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"Pharmacognostic Evaluation and Phytochemical Profiling of *Cyphostemma setosum* (Roxb.) Alston: A Promising Medicinal Plant"

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ABSTRACT

Pharmacognosy is a constituent scientific discipline of pharmacy which has been in use for more than 200 years, and it applies to research on natural product medicines. Pharmacognosy includes processes such as identification, authentication, production, processing, standardization, and many more natural medicinal essentials. The plant *Cyphostemma setosum* (Roxb.) is a succulent climber clothed with scattered grandular bristly hairs, it is belonging to the family Vitaceae, it is known for its ethnomedicinally, folkloric treatment of ailments. The surface studies showed the leaf is amphistomatic with tetracytic and anomocytic. The trichome are multiseriate capitate hair both surfaces, which is a significant character for identification of this plant. In transverse section, the leaf is prominently ribbed abaxially and ridged as a cone towards adaxial side at midvein. Mesophyll is undifferentiated, interspersed with raphide sacs and spareocrystalliferous idoblasts. The ground tissue of midvein consists parenchyma and collenchyma tissues. Petiole in transverse section is oval to subspherical in outline. The powder microscopic and organoleptic characters are also presented in this study. Phytochemical analysis involves both qualitative and quantitative analysis the extraction was carried out by Soxhlet apparatus, the extraction process was carried using different solvents successively in the order of increasing polarity, while qualitative analysis is concerned with the presence or absence of a phytochemical, quantitative analysis accounts for the quantity or the concentration of the phytochemical present in the plant sample. The HPTLC with dual wavelength UV (254/366 nm) was used for the analysis of phytochemical variation in leaf methanolic extracts of *C. setosum.* The FTIR analysis also carried out to identify the function groups. The findings of this study facilitate pharmacognostic standardization of the plant material and add clues in the preparation of herbal monographs for Phyto pharmacopeia.

INTRODUCTION

The screening of commercial variations, adulterants, substitutes, and other drug quality control approaches is facilitated by pharmacognostic the process of assessment. The physical and biological properties of crude pharmaceuticals can be better understood with the help of this reliable and user-friendly instrument. A wide range of diseases can be treated or avoided with the help of medicinal plants due to their many therapeutic properties. Nowadays, medicinal plants have a plethora of bioactive compounds that play a major role in healthcare. The accessibility, low cost, and lack of risk associated with herbal treatments are the three main arguments in favor of their use. However, it is critical to guarantee the product's efficacy and quality (Gajula *et al.*, 2022). Natural medicines have been used since time immemorial to improve human and veterinary health. This human need for medicines is so extensive as to be thought a deep evolutionary behavior (Hardy, 2021). The success of modern medical science has largely depended on drugs originally obtained from natural resources, which is the focus of pharmacognosy, a profile discipline of the pharmaceutical sciences dedicated to study the physical, chemical, biochemical, and biological properties of drugs, drug substances, or potential drugs of natural origin. The goal of our discipline is to discover sources of natural drugs and products of their metabolism from plants, microorganisms, fungi, algae, and animals for the treatment of diseases.

In the twenty-first century, pharmacognosy has become a multidisciplinary, high-tech science of natural medicines in terms of assaying their purity, potency, and consistency, especially in its methodology of using faster and more effective analytical methods, high-throughput screening, target-based drug discovery, and *in silico* methods for virtual ligand screening. Molecular pharmacognosy (Alamgir 2018), genomic pharmacognosy (Yang *et al.*, 2019), and metabolomic pharmacognosy (Allard *et al.*, 2018) have been contemplated as the most promising approaches of pharmacognosy research to meet emergent trends in molecular biology, biotechnology, and analytical chemistry of natural medicines. For example, nowadays DNA barcoding represents an essential addition to the wide range of robust methodologies used to identify and authenticate natural drugs and their pharmaceutical products (Gesto-Borroto *et al.*, 2021).

