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Phytochemical and Bioactive Components of *Lepidagathis barberi* Gamble. a steno-endemic plant.

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Abstract:

This study presents a comprehensive investigation of physicochemical, heavy metal analysis of soil, ash properties, macroscopic and microscopic studies, fluorescence analysis, phytochemical analysis, GCMS, FTIR analysis, nutritional, mineral, vitamin, organic acid analysis and bioactive parameters (Total phenol, tannin and Flavonoid) of the whole plant powder of Lepidagathis barberi were determined by using standard protocols. Soil collected from the plant growing area shows brown and sandy loamy in texture. The value of soil pH is (6.48±0.10), temperature (16.5oC), water holding capacity (10.90 ± 0.15), 1.40 ± 0.03 mg/kg bulk density, 2.15 ± 0.00 moisture, and 1.10±0.10 dsm-1 electrical conductivity. The soil sample shows the presence of various essential elements like C, N, P, K, Ca and Mg. Heavy metal concentration shows the presence of Cu, U, Fe, Pb, M, Ni, Ag, Pt, Zn, Fe, Ga and absence of As, Cd, Cr, and Sb. Ash properties of the plant sample revealed loss on drying 14.20%, total ash 17.35%, acid insoluble ash 19.20% and water soluble ash 13.40%. Macroscopic and microscopic studies show the taxonomical and anatomical studies of the whole plant part. In fluorescence analysis, colors like light brown, dark yellow, greenish brown, white, and dark brown were emitted under UV light at 366nm. In phytochemical analysis, isopropanol extract showed the presence of resin, carboxylic acid, tannins, flavonoid, carbohydrates, glycosides, saponification, protein, phenol, saponin, gum, flavonoglycosides, and alkaloids qualitatively compared to the other solvents. GC-MS analysis showed 26 major peaks with the retention time varying from 2.33 to 31.32 minutes were recorded. FTIR analysis showed 31 distinct peaks, indicating the presence of bioactive compounds, namely flavonoids, phenolics, alcohols, esters, alkanes, and aromatic compounds. Nutritional analysis showed the presence of ash 0.9%, crude fiber 50%, moisture 50%, protein 30.8mg/g, reducing sugar 30.1mg/g, and lipid 1.7mg/g. Mineral analysis showed the presence of sodium

(24.62 mg/L), potassium (0.43 mg/L), phosphorus (0.05 mg/L), and a notable amount of zinc (10.7 mg/kg). Vitamin analysis showed the presence of Vitamin D_2 (2.02 mg/kg), Vitamin E (72.50 mg/kg), Vitamin K_1 (80.27 mg/kg), Vitamin A (198.31mg/kg), and Vitamin C (1.2 mg/g). Organic acid confirmed the presence of oxalic acid (91.25 mg/kg), tartaric acid (92.63 mg/kg), and pyruvic acid (4.81 mg/kg), while malic acid, acetic acid, and citric acid were below detectable limits. The total phenol content was about 24.3 mg/g gallic acid equivalents, the Total tannin content was 81.04mg/g tannic acid equivalents, and the Total flavonoid content was 30.1 mg/g quercetin equivalents, respectively. The present findings indicate that Lepidagathis barberi holds promising phytochemical, nutritional and bioactive properties, suggesting its potential for future development as a medicinal plant; however, further detailed studies are necessary to validate its therapeutic applications.
Keywords: Bioactive compounds, Lepidagathis, Phytochemical, Therapeutic, Vitamins

1.Introduction

Medicinal plants are the richest resource of drugs of traditional systems of medicine, modern medicine, pharmaceutical intermediates and chemical entities for synthetic drugs (Devi,2023). Medicinal plants have an important place in health welfare programs across the globe. Medicinal plants are in demand because they produce a wide variety of drugs. It has been reported that about 80% of the world's population. Depends on herbal medicines for the treatment of various diseases (WHO, 2022). Medicinal plants typically contain several different chemical compounds that may act individually or synergistically to improve health (Jiao et al.,2022). This is one of the main reasons for research on medicinal plants. Herbal medicines have minimum or no side effects and are considered as safe, affordable and available (Tenywa et al.,2023). Many of the plant materials in rural areas are relatively cheap and used in traditional medicine. Several metabolities are produced by plants which comprise an important source of pharmaceutical products. Plants remain the principal source of pharmaceutical agents used in traditional medicine and natural products (Onyegbule et al.,2023). Bioactive constituents isolated from medicinal plants have an important role in the development of novel pharmaceutical agents against different diseases and infections (Chaudhary and Janmeda,2023).

The genus Lepidagathis (Acanthaceae), including Lophostachys Pohl, comprises ca. 150 species and is distributed across the pantropical Countries. In India, it is represented by 37 taxa, of which, 21 are endemic (Bramhadande & Nandikar, 2023). Genus Lepidagathis have not been extensively studied for their biological activities. Regarding bioactivity, there is less scholarly research accessible (Ponnusamy and Balakrishnan, 2023). Lepidagathis barberi is endemic to southern peninsular India which grows on dry sandy gravelly terrain of wastelands or scrub jungles at elevations ranging from 110-400nm and distributed on Tamil Nadu (Dindigul, Erode, Madurai, Theni, Thoothukudi and Virudhunagar districts) (King et al., 2022). This species shares the morphological similarities with Lepidagathis pungens but differs in erect to decumbent habit, glaucous stem, narrow elliptic-oblong leaves, and compressed ovoid spike with convergent bracts. Lepidagathis also shows some pharmacologically important activities such as antipyretic, antiurolithiatic, anti-inflammatory, analgesic, antiemetic, hypo-glycaemic, wound healing, immunosuppressive, and boosting fertility (Kadam et al.,2023). Plants from the Lepidagathis class have traditionally been used to treat polyuria, fever, mouth ulcers, diarrhea, uterine problems, and polyuria. They also have antioxidant, antiviral, hepatoprotective, anti-cancer, and anti-platelet aggregation activities. Lepidagathis sp. is also used to treat skin infections, headaches, jungle fever, cardiovascular diseases, and stomach problems (Ponnusamy and Balakrishnan,2023).

In this context, the present study aims to systematically investigate the physicochemical parameters, heavy metal content, ash values, extractive potential, macroscopic and microscopic studies, fluorescence analysis,

phytochemicals, nutritional analysis organic acids, mineral and vitamin composition, and bioactive properties of L. barberi.

2.Materials and methods:

Collection of plant materials

The experimental plant, L.barberi, was collected from G.Venkataswamy Naidu College campus, Kovilpatti, Tamil Nadu, India. Elevation is about 130m (Mean Sea Level with 9.1719° N latitude and 77.8726° E longitude). The plant specimens were identified and botanically authenticated by BSI, Coimbatore, with accession no.651. A voucher specimen was deposited in the herbarium of S.T.Hindu College, Nagercoil. Specimens of the experimental plant species were collected and dried at room temperature (30±2oC) for about two weeks to get a constant weight. The dried plant materials (as the whole plant) were powdered by a mechanical device and stored for further analysis.

Preparation of plant extracts for preliminary phytochemical screening

Dry powder of the whole plant samples was extracted with different solvents such as isopropanol, ethanol, ethyl acetate, petroleum ether, chloroform, and water at a 20 % (w/v) level using a soxhlet apparatus. The extracts were concentrated and used for qualitative phytochemical analyses.

Physicochemical properties of soil samples

Sample collection and preparation

Five representative samples were collected at a depth of 1-10cm, using a hand trowel, from each of the three places of study area where L. barberi grows and then combined to form a homogenized composite sample of each study area. The homogenised samples were air dried for seven days, grind in a clean mortar and pestle and sieved to pass through a 2mm alumina mesh, then preserved in washed clean plastic bottles for analysis (Buszewski et al., 2000; Nomeda, 2004). While collecting soil samples the upper layer of vegetation, surface litter, stones stubble if any were cleared away and then layer of soil immediately below (0-20 cm) was collected in polythene bag.

Sample analysis

Available phosphorus in soil was extracted by using the Bray and Kartz method. The extract was estimated colorimetrically following the blue color method using ascorbic acid and extract was analyzed by a spectrophotometer at 882 nm (Mamun et al., 2011), available potassium and sodium in soil was determined by flame photometric method after the soil was extracted with 1M ammonium acetate at pH 7, After removing the excessive ammonium, the soil was extracted with 100 g L-1 NaCl solution and the supernatant was used to determine the Cation Exchange Capacity (CEC) using the Kjeldahl distillation and titration method (Akbar et al., 2011). Exchangeable cations (calcium and magnesium) of soil samples were determined by classical routine method by complexometeric titration using EDTA as described in Huq and Alam, 2005. Total nitrogen of soil samples were determined by Kjeldahl's method following concentrated sulfuric acid (H2SO4) digestion as suggested by Jackson (1962). Particle size analysis was done by Hydrometer method, percentage of moisture present in the air dried soil was determined by drying method (Allen et al., 1974). Textural classes were determined by Marshall's Triangular Co-ordinates, as designed by the USDA (1951).

The collected samples were analyzed for major physical and chemical soil quality parameter like soil pH, electrical conductivity (EC), organic carbon (OC), available nitrogen (N), phosphorus (P), potassium (K) (Dahama,2002, Daji, 1998, Gaibe et al.,1976, Hausenbuiller,1976, Hudson, 1994, Jackson, 1973, Johnston,1986).

Heavy metal analysis:

The soil samples were dried at a room temperature for 7 days and sieved (2 mm sieve). The dried samples were subjected to a cool extraction with 0.5 M HNO3 for 30 minutes. Pb, Zn, Cu, Mg, Fe and Ca were determined by acetylene-air flame atomic absorption spectrophotomectry and Cd was determined by graphite furnace atomic absorption spectrophotometry (Buszewski et al., 2000).

Physicochemical properties of the plant sample

The percentage of loss of weight on drying, total ash, acid insoluble ash, water-soluble ash, and residue on ignition were obtained for L.barberi by employing standard methods of analysis as described in Pharmacopoeia of India (Anonymous, 1966).

Pharmacognostic Studies

Macroscopic Studies:

Mature and healthy whole plant L.barberi is collected to study the morphological characters. By using a hand lens in the field and a dissection microscope in the laboratory, the macroscopic characters of the plants were recorded.

Microscopic Studies:

The microscopic study was carried out by sectioning the root, stem, and leaf using a microtome. The thin sections were further washed with running water, stained with safranin for clear observation and confirmation of lignifications. Microscopic examination was done at magnifications of 10× and 40× (Tenywa et al.,2023).

