Rats. *Phytother. Res.* 22, 124–126