In ancient times there was no need of drug identification because people were aware of plants surrounding them. There was Guru Shishya Parampara, the knowledge of medicinal plant identification was transferred from generation to generation. Industrialization leads to extinction of plants leading to end of this tradition. After this, during medieval period drug identification is explained based on morphological characteristics, origin, and action of drug (Nama Rupa gyanam)(Sharma, 2013). India possesses a rich, active, and historically cultural history. In this culture and tradition, health and healing are highly valued concepts. India is the world leader in producing the most medicinal herbs, earning the nickname "the botanical garden of the world" (Pratap *et al.*, 2018; Pratap *et al.*, 2009a; Pratap, 2009b; Sekhar *et al.*, 2011). Plants are known for their vast resource of secondary metabolites which are constitutively produced. These include variety of phytochemicals broadly characterized as alkaloids, phenols, terpenoids, steroids, glycosides, and their derivatives. Secondary metabolites are known to possess pharmacological and therapeutic activities and hence explored to find potent chemical moiety, the Plants have been always used as medicine by mankind to treat health-threatening diseases and still popular to obtain new drug candidates as it is the oldest medical practice for humans. The use of botanical natural health products is on the increase all over the world. It is known that almost 80% of the populations in developing countries rely on the traditional medicine, mainly composing herbal prescriptions (Mahady, 2001, Yadav, 2017).

In present scenario, deficiency and unavailability of authentic drug has resulted in adulteration and substitution. It has become a burning issue which hinders safety and efficacy of drug. So, there is need of standardization of raw drug using present day techniques.

Cyphostemma setosum (Roxb.) Alston. (Vitaceae), the prostrate herb distributed in the lower hills of Palani, the Western Ghats, Tamil Nadu is prescribed by the Puliah tribal community very commonly for controlling diabetics in this region (Pullaiah, 2006; Sujatha and Mariya Selvam, 2015), ulcer and wounds (Nandagopalan *et al.*, 2011; Durairaj and Annamalai, 2013), leaf used as stimulant, indolent tumors and applied externally to assist for the expulsion of guinea worms (Vaidyanathan et al., 2013), boils for healing (Datta, 2009), intestinal worms(Shanmugam *et al.*, 2012; Salai Senthilkumar et al., 2014). Antinociceptive effect (Govindarajan, et al., 2023), Rheumatism and Dysentery (Nallella Sreeramulu *et al.*, 2013), Spinal pains (Prabhakar *et al.*, 2015), antioxidant and antiulcer activity (Chinnamaruthu *et al.*, 2013; Jayachitra *et al.*, 2018), antiplasmodial (Nafuka and Mumbengegwi, 2013), the leaves are use to cure jaundice (Chute and Tiwari, 1999); and also used for washing cattle (Murthy *et al.*, 2007).

Material and methods

Collection and Authentication of Plant Material

The leaves of *Cyphostemma setosum* were collected from Kawal Wildlife Sanctuary (Kawal Tiger Reserve) is a nature preserve located at Jannaram mandal of Mancherial District (Old Adilabad district) in the Telangana state of India, during July / August in the year 2023. The plant was authenticated by Botanical Survey of India, Deccan Regional Centre, Hyderabad, Telangana (accession number- BSI/DRC/2023-24/Identification/404), and the specimen was preserved at Herbarium, Hyderabadensis, Department of Botany, O.U, Hyderabad, Telangana India.

Drying

After cleaning the leaves, they were cut into pieces (0.6—1.4×1×0.3 cm_3) with the help of scissors and knives. They were left for shade drying on the newspapers for ten days. After that, the fruit pieces were dried in a hot air oven at 40 °C for an hour just before starting the extraction process to remove the moisture content.

Successive Extraction Using Soxhlet Apparatus

the fresh leaves of *C. setosum* have been obtained and carefully cleaned with the use of running water to remove any dirt or particles in order to employ this approach to develop extracts from the leaves. The Soxhlet equipment was used for the subsequent extraction. After placing the 20 grams of powdered *C. setosum* leaves in Wattman's No. 1 filter paper in a Soxhlet thimble, 200 milliliters of solvent were added to a round-bottom flask (still pot). a variety different kind of solvents, such as pet ether, chloroform, ethyl acetate, and methanol, were used to extract the leaves. To guarantee that the powder had a uniform size, the dried leaf powder was first ground using a mechanical grinder and then sieved. Using the Soxhlet apparatus, the leaf powder was extracted in stages using pet ether at 60 °C, chloroform at 61 °C, ethyl acetate at 77 °C, and methanol at 65 °C. In order to increase the rate at which new solvent could be cycled, the extraction temperatures were changed to match the solvent's boiling points. For hot continuous and recurrent extraction, six hours were allocated to each solvent. The resultant extracts underwent further concentration and evaporation in a 45 °C oven. Afterwards, the dried extracts were utilized to perform phytochemical screening. (Raman, 2006; Anonymous, 2015; Gajula *et al.*,2002; Kamalakar *et al.*, 2014).