Fluorescence properties:

The coarsely powered dried whole plants of L.barberi were studied initially under daylight and also under ultraviolet radiation. Later, about 1 gm. of the root powder was treated with 10ml of various reagents such as solvents like water, ethyl acetate, chloroform, methanol, and petroleum ethyl; alkaline solutions like aqueous and alcoholic 1 N hydrochloric acid, 50 % sulphuric acid, and 50 % nitric acid, and left overnight. The next day, the residue was removed, and the filtered solution was examined initially under daylight and then under ultraviolet radiation in a dark room for its characteristic fluorescent properties. The ultraviolet lamp with transmitting radiation in the range of 3600 to 4200 Angstrom units was used in this study. Standard pharmacognosy books were referred to, and the methodology reported by Kokoski et al., (1958) was followed.

Phytochemical studies:

Phytochemical tests were done following standard protocols (Harborne, 1998; Trease & Evans, 2002) with different solvents

Detection of Carboxylic acid (Effervescence test)

To 1mL plant extract, 2 mL of sodium bicarbonate solution is added. Colour changes that occur indicate the presence of carboxylic acid.

Detection of Tannins (HCL Test)

To 2mL of plant extract, 2-3mL of 10% HCL is added and boiled for 5-6 min. Formation of red colour indicates the presence of tannins.

Detection of Steroids (Salkowski's test)

To 0.5 mL extract, 5mL of chloroform is added and an equal amount of conc. H2SO4 was added. In the upper layer, a red colour and in the lower layer, yellow with green colour formation indicates the presence of steroids.

Detection of Flavanoids (Ammonia Test)

To 0.5 mL extract, 4mL of 1% ammonia was added, and to this, 1mL of conc. H2SO4 was added. The formation

of yellow colour indicates the presence of flavonoids.

Detection of Glycosides (Born Trageru's Test)

Take 2mL of hydrolysate, 3mL of chloroform was added, shaken vigorously, then the chloroform layer was separated. Then a 10% ammonia solution was added. The formation of pink colour indicates the presence of glycosides.

Detection of Proteins (Bradford Method)

To 500µl of plant extract, 5mL of the Bradford reagent was added, and the mixture was incubated in the dark for 10 to 15 min. Taken the OD at 575nm.

Detection of Phenol (Ferric Chloride Test)

To 50 mg of extract, 5mL of distilled water was added, and a few drops of 5% ferric chloride solution were added. The formation of dark green colour indicates the presence of phenol.

Saponin Test (Foam test)

To 50 mg of plant extract, 20 mL of distilled water was added and shaken vigorously for 15 min; the formation of a 2 cm layer of foam indicates the presence of saponins.

Test for Alkaloids - Mayer's test

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

Saponification test

To 1 or 2 mL of 10 N sodium hydroxide, 2 mL of extract is added and boiled for 2 minutes. The formation of soap or fat indicates a positive test for saponification.

Gum Test (Alcohol test)

The 100 mg of plant extract was dissolved in 2 mL of distilled water. 2mL of absolute alcohol with constant stirring. White colour cloudy precipitate indicates gums & mucilage.

Detection of flavanoglycoside (Shinoda's test)

The 50 mg of plant extract was dissolved in 5mL of ethanol. Added a few drops of magnesium sulfate & a few drops of conc. HCL. The formation of pink colour indicates the presence of flavanoglycoside.

Detection of Carbohydrates (Benedict's test)

To 0.5 mL of extract, 0.5 mL of Benedict reagent was added and boiled for 2 min. Color changes and precipitates are formed. It indicates the presence of carbohydrate.

Detection of resins

To 0.5 mL of plant extract, 3 mL of $CuSO_4$ solution is added. Shaken for about 1-2 min, the formation of green colour precipitate indicates the presence of resins.

Biuret test

To 2mL of extract, 1 drop of 2% $CuSO_4$ solution. Add 1 mL of 95 % ethanol add 2 to 3 sodium hydroxide pellets. Formation of pink colour indicates the test is positive.

Gas Chromatography–Mass Spectrometry (GC-MS analysis)

GC-MS analysis of the ethanol extracts of whole dry powdered material of Lepidagathis barberi was performed using a Perkin Elmer GC Clarus 500 system comprising AOC-20i auto-sampler and a Gas Chromatograph interfaced to a Mass spectrometer (GC-MS) equipped with an Elite – 5MS (5 % Diphenyl/95 % Dimethyl Poly Siloxane) fused capillary column (30 x 0.25 µm IDx0.25 µm df). For GC-MS detection, an electron ionization system was operated in electron impact mode with an ionization energy of 70 eV. Helium gas (99.999 %) was used as carrier gas at a constant flow rate of 1.491 ml/min, and an injection volume of 2 µl was employed (split

ratio of 10:1). The injector temperature was maintained at 250oC, the ion-source temperature was 200oC, and the oven temperature was programmed from 110oC with an increase of 10oC/min to 200oC, then 5oC/min to 280oC, ending with a 9 min isothermal at 280oC. Mass spectra were taken at 70eV, a scanning interval of 0.5 seconds, and fragments from 45-450 Da. The solvent delay was 0 to 2 min, and the total GC/MS running time was 36 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. The mass detector used in this analysis was Turbo-Mass-Gold-Perkin Elmer, and the software adopted to handle mass spectra and chromatograms was GC-MS solution ver-2.53.

Identification of phytocomponents

The relative percentage amount of each component was calculated by comparing its average peak area to the total peak area. The detection employed the NIST (National Institute of Standards and Technology) Version 25.3 – Year 2005 library. The compound prediction is based on Dr. Duke's phytochemical and Ethnobotanical Database (2008) by Dr. Jim Duke of the Agricultural Research Service. Interpretation of GC-MS was conducted using the database of NIST which has more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight, and structure of the components of the test materials were ascertained.

Fourier Transform Infrared Spectrometer (FTIR analysis Cakmak et al., (2006)

Fourier Transform Infrared Spectrometer (FTIR) is the most powerful tool for identifying the type of chemical bonds / functional groups present in the phytochemicals. The wavelength of light absorbed is a salient feature of the chemical bond, as can be seen in the annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bond in the compound can be determined. Dried powder of ethanol extract of Lepidagathis barberi was used for FTIR analysis. 10 mg of dried extract powder was encapsulated in 100 mg of KBr pellet to prepare translucent sample discs. The powdered sample of each extract was loaded in the FTIR spectroscope (Shimadzu, Japan), with a scan range from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}. The analysis was repeated thrice, and the mean ± SD was recorded.

Nutritional analysis:

Determination of Total Ash (Kadam, 2012)

Procedure

Weigh about 1 g of the test sample into an incineration dish that has been previously heated for at least 30 minutes in a muffle furnace at 550 °C, cooled in a desiccator, and weighed to the nearest 0.001 g. Place the dish containing the sample on a hot plate or gas burner and heat gently until the material is fully carbonized. Transfer the dish to the muffle furnace, maintained at 550 °C, and keep it there for three hours. Inspect the ash visually for any remaining carbon particles; if present, return the dish to the furnace for an additional hour. If carbon still remains or is suspected, allow the dish to cool, moisten the ash with distilled water, evaporate to dryness in an oven controlled at 103 ± 2 °C, and ash again at 550 °C for one hour. Finally, cool the dish in a desiccator to room temperature and weigh rapidly to the nearest 0.001 g. The ash obtained may be retained for further determination if required. Duplicate determinations should be carried out for accuracy.

The crude ash, expressed as a percentage test sample, is equal to by mass of the 100

$$(m2 - mo) \times$$
$$ml — MO$$

where,

m. is the mass, in grams, of the empty dish;

ml is the mass, in gram's, of the dish containing the test portion;
m2 is the mass, in grams, of the dish and the crude ash.
Take as the result the arithmetic mean of the two determinations, provided that the requirement for repeatability is satisfied. Report the result to the nearest 0,1 % (m/m).

Repeatability

The difference between the results of two determinations carried out simultaneously or in rapid succession by the same analyst shall not exceed:
0,3 (absolute value) for crude ash yields lower than 3 % (m/m);
10% of the mean value for crude ash yields from 3 to 5 % (m/m);
0,5 (absolute value) for crude ash yields from 5 to 20% (m/m);
2,5 % of the mean value for crude ash yields from 20 to 40 % (m/m);
1 (absolute value) for crude ash yields of 40 % (m/m) or more.

Determination of crude fibre (Weende method)

Procedure

The material was ground and 1 g of it is extracted with petroleum ether to remove fat (initial boiling temperature 35-38°C and final temperature 52°C) and dried at 80qC to constant weight. If fat content is below 1%, extraction is not required. Dried material (2 g) was boiled with 200 mL of 1.25% (w/v) sulphuric acid for 30 min with bumping chips, filtered through muslin cloth and washed with boiling water until washings are no longer acidic. Then, the material was boiled with 200 mL of 1.25% (w/v) sodium hydroxide solution for 30 min, filtered through muslin cloth again and washed with 25 mL of boiling 1.25% (w/v) H2SO4, 50 mL x 3 portions of water and 25 mL alcohol.The residue was removed and transferred to ashing dish (preweighed dish W1). Again, the residue was dried for 2 h at 130±2°C. Then, the dish was cooled in a desiccator and weighed (W2), ignited for 30 min at 600±15°C. Finally, the material was cooled in a desiccator and reweighed (W3).

$$\text{Crude fibre (\%)} = \frac{(W2-W1)(W3-W1)\times 100}{W}$$

Where W is the mass of sample.

Determination of Moisture content (Tandon, 2005)

The moisture content of the sample was determined by the following method suggested by (Tandon, 2005) with little modifications. The sample 1.00 g was transferred into a sterilized beaker. The beaker was closed with a lid and kept in a hot air oven at 500C for four hours. Then, it was cooled in desiccators and weighed.

$$\text{Moisture (\%)} = \frac{100\ (B - C)}{B - A}$$

Where,
A - Weight of empty Beaker (g)
B - Weight of empty Beaker with sample before drying (g)
C - Weight of empty Beaker with sample after drying (g)

Estimation of Protein by the Bradford method:

Procedure

1. Added 10 µl of each standard solution and test samples to the ELISA titer plate.
2. Then, 100µl of Bradford's reagent was added to the standards and test samples.
3. All the samples and standard were done in triplicates to avoid any error.
4. The plate was incubated for a minimum of 10 minutes in the dark.
5. The absorbance was measured at 595nm in a microplate reader.
6. From this, the value of the unknown concentration is found out

Estimation of Reducing Sugar by Benedict's Method:

Standard glucose solution

glucose 10 mg/ml

Benedict's quantitative reagent

100 ml of solution acetate, 37.5 g of sodium carbonate, and 62.5 g of potassium thiocyanate were dissolved in 300 ml of distilled water by warming gently and filtered. 9 g of copper sulphate is dissolved in 50 ml of water, added with continuous stirring. 2.5 ml of potassium ferricyanide is added and the volume is made upto 500 ml with water.