Calculation of percentage yield

The dried extracts obtained through all of the solvents have been weighed and the final yield was calculated based on the weight of the plant material after it had been thoroughly evaporated in the absence of moisture.

$$\text{Percentage of yield } (\%) = \left(\frac{\text{Dry weight of extract}}{\text{Dry weight of a plant}} \right) \times 100$$

Phytochemical screening of *C. setosum*

The leaf extract was subjected to the preliminary phytochemical screening for the presence of secondary metabolites. Phytochemical tests were carried out adopting standard procedures. Tests were performed for alkaloids, flavonoids, saponins, steroids & terpenoids, phenolic compounds, tannins, cardiac glycosides, glycosides, coumarins, anthraquinones, quinones, and resins. The various tests have been conducted qualitatively to find out the presence or absence of bioactive compounds (Singh & Kwalyambumu, 2024; Adil, *et al.*,2024; Kumar *et al.*, 2023; Fisher, 1968; Gahan, 1984; Kokate, 1999; Ramakrishnan, *et al.*, 1994; Ruthmann, 1970; Sasikumar *et al.*, 2014; Trease & Evans, 1982; Wagner *et al.*, 1996; Yasuma & Ichikawa, 1953).

1. **Detection of Alkaloids:** Extracts were dissolved in dilute Hydrochloric acid and filtered
   Mayer's Test: To a 2ml ml filtrate two to three drops of Mayer's reagent were added by the side
2. **Test for Flavonoids:** To the test solution magnesium turnings and a few drops of concentrated HCl were

added and boiled for five minutes. A red color indicates the presence of flavonoids.

3. **Test for Saponins:** About 0.5g of the powdered drug was boiled gently for 2min with 20mL of water and filtered while hot and allowed to cool. 5mL of the filtrate was then diluted with water and shaken vigorously. A frothing indicates the presence of saponins.
4. **Detection of Steroids & Terpenoids**: Liebermann-Burchardt test: To 1ml of extract, 1ml of chloroform, 2 to 3ml of acetic anhydride, and 1 to 2 drops of concentrated sulfuric acid was added. The appearance of the dark green color showed the presence of steroids.
5. **Test for Phenolic compounds: a** small quantity of powdered sample was tested with the following reagents and the color produced indicates the presence of phenolic compounds.
   a. 5% Ferric chloride solution- Deep bluish-black colour.
   b. Lead acetate solution-white precipitate.
6. **Test for Tannins:** A small quantity of the powdered drug was extracted with water. To the aqueous extract, a few drops of ferric chloride solution were added. A bluish-black color indicates the presence of tannins.
7. **Detection of Glycosides**
   The test solution was prepared by dissolving the extract in alcohol or boiling it with a hydro-alcoholic solution.
   a) Baljet's test
   The test solution was treated with 2% sodium picrate. The appearance of yellow to orange color indicated the presence of glycosides.
   b) Legal 's test
   The test solution was treated with pyridine and made alkaline; the addition of 2% sodium nitroprusside gave pink to a red colour indicating the presence of glycosides.
   c) Keller-Killiani test
   The extract (100mg) was dissolved in 1ml of glacial acetic acid containing one drop of ferric chloride solution. This was then underlayer with 1ml of conc. H_2SO_4. A brown ring obtained at the interface indicates the presence of glycosides.
8. **Detection of Cardio glycosides:** Qualitative analysis of cardiac glycosides in the extracts was performed with the Keller Killiani test. 1 mL of acetic acid and 2 drops of ferric chloride were added to 2 mL of extract, then 2 mL of sulfuric acid (concentrated) was added and the color change was observed. Reddish-brown color formation was deemed to be a positive test for cardiac glycosides.
9. **Detection of Coumarins**: 3ml of 10% NaOH was added to 2ml of aqueous extract. The yellow coloration of the contents indicated the presence of Coumarins.
10. **Test for Phytosterols:** A few drops of concentrated sulphuric acid were added to the extract solution, shaken well, and set aside. The lower chloroform layer of the solution turning red indicates the presence of phytosterols.
11. **Detection of Quinones:** Dilute NaOH was added to 1ml of crude extract, blue-green or red coloration indicated the presence of quinones.
12. **Detection of Resins:** To 2 ml of extract, 5-10 drops of acetic anhydrate were added, dissolved by gently heating, and then 0.5ml of sulphuric acid was added. The bright purple color was produced indicating the presence of resins.
13. **Detection of leucoanthocyanins**
    1ml of crude extract was added to 1ml of isoamyl alcohol. Upper layer turned red in color indicating the presence of leucoanthocyanins.
14. **Detection of anthraquinone**
    To 1gr of the powdered plant material, chloroform was added and shaken for 5mints. Contents were filtered and to the filtrate, 5ml of was ammonia solution was added and agitated gently. A bright pink color in the upper aqueous layer indicated the presence of anthraquinone.
15. **Detection of fixed oils**
    A small quantity of extract is pressed between two filter papers. Oil stain on the paper indicates the presence of fixed oils.
16. **Detection of Gums and Mucilage's**
    Dissolve 100mg extract in 10mL distilled water + 25mL absolute alcohol (constant stirring) White or cloudy precipitate indicates the presence of Gums and Mucilage's.

Quantification of phytochemical contents from *C. setosum*

Quantification of total content of alkaloids

1 mg of the plant extract was dissolved in dimethylsulphoxide and added 1ml of 2N HCl and filtered. This solution was transferred to a separating funnel, 5ml of bromocresol green solution then 5ml of phosphate buffer were added. The mixture was shaken with 1, 2, 3 and 4ml of chloroform by vigorous shaking and collected in a 10ml volumetric flask and diluted to the volume with the chloroform. A set of reference standard solutions of atropine (20, 40, 60, 80 and 100µg/ml) were prepared in the same manner as described already. The absorbance for standard solutions and test solutions were determined on the reagent blank at 470nm with an UV/Visible spectrophotometer. The content of alkaloids was expressed as mg of AE/g of plant extract (Tan, 2018).

Total content of flavonoids quantification.

Colorimetric assay was used to determine the total content of flavonoid using aluminum chloride for the reaction, the plant extract of 1 ml and distilled water of 4 ml was taken in a 10 ml of flask. 0.30 ml of 5 % sodium nitrite and after 5minutes, 0.3ml of 10 % aluminium chloride was mixed in the flask. 5minutes later, 2 ml of 1M NaOH was treated and diluted using 10 ml distilled water. A set of standard solutions of quercetin (20, 40, 60, 80 and 100µg/ml) were prepared as mentioned earlier. The absorbance was measured for test and standard solutions using reagent blank at 510nm wavelength by UV-Visible spectrophotometer. The total content of flavonoid was denoted as mg of QE/g of extract.

Quantification of total content of phenolic compounds

The phenolic compounds concentration in extract was quantified by Spectrophotometry method. Folin-Ciocalteu method was employed for the quantification of total phenolic content. The reaction mixture contains 1 ml of plant extract and 9ml of distilled water. 1 ml of Folin-Ciocalteu phenol reagent was treated with the mixture and well shaken. After 5minutes, 10 ml of 7 % Na2CO3 solution was treated with the mixture. The volume was 25ml. A set of gallic acid standard solutions (20, 40, 40, 60, 80 and 100µg/ml) were prepared as earlier. Incubated for 90 min at 30°C and absorbance was analyzed for test and standard solutions with reagent blank at 550 nm with using UV-Visible spectrophotometer. The content of total phenolic compound was denoted as mg of GAE/gm of extract (Kalita Pallab *et al.*, 2013).

Quantification of tannin total content

Folin-Ciocalteu method was used to quantify the tannin total content. About 0.1ml of plant extract was added in 10 ml of volumetric flask containing the distilled water of 7.5ml and Folin-Ciocalteu phenol reagent of 0.5ml, 35% Na2CO3 solution of 1 ml and diluted to 10ml using distilled water. The reagent mixture was well shaken and kept at 30°Ctemperature for 30min. A set of gallic acid solutions (20, 40, 60, 80 and 100µg/ml) were prepared as mentioned earlier. Absorbance of standard and test solutions was analyzed with blank at 725nm wavelength using UV-Visible spectrophotometer. The tannin total content of tannin was expressed as mg of GAE/g of extract(Shubhangi *et al.*, 2023).