Procedure

1. Added 10 µl of each standard solution and samples to the 96-well plate.
2. Benedict's reagent (200 µl) was added to the standards and samples.
3. All the samples and standards were done in triplicate to avoid any error.
4. The plate was heated for 10 minutes.
5. The absorbance was measured at 595nm in an ELISA reader.
6. From this, the value of the unknown concentration is found.

Estimation of Lipid by the Vanillin method:

For cholesterol estimation, the solvent was prepared by mixing chloroform and methanol in a 2:1 ratio. A standard cholesterol stock solution (10 mg/ml) was prepared by dissolving cholesterol in this solvent, and varying volumes were used to create a range of standard concentrations. The test samples were dissolved in water at a predetermined concentration, and different volumes were dispensed into tubes to adjust the sample amounts. For background measurement, 100 µl of concentrated sulfuric acid was added to each tube, incubated at 90 °C for 10 minutes on a dry heating bath, cooled to room temperature, and the background absorbance was measured at 540 nm. For color development, a sulfo-phospho-vanillin reagent was prepared by dissolving 0.2 mg of vanillin per ml of 17% phosphoric acid. After the background reading, 50 µl of this reagent was added to each tube, mixed, and the absorbance was measured again at 540 nm after 5 minutes of color development using a microplate reader.

Mineral analysis:

Determination of Calcium (EDTA titrimetric method)

Apparatus required: Burettes, pipette, conical flask, beakers, and droppers.

Reagents: Sodium hydroxide (8%), Murexide indicator (ammonium purpurate), Standard EDTA titrant, 0.01M

Procedure: A known volume (50ml) of the sample is pipetted into a clean conical flask, to which 1ml of sodium hydroxide and 1ml of iso-propyl alcohol is added. A pinch of murexide indicator is added to this mixture and

titrated against EDTA until the pink color turns purple.

Determination of Potassium (Flame photometric method)

Apparatus required: Flame photometer, lab glassware, and Whatman filter paper.

Reagents: Deionised distilled water, Stock potassium solution, Working Potassium solution

Procedure: The filter of the flame photometer is set at 766.5nm (marked for Potassium, K) and the flame is adjusted for blue colour. The scale is set to zero and maximum using the highest standard value. A standard curve of different concentrations is prepared by feeding the standard solutions. The sample is filtered through the filter paper and fed into the flame photometer. The concentration is found from the standard curve or by direct reading.

Determination of Sodium (Flame photometric method)

Reagents: Deionised water, Stock sodium solution, Working Potassium solution

Procedure

The filter of the flame photometer is set to 589nm (marked for Sodium, Na). By feeding distilled water, the scale is set to zero and maximum using the standard of the highest value. A standard curve between concentration and emission is prepared by feeding the standard solutions. The sample is filtered through filter paper and fed into the flame photometer, and the concentration is found from a graph or by direct readings.

Phosphorus:

An Industrial dye wastewater sample of 5 mL was taken from the clean test tube and mixed with the Soil Doctor-P capsule. The solution was mixed thoroughly until the chemical was dissolved. Then, four drops of TCA reagent were added carefully and mixed well. The tube was kept at room temperature for 20 minutes for colour development.

Determination of Zinc (Colorimetric method)

Materials Required

Copper sulphate solution, Ammonium citrate solution, α-Nitroso-β-Napthol solution, Chloroform, Alizarin indicator solution, Ammonium hydroxide solution, Zinc acetate, Hydrochloric acid solution, Bromine water, Methyl red indicator solution and Phenol red indicator solution.

Procedure

To determine zinc by the colorimetric method, dilute 10 ml of the test solution to about 40 ml, add two drops of methyl red indicator and 1 ml of copper sulphate solution, and neutralize with ammonium hydroxide. Adjust the solution with hydrochloric acid to reach about 0.15 N acidity, keeping the pH between 1.9 and 2.1. Pass hydrogen sulphite gas through the solution until precipitation is complete, then filter through a fine filter paper washed with hydrochloric acid and water. Rinse the residue, combine the washings with the filtrate, boil to remove hydrogen sulphite, add bromine water, and continue boiling to remove excess bromine. After cooling, neutralize to phenol red with ammonium hydroxide, add a small amount of hydrochloric acid, make up to volume, and take an aliquot containing 4–20 µg of zinc. Adjust the aliquot to about 20 ml with water in a separating funnel, add ammonium citrate and α-nitroso-β-naphthol solutions, shake, separate, and discard the solvent layer. Wash the aqueous layer with chloroform to remove excess reagent, adjust the pH to about 8.0–8.2, then add dithizone solution and carbon tetrachloride, shake, and separate. After discarding the aqueous phase and washing down the funnel sides, transfer the zinc back into the aqueous phase using dilute hydrochloric acid. Adjust the pH of the aqueous layer to 8.8–9.0 with ammonium citrate solution and measure the amount of dithizone needed by titrating a standard zinc solution under identical conditions. Multiply the volume of dithizone required for the standard by 1.5 and add this amount to the test sample. Extract the zinc-dithizone

complex into carbon tetrachloride, pipette an aliquot, dilute, and measure absorbance at 540 nm. Compare the result with a calibration curve prepared using standard zinc solutions and process a blank in parallel. Finally, calculate and report the zinc content as a percentage of zinc in the sample.

Vitamin Analysis:

HPLC-Analysis of vitamin D2, E, K1, A (Fat Soluble Vitamins) - (Ramadan & Morsel, 2002) and (Gama et al., 2009)

Standard and Sample preparation:

Working standards ranging from 5 to 100 ppm were prepared, and all stock standard solutions were stored at 4 °C when not in use; stock solutions of fat-soluble vitamins were kept protected from light. For analysis, 5 g of the sample was weighed and extracted with 20 ml of an acetone–hexane mixture (1:1, v/v) for β-carotene determination, while another 5 g portion was extracted with 20 ml of an acetonitrile–methanol mixture (1:1, v/v) for the estimation of other vitamins. All prepared sample solutions were stored in the dark and diluted if necessary. Before injection, the solutions were filtered through a 0.45 µm filter.

Vitamin C (water-soluble vitamin) Spectrophotometric method:

The vitamin C content was determined immediately after sample preparation. For the analysis, 20 ml of oxalic acid solution, 0.2 ml of 0.01% methylene blue solution, and 1 ml of acetate buffer (pH 4.2) were taken in a 50 ml beaker. To this, 1 ml of the freshly prepared and heated test sample (ripened sample maintained at 50 °C) was added. Absorbance was measured at 540 nm using a microplate reader at intervals of 5, 10, 15, 20, 25, and 30 minutes of boiling. The same procedure was followed for semi-ripened and unripened fruit samples, using both diluted and fresh juices.

Quantification of Organic Acid by HPLC (Claessens, 2001)

Sample Preparation

In 5g of sample, add 25 mL of warm milliQ water and vortex for 5 minutes. Then the samples were kept in a sonicator for 30 minutes, then centrifuged at 4500 rpm for 10 min. Then the supernatant was filtered through a 0.4 µm filter and injected into the HPLC system.

Bioactive parameters:

Determination of total phenol content (Folin Ciocalteu's method)

The total phenolic content was determined using the Folin–Ciocalteu's colorimetric method. Aliquots of 1 ml of each sample extract and standard gallic acid solutions (ranging from 0.007 mg/ml to 1 mg/ml) were transferred into test tubes, followed by the addition of 5 ml of distilled water and 0.5 ml of Folin–Ciocalteu reagent. The mixture was shaken well and, after 5 minutes, 1.5 ml of 20% sodium carbonate solution was added. The volume was then made up to 10 ml with distilled water and incubated at room temperature for 2 hours, allowing an intense blue color to develop. Absorbance was measured at 750 nm using a UV-Visible spectrophotometer against a reagent blank prepared with solvent. All measurements were carried out in triplicate, and a calibration curve was prepared using standard gallic acid solutions. The total phenolic content of the sample was expressed as mg of gallic acid equivalents (GAE) per 100 g of dry mass (Bhalodia et al., 2011; Patel et al., 2010).

Determination of total tannin content (Folin Ciocalteu's method):

The tannin content was determined using the Folin–Ciocalteu's colorimetric method. Approximately 0.1 ml of the sample extract was transferred to a 10 ml volumetric flask containing 7.5 ml of distilled water, followed by the addition of 0.5 ml of Folin–Ciocalteu phenol reagent and 1 ml of 35% sodium carbonate solution. The

volume was made up to 10 ml with distilled water, the mixture was shaken well, and then allowed to stand at room temperature for 30 minutes. A set of standard tannic acid solutions (0.07 to 1 mg/ml) was prepared similarly. Absorbance of the test and standard solutions was measured at 700 nm using a UV-Visible spectrophotometer against a blank. All measurements were performed in triplicate, and the tannin content was calculated and expressed as mg of tannic acid equivalents per gram of dried sample.

Determination of total flavonoid content:

The total flavonoid content was estimated using the aluminium chloride colorimetric assay. For this, 1 ml of each sample aliquot and 1 ml of standard quercetin solution (50 mg/ml) were transferred into test tubes, followed by the addition of 4 ml of distilled water and 0.3 ml of 5% sodium nitrite solution. After standing for 5 minutes, 0.3 ml of 10% aluminium chloride solution was added. At the sixth minute, 2 ml of 1 M sodium hydroxide solution was added, and the final volume was adjusted to 10 ml with distilled water. The mixture was mixed well, resulting in the development of an orange-yellow color. Absorbance was measured at 510 nm using a UV-Visible spectrophotometer, with distilled water as blank. Quercetin was used as the standard, and all samples were analyzed in triplicate. A calibration curve was prepared using standard quercetin solutions, and total flavonoid content was calculated and expressed as mg of quercetin equivalents per 100 g of dry mass.(Patel et al., 2010; Pallab et al., 2013; Satish Kumar et al., 2008; Patel et al., 2012).

3. Results and discussion:

Systematic Position

Class: Dicot
Subclass: Gamopetalae
Series: Bicarpellatae
Order: Lamiales
Family: Acanthaceae
Genus: Lepidagathis
Species: barberi
Author citation: Gamble

Etymology: The specific epithet ‘barberi’ was named in honour of Dr. C.A.Barber (1860-1933), a British botanist and the founder Director of Sugarcane Breeding Institute, Coimbatore (King et al.,2022).

Physicochemical properties of the soil:

The physicochemical properties of the soil sample collected from the area where L. barberi grows are shown in Table 3.1. The soil sample is sandy loamy in texture, which ensures good aeration and drainage. It is brown in colour due to the presence of organic matter, and the pH is 6.48 ± 0.10, indicating that it is neutral soil, neither acidic nor alkaline. The temperature of the soil sample was 16.5 °C. The soil shows moderate water holding capacity (10.90 ± 0.15%), which provides favourable conditions for root activity, water absorption, and microbial processes essential for plant health. The bulk density (1.40 ± 0.03 g/cm³) indicates moderate soil compaction with good aeration and drainage, supporting healthy root growth and nutrient availability. The moisture content (2.15 ± 0.00%) reflects good drainage, ensuring sufficient air in the root zone and preventing waterlogging. The electrical conductivity (1.10 ± 0.10 dS/m) indicates moderate soluble salts without risk of salinity stress, supporting healthy growth. The soil sample shows the presence of various essential elements such as organic carbon (4.30 ± 0.05%) and total nitrogen (21.20 ± 0.06%), as well as available phosphorus, potassium, calcium, and magnesium (50.25 ± 0.08 mg/kg, 120.50 ± 0.04 mg/kg, 33.10 ± 0.10% and 10.10 ± 0.09%, respectively). These levels indicate good soil fertility, which was found to be lower from the findings of Jency

et al., 2023, Mamum et al., 2011. The values presented above are within the permissible limits prescribed by WHO.

Table 3.1: Physicochemical properties of the soil sample collected from L.barberi growing area.

Sl. No.	Parameters analyzed	Observation	WHO permissible limits
1	Colour	brown	-
2.	Textural class (USDA)	Sandy loamy	-
3.	pH	6.48±0.10	6.5-8.5
4.	Temperature (^{0}c)	16.5^o C	-
5.	Water holding capacity (%)	10.90±0.15	-
6.	Moisture %	2.15± 0.00	
7.	Bulk density (mg/kg)	1.40±0.03	-
8.	Electrical conductivity (dsm-^{1})	1.10±0.10	4
9.	Organic carbon (%)	4.30±0.05	> 0.86
10.	Total nitrogen (%)	21.20±0.06	> 80
11.	Available phosphorous (mg/kg)	50.25±0.08	>7
12.	Available potassium (mg/kg)	120.50±0.04	>80
13.	Available calcium (%)	33.10±0.10	4.00
14.	Available magnesium (%)	10.10±0.09	3.00

Heavy metal concentration in the soil sample

The soil sample collected from the area where Lepidagathis barberi grows was analysed for heavy metal content. The results revealed the presence of Copper (1.50 mg/g), Uranium (0.20 mg/g), Ferrous (140.25 mg/g), Lead (12.50 mg/g), Manganese (0.21 mg/g), Nickel (2.50 mg/g), Silver (0.10 mg/g), Platinum (1.30 mg/g), Zinc (11.20 mg/g), Iron (25.80 mg/g), and Gallium (0.40 mg/g) indicates that the soil provides key elements required for plant metabolic processes, enzyme activation, and overall growth. Toxic elements such as Arsenic, Cadmium, Chromium, and Antimony were not detected in the sample. None of the heavy metals analyzed in the soil sample exceed WHO permissible limits which was similar to the previous result of Parvez et al.,2023, Addis and Abebaw, 2017. The soil appears to be within safe ranges for all tested heavy metals. but further monitoring is recommended to ensure that any potential accumulation does not affect the plant's suitability for medicinal use. The data is presented in Table 3.2

Table 3.2: Estimation of heavy metal concentrations in soil sources of L.barberi growing area.

S.No.	Heavy metals analysed	Concentration of heavy metals in samples tested (mg/gm soil)	
		mple from *L. barberi*	WHO permissible limit
	Arsenic (As)	NP	**100.00**
	Cadmium (Cd)	NP	**0.20**
	Chromium (Cr)	NP	**100.00**
	Copper (Cu)	1.50	**5.00**
	Uranium (U)	0.20	**1-10**
	Ferrous (Fe)	140.25	**200.00**
	Lead (Pb)	12.50	**25.00**
	Antimony (Sb)	NP	**36**
	Manganese(M)	0.21	**5.00**
	Nickel (Ni)	02.50	**25.00**
	Silver(Ag)	0.10	**0.20**
	Platinum (Pt)	01.30	**2.93-13.68**
	Zinc (Zn)	11.20	**30.00**
	Iron (Fe)	25.80	**40.7**
	Gallium (Ga)	0.40	**0.80**

NP- not present

Ash properties of the whole plant extract

Ash values are useful for determining the authenticity and purity of the sample and are important qualitative standards (Natikar et al., 2019). The ash properties of the whole plant powder of L. barberi are presented in Table 3.3. The ash values of the plant sample show that the acid insoluble ash (19.20 ± 0.02%) was found to be higher followed by total ash (17.35 ± 0.04%), loss on drying (14.20 ± 0.10%), and water soluble ash (13.40 ± 0.05%). The higher amount of acid insoluble indicates the presence of contamination of siliceous earth material. which is similar to the studies of Menpara et al., 2014, Kadam et al.,2013, Siraj et al.,2020, Choudhary et al.,2024. These percentages indicate that the whole plant powder holds good mineral content, supporting its potential for therapeutic applications.

Table 3.3: Ash properties of L. barberi whole plant dry powder samples collected from study area.

S.No.	parameters	value
1.	Loss on drying (%)	14.20 ± 0.10
2.	Total ash (%)	17.35 ± 0.04
3.	Acid insoluble ash (%)	19.20 ± 0.02
4.	Water soluble ash (%)	13.40 ± 0.05

Qualitative Solubility Test of L. barberi Powder

The qualitative solubility test revealed that the powdered whole plant of L. barberi was soluble in water, alcohol

(95% ethanol), and dilute hydrochloric acid. This indicates that the plant material contains a wide range of phytochemical constituents that can be extracted in both polar and mildly acidic solvents, supporting its potential for diverse medicinal and pharmacological applications (Table 3.4 and plate 3.1).

Table 3.4 : Qualitative Solubility Test of L. barberi Powder

S.No	Name of the sample	Result		
		Water soluble	Acid soluble	Alcoholic soluble
1.	*L.barberi*	Soluble	Soluble	Soluble

Plate 3.1: Extractive value of powdered whole plant sample of L. barberi

Studies on the pharmacognostic properties of Lepidagathis barberi:

i. Macroscopic studies:

The selected L. barberi is an endemic medicinal plant. It is a flowering plant commonly known as Holly-leaf Foxglove (Karappan poondu in Tamil). It belongs to the family Acanthaceae with more than 100 species, and it is native to Karnataka and Tamil Nadu. It is distributed in tropical and subtropical regions of the world with 148 species. It is represented by 30 species and 7 varieties in India, of which 18 species and 1 variety are endemic to the country. So far, 12 species of Lepidagathis have been reported from Tamil Nadu, which includes four strict endemics, namely L.barberi, L.diffusa, L.pungens, and L.spinosa. It is subjected to macroscopic analysis to study the external features (King et al., 2022), plate 3.2.

a. Root

Root is cylindrical, tapering, elongated, with woody rootstock; branches up to 30 cm long, colour (outer) dark brownish, size 3-5 cm long, surface yellowish to dark brown, having strong fibres, longitudinal fissure. Lenticels are absent (King et al., 2022).

b. Stem

Stems are well-branched, cylindrical, 4-angled, pubescent, and green to purplish when young and turn ash coloured when old, cylindrical, branches white. Internodal distance 5-25mm (King et al., 2022).

c. Leaf

Leaves sessile, 1.2 X 0.5-1 cm, isophyllous, opposite-decussate, oblong to narrowly elliptic, 8-22 x 4-9 mm, obtuse or truncate at base, 3-6 pairs or spinose- denate with cilia at margins, acute with a spinose process at apex, glabrous throughtout, midvein broad at base, lateral veins 3-6 pairs, obtuse or truncate at base (King et al., 2022).

d. Flower

Inflorescence a spike, compactly ovoid due to adpressed/ converged fertile bracts, alternately arranged on the axils of leaves, 8-16mm long. Bracts: sterile bracts, 4 arranged in two pairs, heteromorphic, lance-ovate to ovate,

entire or pilose at margins, caudate with a spinose process at apex. Corolla bilabiate, 10-14mm long, pinkish white with many purplish brown markings on expanded portions of tube inside and yellow dots or patches on the palate, tube 5.8-7.5mm long, stamens 4, didyanamous, silaments with purple stripes, glabrous: anticous, filaments 3-3.3mm, longitudinally dehiscent, Flowering and fruiting occurs from June- january (King et al., 2022).

e. Fruit

Capsules compressed, 2 seeded, Ovary globose, 0.9-1mm, glabrous, 2-loculed, ovules 2 in each locule, nectary disk cupulate, style 5.7-7mm long, bristled hairy with glands at base, stigma entire, capsules ovoid in face view, 4-5mm x 2.3-2.mm, glabrous, yellowish seeds, 1 or 2, fertile, ovoid, 2.3-3.3 x 1.6-2mm, flat, densely clothed with hygroscopic white hairs (King et al., 2022).

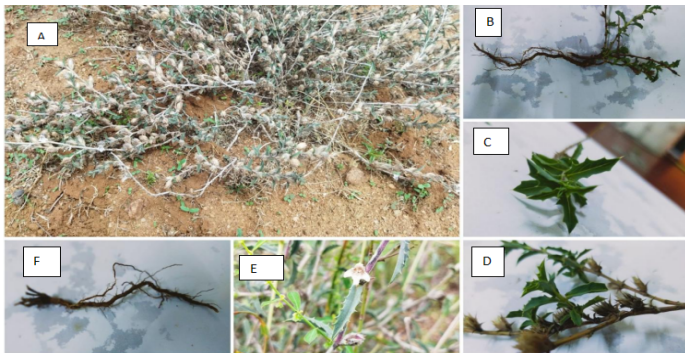


Plate: 3.2 Macroscopic structure of Lepidagathis barberi: A)Habitat, B) Small twig, C) Leaf, D)Inflorescence, E)Flowering twig in face view, F) Root

ii. Microscopic (anatomical) studies

The experimental plant is subjected to microscopic studies to investigate the anatomical features. This study is necessary for the identification and authentication of the selected plants.