Fourier transform infrared spectroscopy (FTIR)

A dry leaf powder of *C. setosum* was taken and subjected to Fourier transform infrared (FTIR, CFRD, Osmania University, Hyderabad, Telangana) spectroscopy measurement using the potassium bromide (KBr) pellet technique diffuse reflection mode at a resolution of 4cm-1. The powder was mixed with KBr and exposed to an infrared source of 500 to 4000 cm-1. A similar process was used for the FTIR studies of *C. setosum* extract before and after bio reduction (Straková *et al.*, 2020; Pakkiri Samy *et al.*, 2017; Sravan Kumar *et al.*, 2015). The frequency of the vibrational peak (v) depends on two factors i.e., force constant and reduced

mass, which can be explained by the following equation (Ahmed *et al.*, 2021).

$$v = 1/2\pi c \sqrt{(k/\mu)}$$

Here, **c**- is the speed of light,

k- is the force constant

μ- is the reduced mass

High-Performance Thin Layer Chromatography (HPTLC) analysis

An aluminium silica gel plate 60 F254 (5 x 10 cm with 0.25 mm thickness; Merck, Darmstadt, Germany) was coated with the concentrated methanolic extract using a 25μL syringe. Methanol was used for washing the plates prior they were used. The next thing to do was to insert the mobile phase into the TLC development chamber using filter paper. The solution samples and reference solutions were administered in bands with a width of 6 mm and a spacing of 10 mm using an automatic operation TLC Sampler 4 applicator (CAMAG, Muttenz, Switzerland, supplied by Anchrom Technologists, Mumbai) that was fitted with a 25-μL Hamilton syringe with nitrogen flow. The flow velocity was kept constant at 15 microliters per second, while the spacing between bands remained at 20 millimetres per second. The slit size was maintained at 4 × 0.20 mm and the scanning speed was 20 mm/sec. A saturated Toluene mixture made up the mobile phase. Ethyl acetate, methanol, acetic acid, chloroform, and methanol (8:2 v/v) served as the mobile phase in the chromatography that separated the phytoconstituents. The optimal chamber saturation period was 20 minutes at room temperature, and the chromatographic run length was 8.5 cm. Following development, the TLC plates were air-dried in a vented wooden room using a hot air dryer. The CAMAG TLC Scanner III was used to conduct densitometric scanning at three different wavelengths: 254 nm and 366 nm, with a slit size of 4 × 0.20 mm and a scanning speed of 20 mm/sec. The WinCATS programme (version 1.4.3). CAMAG) ran all the instruments that used the intensity of diffusely reflected light to determine the chromatographed compounds. An illuminator, Reprostar 3, and digital camera Power Shot G2 (Canon, Tokyo, Japan) were used for photographic evidence within the Digi Store 2 system (CAMAG). The radiation source, a deuterium lamp, emitted a UV spectrum within 200-400 nm.(Ananya *et al.*, 2024; Bhargava *et al.*, 2021).

Macroscopic evaluation

Observed macroscopic characteristics of a fresh *C. setosum* leaf included the characteristics of the lamina, the presence of the petiole, and the leaf base. Venation, shape, texture, apex, phyllotaxis, petiole, margin, and surface distinguish laminae. Other distinguishing characteristics comprise (Cooke, 1903; Anon. 2008; Kirtikar & Basu, 2012).

Microscopic evaluation

Before the leaves are encased in paraffin wax, they are boiled, fixed in F.A.A. (Formaldehyde-Acetic Acid-Alcohol), and dehydrated with a sequence of xylene and alcohol. The leaves are then encapsulated with paraffin wax. To analyse sections cut at 10–12 m with an Optica rotating microtome, crystal violet and basic fuchsin combination staining was conducted, followed by Canada balsam mounting (Johansen 1990). The microscopical examinations conducted included both quantitative and qualitative evaluations. The microphotographs were captured using a digital Sony camera and a trinocular Olympus BX-53 microscope.

Qualitative microscopy

Using a microscope, the surface and cross-section of the leaf were observed and analyzed in great detail. According to the method, the segment was stained with a 1:1 mixture of chloroglucinol and hydrochloric acid. Numerous microscopic structures were meticulously analyzed and photographed (Pandya *et al.*, 2010; Kokate, 2005; Ali, 2008).