Root:

The transverse section of L. barberi root are shows a single epidermis with cuticle. Stele diarch, pith is present. Secondary growth is anomalous, producing bands of included phloem that give an impression of growth rings. Wood with small and short vessels. Vessels are scattered, solitary, paired, or in series of 3-4. Small patches of thick-walled paratracheal parenchyma were produced. Ray uniseriate or biseriate; uniseriate being more frequent. Endodermis distinct. Cortex narrow, parenchymatous, cells getting stretched with growing girth (plate 3.3).

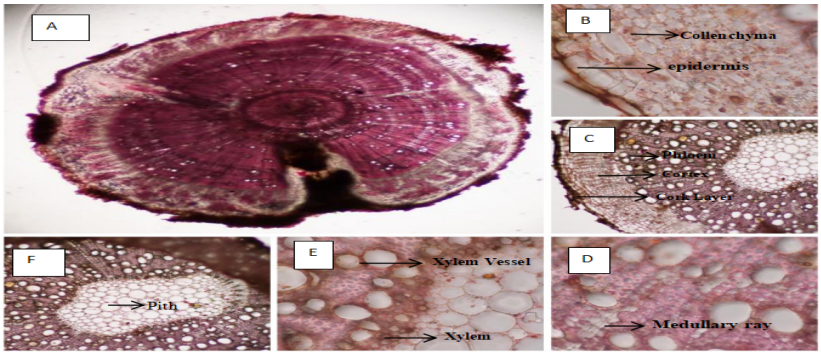


Plate 3.3: Microscopic structure of root: A) T.S of the root, B) T.S of the root- cortex region C) T.S of the root- portion enlarged, D) T.S of the root- portion enlarged showing medullary ray, E) T.S. of the

root-xylem, F) T.S.of the root-pith region

Stem:

The transverse section of the young stem (plate 3.4) is roughly quadrangular with four narrow wings. Wings forming pairs on the dorsiventral side. Single-layered epidermis showing chlorophyllose bands with stomata and non-chlorophyllose bands. Stomata diacytic, hemi-bicyclic, and bicyclic. A solitary cystolith is reported. Collenchymatous hypodermis is seen. Wings filled with collenchyma up to half, followed by chlorenchyma. Narrow cortex, 2-3 layered, parenchymatous, cells enclosing small intercellular spaces. Distinct endodermis is present. Endodermal cells are squarish. Pericycle is not distinct. Internal phloem in the form of a continuous band encircling the pith. Phloem cells are opposite the protoxylem with stored food. Pith is small, containing raphides and styloids. Secondary growth is anemalous. Cambium produces a continuous phloem cylinder of thick-walled cells to the outer side of secondary xylem with patches of included phloem and thick-walled paratracheal parenchyma to the inner side. Vessels scattered, solitary or paired, cylindrical or quadrangular-tailed; tails short or long present on one end. Both ends, broader vessels without tails. Perforation mates horizontally to slightly oblique. Vessels are extremely small. Ray is uni and biseriate. To keep pace with growing girth, a few cells of inner phloem ring divide to add secondary parenchyma; as a result, a parenchymatous ring with scattered patches of the inner phloem is produced. Some pith cells become lignified, and variously shaped crystals of calcium oxalate (raphides and styloids rectangular) are present. Cork cambium deep-seated, orginating from endodermis, producing secondary parenchymatous cortex to the outer side, while to the inner side almost no cells are produced. Some of the cortical cells also divide tangentially, producing secondary cortical parenchyma to keep pace with the growing girth of wood.

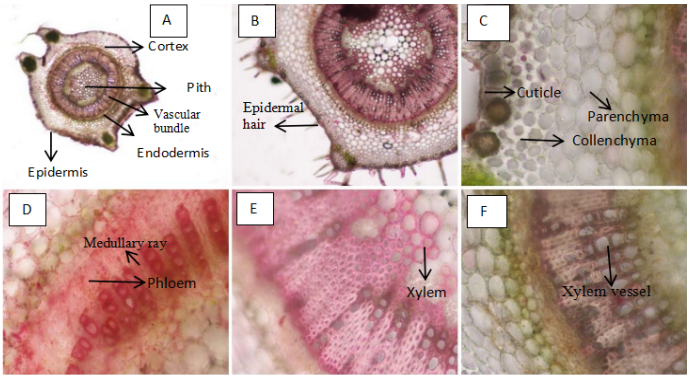


Plate 3.4: Microscopic structure of the stem: A) T.S. of the stem, B) T.S of the stem- Lignified elements, C) T.S of the stem: Portion enlarged, D,E,F) T.S of the stem: xylem and phloem.

Leaf:

Transverse section of L.barberi showed a single layered epidermis, cells shallow, sinuous, cutinised & cuticularised. Diacytic stomata, bicyclic & hemi-bicyclic. Large lithocysts are reported in epidermis containing cystoliths of various sizes. Along the margin of veins, cystoliths form continuous chains parallel to the midrib of veins, while in the intercostal areas, they are horizontally scattered or in horizontal chains. Mesophyll is homogenous, consisting of palisade tissue only. Upper 2-3 layers of elongated cells compactly placed and densely filled with chloroplasts. Lower palisade layers of short cells with fewer chloroplasts. Vein bundles embedded in mesophyll, bundle sheath parenchymatous. Around the bundle sheath, palisade cells are concentrically arranged. Mesophyll is continuous in the margin; however, the lowermost layer of cells is devoid of chloroplasts. 1-2 vein bundles present in the margin. The midrib is ridged on the upper side. Epidermis

cutinised, cystoliths are solitary. Collenchymatous hypodermis fills the ridge. Parenchymatous ground tissue is present with small intercellular spaces. The Mesophyll continues in the midrib, reaching up to the vascular bundle on both the lateral sides. Vasculature in the form of central crescent vessels arranged in series, separated by thin-walled cells. Internal phloem in the form of a continuous band (plate 3.5).

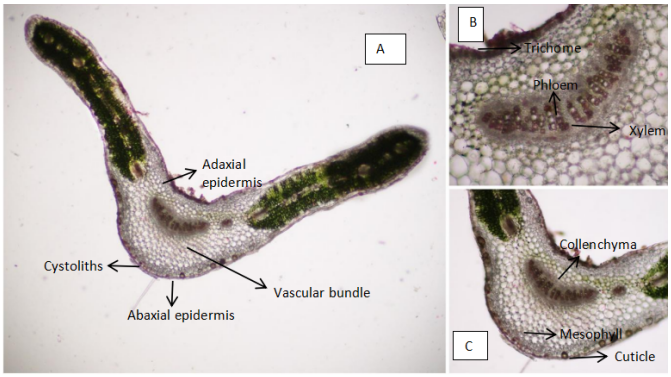


Plate 3.5: Microscopic structure of the leaf: A) T.S of the leaf, B&C) T.S of the leaf- midrib enlarged

Fluorescence properties:

Fluorescence studies helps in the identification of drugs that are more or less difficult to distinguish (Kasthuri and Ramesh, 2018). The fluorescent behaviour of the whole dried plant powder of L.barberi was studied using extracts of different solvents under daylight and UV-light at 366 nm, and the results are presented in Table 3.5. Dry powder of the whole plant sample of L.barberi emitted brown, dark green, light yellowish, green, and blue colours in daylight. Under UV- light, the whole plant sample of the selected plant appeared light brown, dark yellow, greenish brown, white, and dark brown in colour after being treated with various reagents under UV light 366nm. These distinct color changes under both lighting conditions indicate the presence of diverse phytochemical classes, such as flavonoids, alkaloids, tannins, saponins, phenolic, and essential oils. The color variations reflect the interaction of the plant's phytoconstituents with specific solvents and reagents, each class contributing to the therapeutic and diagnostic potential of the plant. The grade and purity of the plant powder of L. barberi were determined based on the colour reactions with specific reagents, which showed no indication of any harmful or toxic constituents.

Table 3.5: Fluorescence analysis of the dry powder of the whole plant powder of Lepidagathis barberi

Solvents used	*L. barberi* whole plant sample	
	Daylight	UV-light(366 nm)
Drug powder (DP)	Brown	Light brown
DP + Dist. water	dark brown	Brown
DP + Ethyl acetate	yellow	Dark yellow
DP + Isopropyl	Dark green	Greenish brown
DP + Ethanol	Light yellowish	white

DP + Petroleum ether	Green	Greenish sky blue
DP + NaOH(Aqueous)	brown	Greenish brown
DP + 1N NaOH (Ethanolic)	brown	Greenish brown
DP + 50% H2SO4	blue	brown
DP + 50% HNO3	brown	Dark brown

Studies on the phytochemicals in the experimental plant

Phytochemical screening was investigated using different solvents, including isopropanol, ethanol, ethyl acetate, petroleum ether, aqueous, and chloroform extracts of the whole plant Lepidagathis barberi. The data are presented in Table 3.6. Among all the solvents tested, the isopropanol extract showed the highest number of positive results, indicating a greater extraction efficiency for various phytochemicals compared to ethanol, ethyl acetate, petroleum ether, aqueous, and chloroform extracts. Specifically, the isopropanol extract of L. barberi showed the presence of resins, carboxylic acids, tannins, flavonoids, glycosides, saponification, protein, phenol, saponin, gum, flavanoglycosides, and alkaloids campared to the other solvents. The Biuret reaction was positive only in the ethyl acetate extract. Steroids and Biuret tests showed negative results. Only the ethyl acetate extract tested positive for steroids. Flavonoids are known to have antioxidant effects, inhibiting the initiation, promotion and progression of tumors. Tannins possess antiviral, antibacterial and antitumor activity (Adil et al.,2024). Flavonoids and phenols possess anti-allergic, antibacterial and antioxidant properties, while saponins have been reported to exhibit plant defense, anti-inflammatory and antiviral activities (Obi and Okwute,2023). Steroids have been reported to have antibacterial properties. Alkaloids have been associated with medicinal uses for centuries and one of their common biological properties is their cytotoxicity. Several workers have reported the analgesic, antispasmodic and antibacterial properties of alkaloids. Glycosides are known to lower the blood pressure according to many reports (Yadav and Agarwala,2011). Saponins are useful in the treatment of upper respiratory tract inflammations; they also have anti-diabetic and anti-fungal properties. Saponins, often referred to as natural detergents due to their foamy nature, also possess anti carcinogenic properties, immune modulation activities and regulation of cell proliferation as well as inhibition of the growth of cancer cells and cholestero lowering activity (Ajuru et al.,2017). These phytochemicals possess various biological activities, including anti-inflammatory, antibacterial, anticancer activity and antioxidant properties. Overall, the phytochemical profile of L. barberi indicates its potential to serve as a source of beneficial bioactive compounds. This supports its traditional use in medicine and highlights its possible role in improving health and well-being.

Table 3.6: Phytochemical analysis of various extracts of Lepidagathis barberi

S.No.	Phytochemical compounds	Isopropanol	Ethanol	Ethyl acetate	Petroleum ether	Aqueous	chloroform
1.	Resins	+	-	+	-	-	

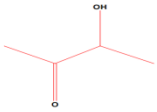
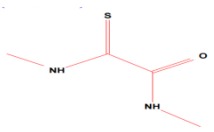
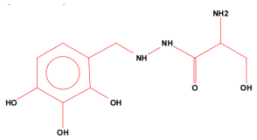
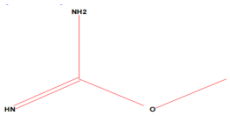
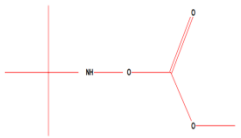
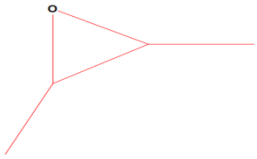
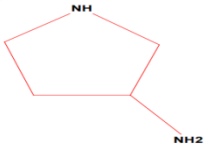
							+
2.	Carboxylic acid	+	-	-	-	-	-
3.	Tannins	+	-	-	-	+	+
4.	Steroids	-	+	-	+	-	-
5.	Flavonoid	+	-	+	-	-	-
6.	Carbohydrates	+	-	-	+	-	-
7.	Glycosides	+	-	+	-	+	+
8.	Saponification	+	-	-	-	+	-
9.	Protein	+	-	-	+	-	+
10.	Phenol	+	-	-	-	-	+
11.	Biuret	-	-	+	-	-	-
12.	Saponin	+	-	-	+	-	-
13.	Gum	+	-	-	-	-	-
14.	Flavanoglycosides	+	-	-	-	-	-
15.	Alkaloids	+	-	-	-	-	-

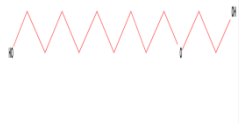
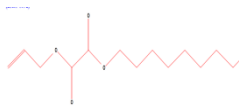
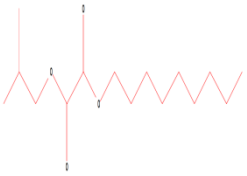
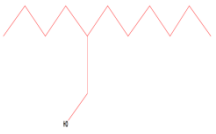
GCMS Analysis:

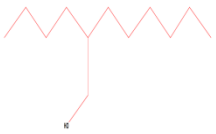
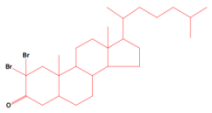
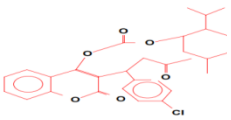
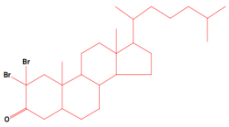
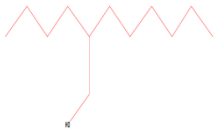
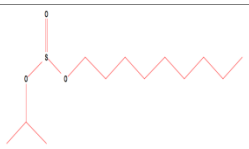
The chemical constituents identified by the GC/MS analysis of the ethanolic plant extract of L.barberi were enumerated along with their molecular formula, retention time, and peak area (Table 3.7 and Figure 3.1). It contains 26 major peaks and with many small peaks, indicating the presence of major compounds. The GC-MS analysis of the ethanolic extract of L. barberi revealed 26 distinct phytochemical compounds, eluting between 2.33 to 31.32 minutes of retention time. The chromatogram shows sharp and intense peaks, especially in the retention time range of 22–30 minutes, indicating the presence of high molecular weight and thermally stable bioactive compounds. The major chemical classes detected include: Alcohols (1-Octanol, 2-butyl-, Nonamethylene glycol), Alkanes (Tetradecane, Hexadecane, Dotriacontane), Esters and Ethers (Oxalic acid, isobutyl nonyl ester), Amides and Amines (Acetamide, 3-Aminopyrrolidine), Halogenated hydrocarbons (1-Iodo-2-methylundecane and 2,2-Dibromocholestanone), Sulfur-containing compounds (Sulfurous acid esters). Hexadecane and Tetradecane was found in the previous study of Keke et al.,2023.Among, 26 compounds, Hexadecance has the largest peak with 25.603% area. These phytoconstituents are reported to possess a wide range of pharmacological activities, including antimicrobial, antioxidant, antifungal, anti-inflammatory, antiepileptic, antiproliferative, and cytotoxic effects. The presence of such diverse bioactive compounds supports the traditional medicinal usage of L.barberi and its potential in natural product-based drug discovery.

Table 3.7: GC-MS analysis of the powdered whole plant L. barberi:

SI.NO	RT	Name of the compound	Molecular formula	Molecular	Structure	Biological activity

				weight		
1	2.338	2-Butanone, 3-hydroxy-	C4H8O2	88		Antimicrobial activity Anticancer activity
2	3.659	Acetamide,n-methyl-2-(methylamino)-2-thioxo-	C4H8ON2S	132		Anti-inflammatory activity, Anti microbial activity, **Antitubercular Activity and** Antifungal activity
3	3.779	Benserazide	C10H15O5N3	257		Anticancer activty, Antifungal activity Neuroprotective effects, Antiparkison agents
4	5.460	O-methylisourea hydrogen sulfate	C2H6ON2	74		Antibacterial activty, Antifungal activity and Antioxidant activity
5	5.610	1,1-dimethylethylamine, n-methoxycarbonyloxy	C6H13O3N	147		Antifungal activity
6	5.800	Oxirane, 2,3-dimethyl-	C4H8O	72		Antimicrobial and Anticancer activity
7	12.553	3-Aminopyrrolidine	C4H10N2	86		Antiepileptic, Agonist properties, Anticancer activity and Antimicrobial activity

8	15.279	Nonamethylene glycol	C11H24O3	204		Antibacterial activity Antioxidant activty
9	15.864	N-Formyl-2-amino-2-methylpropanal	C5H9O2N	115		Antifungal, Anticancer activity, Antioxidant activity
10	20.521	Oxalic acid, allyl nonyl ester	C14H24O4	256		Anti Antimicrobial ity Antioxidan
11	21.331	Hexadecane	C16H34	226		Antibacterial, Anti-inflammatory and Antioxidant activity
12	22.106	Oxalic acid, isobutyl nonyl ester	C15H28O4	272		Antimicrobial activity
13	22.852	Silane, trichlorodocosyl-	C22H45Cl3Si	442		Antimicrobial activity
14	23.577	1-Iodo-2-methylundecane	C12H25I	296		Antimicrobial activity and **Estrogenic Activity**
15	24.277	1-Octanol, 2-butyl-	C12H26O	186		Antimicrobial and Antiproliferative activity
16	24.952	Tetradecane	C14H30	198		Cocarcinogenic activity, diuretic, antituberculosis,Antibacterial and Antifungal activity
17	25.603	Hexadecane	C16H34	226		Antibacterial, Antifungal, antipyretic,

						anthelmentic, antidiarrhea, antidiabetic and Antioxidant activity
18	26.248	1-Octanol, 2-butyl-	C12H26O	186		Antimicrobial and Antiproliferative activity
19	26.958	Nonadecane	C19H40	268		Antibacterial, Antifungal and Antioxidant activity
20	27.093	2,2-Dibromocholestanone	C27H44OBr2	543		No biological activity
21	27.323	2-Isopropyl-5-methylcyclohexyl 3-(1-(4-chlorophenyl)-3-oxobutyl)-c	C30H33O6Cl	524		Antibacterial, Anti-inflammatory, Anti-arthritic effects, Antioxidant and Antimicrobial activity
22	27.613	2,2-Dibromocholestanone	C27H44OBr2	542		Anticancer activity, Anti-diabetic acitivity and Antifungal properties
23	27.789	1-Octanol, 2-butyl-	C12H26O	186		Antifungal activity
24	28.759	Sulfurous acid, nonyl 2-propyl ester	C12H26O3S	250		Antibacterial and Antioxidant activities
25	29.929	1-Iodo-2-methylundecane	C12H25I	296		Potential Role in Cancer Metabolism, Estrogenic properties Antimicrobial activity

26	31.325	Dotriacontane	C32H66	450		Antimicrobial, Antioxidant, Anticonvulsant, Antitubercular activity and Cytotoxicity

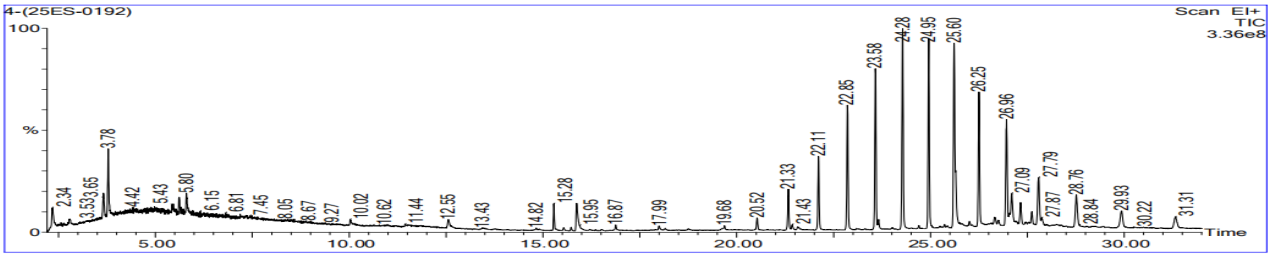


Figure 3.1: Chromatogram for GC-MS analysis of powdered whole plant sample of Lepidagathis barberi

FTIR Analysis:

The FTIR analysis of L. barberi revealed the presence of diverse functional groups, indicating a rich phytochemical composition. A total of 31 distinct peaks were observed in the wavenumber range of 401–4582 cm^{-1}, corresponding to various bioactive compounds. The broad and intense peak around 3410.15 cm^{-1} corresponds to hydroxyl (–OH) stretching, classified as strong, suggesting abundant alcohols, phenols, or flavonoids. Similarly, strong absorption bands for C–H stretching were observed at 2924.09 cm^{-1} and moderately strong at 2854.65 cm^{-1}, indicating alkanes or fatty acid chains. A distinct strong peak at 1666.5 cm^{-1} represents carbonyl (C=O) or alkene (C=C) stretching, confirming the presence of aromatic or amide groups. Peaks at 1056.99 cm^{-1} and 1165.0 cm^{-1} for C–O stretching were medium in intensity, supporting the presence of alcohols, ethers, or glycosides. Aromatic C–H bending at 779.24 cm^{-1} and 879.54 cm^{-1} appeared as medium peaks, indicating aromatic or alkene rings. Skeletal vibrations and metal–oxygen stretching below 700 cm^{-1} appeared weak to medium, reflecting possible inorganic residues or plant matrix vibrations. Overtone and combination bands above 3700 cm^{-1}, such as those at 3772.76 cm^{-1} and 3888.49 cm^{-1}, were weak and likely due to harmonics or instrument artifacts. Weak peaks were also noted at 2098.55 cm^{-1} for alkyne/nitrile stretches and at 2430.31 cm^{-1} for CO_2 overtones. Peak 2924. was found in the previous study of Sownthariya and Shanthi, 2022.These findings confirm the presence of functional groups such as hydroxyls, alkyl chains, carbonyls, alkenes, C–O bonds, and aromatic structures. Overall, the FTIR profile strongly supports that L. barberi contains diverse phytoconstituents, making it a promising candidate for further studies in nanomedicine, antimicrobial applications, and drug formulation. (Table 3.8, Figure 3.2).