Surface view of leaf

Before creating epidermal peels, the skin was scratched and peeled with a razor blade. The peels were then stained with safranine and suspended in glycerin. After being submerged for several hours in a 4% sodium hypochlorite solution, the leaf lost all of its chemicals and colour as a consequence of the treatment. The works from (McVeigh , 1935). Various aspects of bleached leaves, including the lamina, midrib, and petiole, were examined in great detail under a microscope.

Transverse section (T.S.) of Leaf

After submerging the new leaf in water, we randomly divided it along its midrib to produce cross-sections. Fine sections were fixed on a glass plate with glycerin in the absence of a staining agent, and the resulting images were then examined under a microscope. Two components were used to produce the tissue section stain: phloroglucinol and concentrated hydrochloric acid. Trichomes, stomata, and a number of other distinguishing characteristics were observed (Gokhale & Kokate, 2008; Khandelwal, 2007).

Powder microscopy

There was a microscopic examination of desiccated, finely powdered leaves lignified substances can be distinguished from other kinds of substances using a colouring solution (phloroglucinol and hydrochloric acid, mixed in a 1:1 ratio). When viewed through a microscope, a minute amount of foliage that had been reduced to a granular consistency was observed. After mounting the powder in glycerol with 1-2 drops of phloroglucinol solution (0.1% w/v) and concentrated hydrochloric acid, a coverslip was positioned on top. Before the material was examined under a microscope, this was performed. There were indications of trichomes, stomata, epidermis cells, xylem arteries, fibres, and additional structures. As a direct result, photomicrographs of the cellular structures were able to be taken for use in subsequent research (Thitikornpong , 2011).

Quantitative microscopy

Estimation of the stomatal number and stomatal index

Stomatal number is the average number of stomata present in the epidermis of a leaf, measured in millimetres squared. Calculating the stomatal index of a leaf involves dividing the total number of stomata on the leaf by the total number of epidermal cells in a particular location and expressing the result as a percentage. The following algorithm will be utilized to calculate the stomatal index: SI represents the stomatal index, S represents the number of stomata per unit area, and EP represents the number of epidermal cells per unit area. This index is computed by multiplying the ratio between S and S plus EP by 100. A section of the leaf's epidermis was separated into its upper- and lower-layers using forceps. Glycerol was utilized to apply it to the film. The prepared sample was placed on the microscope's stage, and a camera lucida was used to record the epidermal cells and stomata on a piece of black paper divided into four 4 mm2 fields. We counted the stomata and epidermal cells in each region to ascertain their total number. Using the described method, we determined the stomatal index for both the upper and lower epidermis. The stomatal index is the average number of stomata present per unit of skin surface area (Kumar *et al.*,2012).

Estimation of vein termination and vein-islet number

Between the leaf's margin and midrib, leaf area was measured in millimeters per square millimeter. This enabled the determination of variables such as the number of vein-islets and the veinlet termination point. A sketching camera was prepared and Lucida and black paper were inserted within it. In the exact center of the playing field, a square measuring precisely 4 millimeters on each of its four sides was constructed. We traced every vein within the square, all the way to the overlapping islands that formed in the spaces between the four corners (Khan *et al.*, 2016). For four neighboring squares, the average vein islet count and veinlet termination were recorded (Kumar *et al.*,2012).

Estimation of palisade ratio

In a section through the epidermis of a leaf, palisade cells were visible immediately underneath the epidermal cells. The palisade layer that resides beneath the epidermis cells was then reconstructed using a camera lucida. The palisade ratio was calculated by averaging the number of epidermis cells of four distinct types within five groups from five distinct leaf regions (Khan *et al.*, 2016).

Determination of width and length of vessels and fibers

Through the use of Schultze's maceration solution, the xylem arteries were separated from the other histological characteristics. In order to create Schultze's maceration fluid, potassium chlorate was combined with a 50% volume by volume solution of nitric acid, and the resulting mixture was heated in a

water boiler. The above-described macerating solution was applied to a fragment of the leaf to be processed. On occasion, potassium chlorate was utilized in order to dissolve and soften the foliage fibers. By mounting the treated leaf tissue on a microscope slide, agitating it with a needle, and repeatedly flushing it with water, the acid was eliminated. This procedure was performed multiple times. The diameter and length of the arteries were measured and recorded with the aid of a calibrated micrometer eyepiece. This investigation measured the lengths and diameters of fifty distinct xylem vessels and filaments (Karuppaiyan & Nandini, 2006).