Table 3.8.: FTIR analysis of the ethanolic extract of powdered whole plant of L. barberi

S.No	Wavenumber (cm^{-1})	Bond/Vibration	Functional group
1	401.19	M–O stretch	Inorganic/mineral residues
2	462.92	M–O stretch	Inorganic/mineral residues

3	532.35	C–C skeletal bend	Plant biomatrix
4	601.79	Ring deformation	Aromatic/polyphenolic backbone
5	655.8	Ring deformation	Aromatic/polyphenolic backbone
6	779.24	Out-of-plane aromatic C–H bend	Aromatic compounds (flavonoids)
7	879.54	=C–H out-of-plane	Aromatic / Alkene
8	1056.99	C–O stretching	Alcohol
9	1165.0	C–O stretching	Alcohols / Ethers / Glycosides
10	1273.02	C–O stretching	Esters / Amines
11	1381.03	O–H bend	phenolics
12	1666.5	Aromatic C=C	Aromatics / Amides
13	1820.8	Overtone of carbonyl group	Carbonyl compounds (esters/ketones)
14	1851.66	Overtone of carbonyl group	Carbonyl group
15	2098.55	Alkyne / Nitrile	Minor alkynes/nitriles
16	2430.31	CO_2 combination band	Atmospheric CO_2
17	2854.65	C-H stretching	Alkanes
18	2924.09	C-H stretching	Alkanes
19	3410.15	O-H stretching	Alcohol
20	3772.76	O–H	Alcohols / Phenols
21	3888.49	O–H / N–H	Alcohols / Amines
22	4073.66	Harmonic of O–H / N–H	Alcohols / Amines (weak)
23	4135.38	Harmonic of C=O / amide	Carbonyls / Amides (weak)
24	4150.81	Combination of vibrations	Plant matrix vibrations
25	4212.53	Harmonic / noise	Instrument noise
26	4274.26	Vibrational harmonic	Instrument noise
27	4320.55	Vibrational harmonic	Instrument noise
28	4413.13	Noise / weak harmonic	Instrument noise
29	4474.85	Noise / weak harmonic	Instrument noise
30	4521.14	Noise / weak harmonic	Instrument noise

31	4582.87	Noise / weak harmonic	Instrument noise

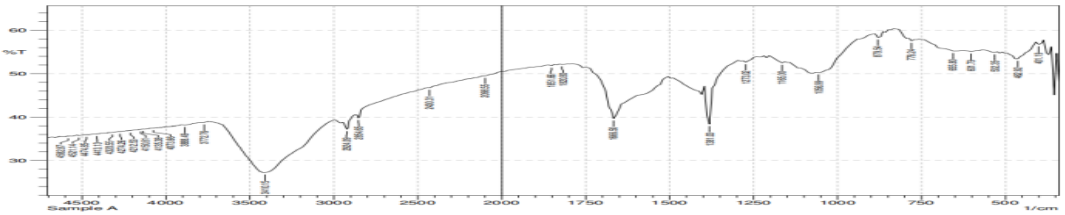


Figure 3.2: Chromatogram for the FTIR analysis of the ethanol extract of powdered whole plant sample of L. barberi

Proximate Nutritional analysis:

The nutritional composition of L.barberi was analyzed to determine its suitability as a natural source of dietary nutrients. The total ash content of the dried plant powder was 0.9 %, indicating a normal level of mineral residue and confirming the absence of excess inorganic contamination. The crude fiber content was found to be 50 %, which shows that the plant is a rich source of dietary fiber. The moisture content was 10 %, confirming good drying and suitable shelf-life for storage without spoilage which was similar to the previous studies of Kishan et al., 2022, Raju et al., 2014 which is lower than the value measured in the present study. In addition, the plant contained a notable protein content of 30.8 mg/g, suggesting it could contribute to daily protein intake. Presence of protein was found similar to the Aborisade et al., 2017 which was lower than the present result. The reducing sugar content was 30.1 mg/g, indicating the presence of simple carbohydrates that can provide quick energy. The lipid content was 1.7 mg/g, showing that the plant has a low fat level, which can be suitable for low-fat diet formulations (Table 3.9).

Table 3.9: Nutritional value of the whole dried plant powder of L.barberi

Sl. No.	Nutritional value	Concentration (%)/mg/g
1	Ash	0.9%
2	Crude fiber	50%
3	Moisture	10%
4.	Protein	30.8mg/g
5.	Reducing sugar	30.1mg/g
6.	Lipid	1.7mg/g

Mineral analysis:

Mineral analysis revealed that calcium was not detected in the extract. However, the plant contained sodium (24.62 mg/L) in higher level compared to other minerals followed by zinc (10.7 mg/kg), potassium (0.43 mg/L), and a notable amount of phosphorus (0.05 mg/L), which is an essential trace element for human health (Table 3.10). Sodium is an essential electrolyte that helps to maintain the balance of water in and around the cell (Batool et al., 2023). Sodium was also find higher in the findings of Sael et al., 2020. Potassium is essential and is required in large amounts for proper growth and plant reproduction. Phosphorous maintain blood sugar levels and normal heart contraction. Zinc is vital in protein synthesis, cellular differentiation and replication, immunity and sexual functions (Achi et al., 2017).

Table 3.10: Mineral analysis of whole plant powder of L.barberi

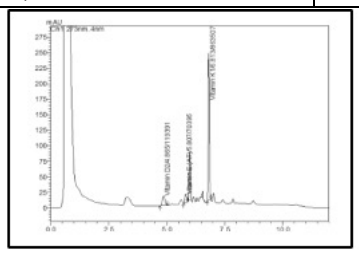
S.NO	Name of the test sample	No. of test minerals	Test o Test sample Mg/L
1.	*L.barberi*	Calcium	0
2.		Sodium	24.62
3.		Potassium	0.43
4.		Phosphorous	0.05
5.		Zinc	10.7

Vitamin analysis:

The analysis of fat-soluble vitamins showed the presence of Vitamin D_2 (2.02 mg/kg), Vitamin E (72.50 mg/kg), Vitamin K_1 (80.27 mg/kg) and Vitamin A (198.31mg/kg) were analyzed by HPLC, Vitamin A was found to be higher which was similar to the previous result of Akubugwo et al.,2007 followed by Vitamin K1, Vitamin E and Vitamin D2, while Vitamin C content was determined separately by colorimetric method at 540 nm because it's water-soluble and unstable in heat. The water-soluble Vitamin C content was found to be 1.2 mg/g, indicating the plant's antioxidant potential (Table 3.11 and 3.12, Figure 3.3). Vitamin C and E are very important antioxidants which protect the cell membranes from oxidative stress/damage caused by free radicals. Vitamin C possesses an antioxidant property and required for maintenance of normal connective tissues, wound healing and also facilitates the absorption of dietary iron from the intestine (Achi et al., 2017).Vitamin C is the most significant nutrient in leafy foods. It is outstanding for its cancer prevention agent properties, and it helps the body in hindering viral disease, bacterial contaminations, and poisonous quality (Saed et al.,2020).

Table 3.11: Vitamin analysis of whole plant powder of L.barberi

S.NO	Name	Ret. Time (min)	Area	Conc.(mg/Kg)
1	Vitamin D2 ((Ergocalciferol))	4.8	119,391	2.02
2	Vitamin E (α-tocopherol)	5.8	70,395	72.50
3	Vitamin K1 ((Phylloquinone))	6.8	863,507	80.27
4	Beta-carotene (Vitamin A)	8.7	275,904	198.31



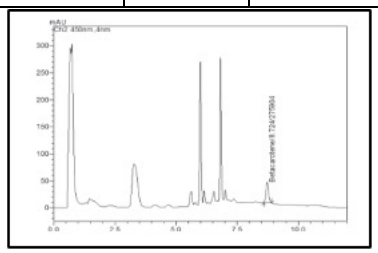


Figure 3.3.: Chromatogram for vitamin analysis of powdered whole plant sample L.barberi

Table 3.12: Total vitamin content of L.barberi

Name of the sample	OD value at 540 nm	Total vitamin C content	Mean value of total vitamin C content (mg/g)
L.barberi	0.193	0.11574	1.2
	0.199	0.12763	
	0.201	0.13159	

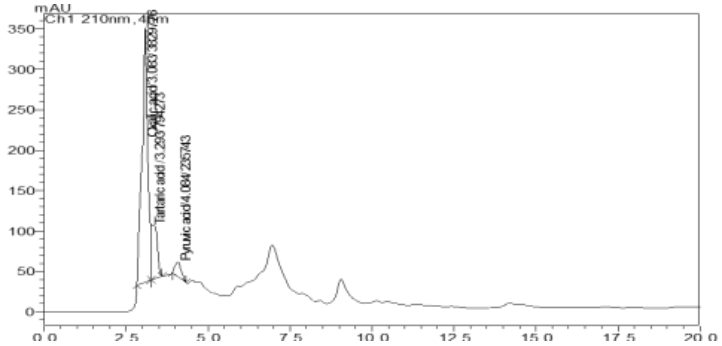
Organic acids:

Organic acids play an important role in plants as flavour, colour, aroma, stability and microbiological control of the products (Ahmed et al., 2022). Organic acid profiling confirmed the presence of oxalic acid (91.25 mg/kg), tartaric acid (92.63 mg/kg), and pyruvic acid (4.81 mg/kg), while malic acid, acetic acid, and citric acid were below detectable limits. These organic acids contribute to the plant's possible antioxidant and preservative properties (Table 3.13 and figure 3.4). Tartaric acid was found higher in Tamarindus indica which was similar to the study Ahmed et al., 2022, Li et al., 2018 in Copis herb.

Table 3.13.: Organic acids in the powdered whole plant sample of L.barberi

S.NO	Name	Ret. Time (min)	Area	Conc.(mg/kg)
1	Oxalic acid	3.0	3,829,776	91.25
2	Tartaric acid	3.3	794,273	92.63
3	Pyruvic acid	4.0	235,743	4.81
4	Malic acid	4.3	-	BDL
5	Acetic acid	5.8	-	BDL
6	Citric acid	7.7	-	BDL

Figure 3.4:Chromatogram for organic analysis of powdered whole plant sample L.barberi



Bioactive parameters:

Total phenol content:

The total phenolic content of the isopropanol extract of L. barberi was determined and expressed as Gallic Acid Equivalents (GAE) per g dry extract by using the equation based on the calibration curve where, y = 0.4372x + 0.3754, R2 = 0.9551. The optical density (OD) was measured at 750 nm, and the OD values ranged between 1.45 and 1.78 for the samples analyzed. The total phenolic content in L. barberi was found to be 24.3 mg/g, indicating that the plant is a good source of bioactive phenolic compounds. Htay et al., 2023 study showed a similar result in stem and bark of Bauhinia purpurea which is lower than our findings. The detailed results are shown in Table 3.14 and figure 3.5. Total phenol content in plants refers to the total amount of phenolic compounds present, which are known for their antioxidant properties and potential health benefits. phenolic compounds are also very important plant constituents because of their hydroxyl groups confer scavenging ability **(Bhalodia et al.,2011).**

Table 3.14 : Total phenol content whole plant powder of L.barberi

Name of the sample	OD value at 750 nm	Total Phenol content	Mean value of total phenol content (mg/g)
L.barberi	1.78	3.21272	24.3
	1.09	1.63449	
	1.45	2.45791	

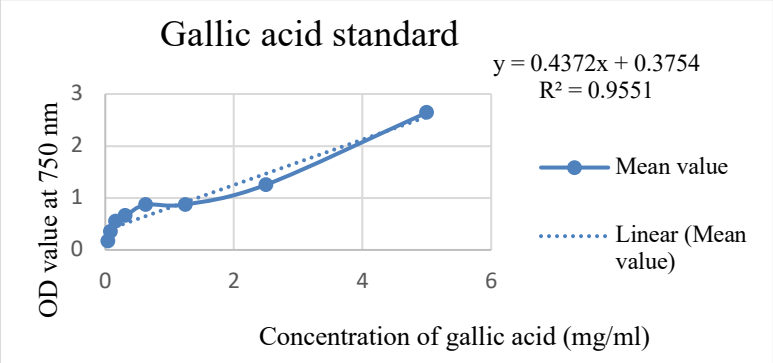


Figure 3.5: Standard Curve of Total Phenol Content

Total Tannin content:

The quantitative estimation of tannin content in isopropanal extract of L.barberi was carried out using the Folin–Ciocalteu method, with absorbance measured at 700 nm and expressed as tannic acid standard per g dry extract by using the equation based on the calibration curve where, y = 0.4923x + 0.4941 , R2 = 0.9736.The optical

density (OD) values for the samples ranged from 0.512 to 0.567. The total tannin content in the extract was found to be 81.04 mg/g, indicating a high level of tannins in the plant. The detailed results are presented in Table 3.15 and figure 3.6. Tannin content was found to be higher which was similar to Sharma et al., 2021. Tannins are natural polyphenols ubiquitously distributed in plants, such as vegetables, fruits and seeds. Tannins are widely used in wine industry for color stabilizer; balancing the complexity in wines, inhibit certain enzymes in infected fruits and act as wine fining agents (Mohammed and Manan, 2015).

Table 3.15: Total tannin content whole plant powder of L.barberi

Name of the sample	OD value at 700 nm	Total Tannin content	Mean value of total tannin content (mg/g)
L. barberi	0.512	3.63599	81.04
	0.567	14.808	
	0.523	5.8704	

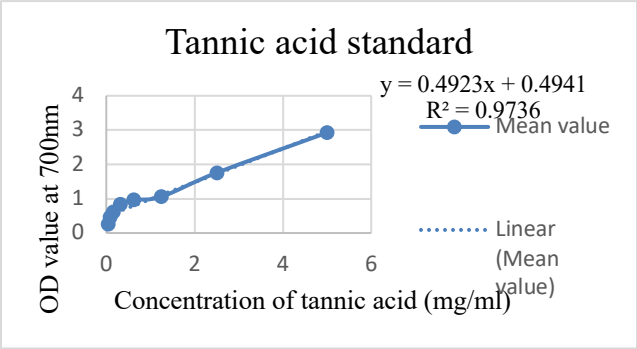


Figure 3.6: Standard Curve of Total Tannin Content

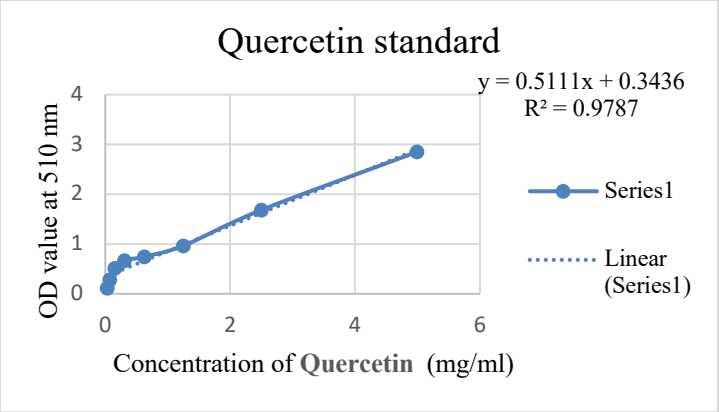
Total Flavonoid Content:

The total flavonoid content in isopropanal extract of L. barberi was determined using quercetin as the standard reference compound. According to this calibration curve, the flavonoid content of whole dry plant extract was determined by using the following equation y = 0.5111x + 0.3436, R2 = 0.9787. The absorbance was measured at 510 nm, and the optical density (OD) values ranged from 1.80 to 1.956. The mean flavonoid content in the extract was calculated to be 30.1 mg/g, indicating a good presence of these bioactive compounds. Previous studies shows the presence of flavanoid in Curcuma xanthorrhiza whiuch was four times higher than our findings (Sukweenadhi et al., 2020). The detailed results are shown in Table 3.16 and figure 3.7. Flavonoids are among the major groups of phenolic compounds with broad spectrum of chemical and biological activities, particularly radical scavenging and antimicrobial activities (Ayele et al., 2022).

Table 3.16 : Total flavonoid content whole plant powder of L.barberi

Name of the sample	OD value at 510 nm	Total Flavonoids content	Mean value of total flavonoids content (mg/g)
L.barberi	1.897	3.03933	30.1
	1.956	3.15476	
	1.8	2.84954	

Figure 3.7: Standard Curve of Total flavonoid Content



4.Conclusions:

The present investigation of Lepidagathis barberi demonstrates that this endemic medicinal plant possesses phytochemical, biochemical and pharmacognostic value. The physicochemical analysis revealed that the soil is sandy loamy, slightly alkaline, with a moderate water holding capacity, and the presence of various essential elements, which are all within safe limits and ideal for healthy plant development. The heavy metal analysis confirmed that toxic metals like arsenic, cadmium, chromium, and antimony are absent, while essential trace elements like iron, zinc, and copper are present within the WHO safety standards, highlighting its safe cultivation environment. The ash properties (including total ash, acid-insoluble ash, water-soluble ash, and loss on drying) indicate good purity and minimal contamination, which are vital markers for herbal quality control. The extractive value showed that the plant is soluble in water, acid, and alcohol, confirming the presence of a wide range of phytochemicals that can be easily extracted for medicinal use. Detailed macroscopic and microscopic studies provided clear diagnostic features such as its characteristic root, stem, leaf, flower, and fruit structures and unique anatomical traits like specialized stomata, cystoliths, anomalous secondary growth, and distinctive vascular arrangements, which help in authenticating the plant. Fluorescence analysis revealed distinct color changes under daylight and UV light with different reagents, further supporting the presence of diverse chemical groups like flavonoids, phenolic, alkaloids, tannins, and saponins. The phytochemical screening confirmed the presence of resins, carboxylic acids, tannins, flavonoids, alkaloids, saponins, glycosides, proteins, and gums compounds which are higher in isopropanol extract.

The GC-MS analysis identified 26 bioactive compounds belonging to various classes such as alcohols, alkanes, esters, amines, sulfur-containing compounds, and halogenated hydrocarbons, which are reported to have antimicrobial, anticancer, antioxidant, and cytoprotective activities. FTIR analysis detected functional groups such as hydroxyls, carbonyls, aromatics, alkanes, and metal–oxygen bonds, which align with the chemical classes seen in GC-MS and explain the plant's rich biological profile. The nutritional analysis showed that L. barberi is a good source of dietary fiber, moderate protein, simple sugars for energy, and low fat, making it a healthy plant-based supplement. The mineral analysis confirmed the presence of beneficial elements like sodium, potassium, phosphorus, and zinc, which are essential for human health. The organic acid profile detected oxalic acid, tartaric acid, and pyruvic acid, adding to its antioxidant properties and possible preservative benefits. The vitamin content was notable for fat-soluble vitamins A, D_2, E, and K_1, whereas Vitamin C, together supporting its antioxidant, immune-boosting, and general health benefits. Finally, the bioactive parameters showed higher level of tannins, followed by flavonoids and phenols,, which scientifically support its strong antimicrobial, antioxidant, anti-inflammatory, and anticancer potential.

These results confirm that Lepidagathis barberi holds significant promise as a source of valuable natural compounds for traditional and modern medicine. Therefore, this study validates its traditional uses and encourages further research to isolate its active principles, understand their pharmacological actions, and develop safe herbal drugs, nutraceuticals, and natural health products. Sustainable cultivation and conservation efforts will also be crucial to preserve this important endemic species for future generations.

5.References:

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