Determination of biocrystals of leaf

After the Ca Ox crystals had been bleached, they were separated so that they could be examined under a microscope. With the aid of photomicrographs, the diameters and patterns of the crystals were analyzed. The formation of crystals in leaves results from a variety of metabolic processes, with the majority of crystals found in leaves being stored in the cytoplasm and cell vacuoles. In the production of crystals, calcium compounds such as calcium carbonate and calcium oxalate are frequently employed. According to the findings of a number of researchers (Cuéllar-Cruz *et al.*, 2020), the plant's cells contain numerous Ca oxalate crystals. Moreover, according to (Mazen *et al.*, 2003), aluminum is the only element present in these prismatic crystals.

Organoleptic parameters

Parameters like color, odor, and taste were evaluated organoleptically.

Observation and results

Plant profile

Cyphostemma setosum (Roxb.) Alston (Family: Vitaceae) Cooke, 1:254 (1903); Anon. 6:408(2008b); Kirtikar & Basu, 3: 852(2012)

Table:1. Taxonomic classification of *Cyphostemma setosum* (Roxb.) Alston

Kingdom	Plantae
Phylum	Streptophyta
Class	Equisetopsida
Subclass	Magnoliidae
Order	Vitales
Family	Vitaceae
Genus	Cyphostemma
Species	*Cyphostemma setosum*

Vernacular names: English: Hairy wild vine**. Hindi:** Harmal. **Marathi:** Khajgolichavel**, Tamil:** Kauri, Pulinaralai**, Telugu:** Barrebachali, Pullabachali

Local name: Barre bacchali

Macroscopic evaluation of the leaf

Whole plant clothed with scattered glandular bristly hairs, stem herbaceous, prostrate, weak, succulent, striate and sulcate, tendrils leaf opposed, forked, long. Leaves succulent, sessile 3 foliolate (The lower sometimes simple); leaflets sub fleshy, 5-7 5 by 3 8-5 cm., shortly petioluled (the petiolule of the terminal leaflet the longest) elliptic or obovate oblong, obtuse, irregularly bristle-toothed or laciniate, glabrous or nearly so above, glandular - hispid on the nerves beneath, pale green, stipules broadly ovate, acute Flowers 2mm., long. contracted in the middle, arranged in leaf -opposed or apparently terminal dichotomous or trichotomous lax divaricate glandular cymes, peduncles 387.5 cm., long, glandular-hispid, pedicels short. Calyx cup shaped, subtruncate Petals hooded at the apex, ultimately reflexed. Style subulate. Berry 6.5-8 mm., diameter, ovoid, glandular - hispid, scarlet **(Fig-1D&2A-F).**

Distribution: Found in dry localities in peninsular India, on dry stony hills up to 800 m., sometimes on black plains, Maharashtra, Andhra Pradesh, Karnataka and Tamil Nadu.

Ethnomedicinal uses in the present investigation: The leaf paste is mixed with coconut oil and applied on piles once a day till cure.

Cancer: Rhizome with seeds of Piper nigrum is taken in equal quantities and ground. 2 spoonsful of paste mixed with a spoonful of honey is administered daily twice for 45 d.

Fig 1 : Macroscopic leaves of *C. setosum*

Fig: 2A-F – Habitata of *C. setosum.*

Microscopic evaluation of the leaf (Surface View)
Leaf – Adaxial surface:
LEAF LAMINA ADAXIAL
Epidermal cell complex: Epidermal cells 5-8 sided, few 4 sided, polygonal isodiametric, few anisodiametric, sides thick, straight, few straight to curved, surface smooth, contents slightly dense **Dist:** Common, all over, except on veins, irregularly arranged, variously oriented **E.C.F. 1615 per sq.mm**
Costal cells: 6-8 sided, polygonal anisodiametric to linear, sides thick, straight to curved, surface smooth, contents slightly dense Dist. On primary, secondary veins, irregularly arranged, parallelly oriented
Stomatal complex: Tetracytic, few anomocytic, subsidiaries 4-5, monocyclic, indistinct, mostly of f-type, few a-type, rarely c-type, guard cells reniform, densely cytoplasmic Dist: Common, all over, except on veins, irregularly arranged, variously oriented. **S.F. 182 per sq. mm., S.1, 9.65.**
Trichome complex: i). Multiseriate capitate hair: Foot: Multicellular, cells juxtaposed, walls thin, contents scanty. Stalk: Multiseriate, walls thin, surface smooth, contents scanty Head: Multiseriate, capitate, 4-8 celled in length, 2 to 7 celled in width, walls thin, surface smooth, contents slightly dense **(Fig;3AB).**
LEAF LAMINA ABAXIAL
Epidermal cell complex: Epidermal cells as described on lamina adaxial except mostly anisodiametric, few linear **E.C.F. 2634 per sq.mm**
Costal cells. As described on lamına adaxial
Stomatal complex: As described on lamina adaxial, except subsidiaries mostly a - type, few f-type and rarely c-type. **S.F. 492 per sq mm., S.I. 16.5**
Trichome complex: i). Multiseriate capitate hair: As described on lamina adaxial **(Fig:3CD).**

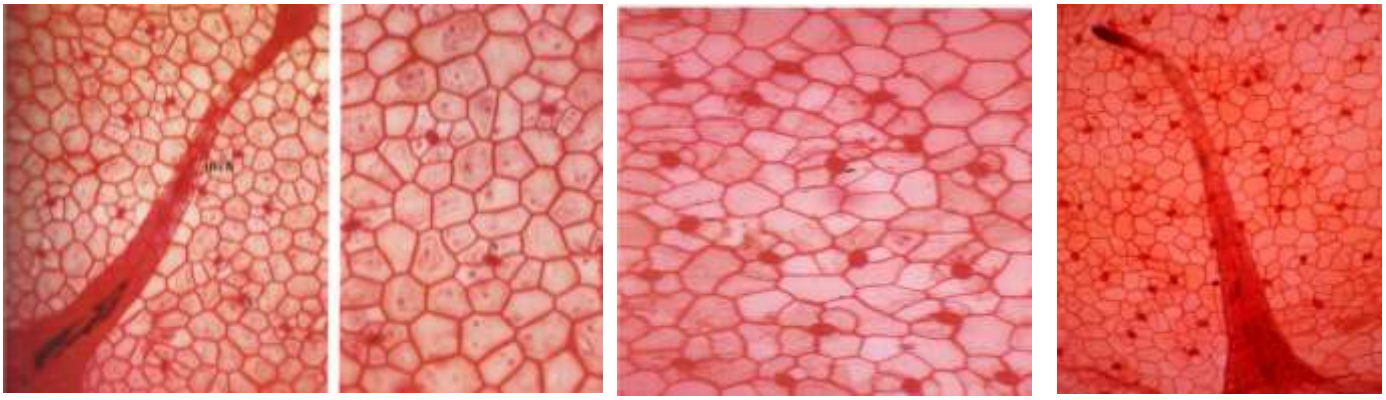

Fig 3A.Leaf adaxial surface showing multiseriate capitate hair X 125; 3B. Leaf adaxial surface with stomata X 158; 3C. Leaf abaxial surface X 226; 3D. Leaf abaxil surface with mch trichome X 134.

Transverse Section of leaf:

T.S. OF LEAF: Leaf is prominently ribbed abaxially and ridged as a cone towards adaxial side at midvein. Secondary veins flat on either sides. Midvein is 1630-1980 (1788) µm and lamina 150-202 (168) µm in thick

EPIDERMIS: 1-layered, cells over the lamina mostly barrel shaped. few oval to spherical, cells adaxially larger, barrel shaped, 3382 (67) µm tangentially long and 27-55(38) µm wide, isodiametric cells 27-55(38) µm in diameter, walls thin, contents scanty, in few dense, cells on the abaxial smaller. elongated cells 33-63(44) µm tangentially long and 14-36(23) µm radially wide and isodiametric cells 27-44(35) µm in diameter, contents scanty, slightly dense in few, cells over the midvein adaxial as on lamina adaxial, abaxially epidermal cells over midvein oval to circular 22-40 (31) µm in diameter, walls thin, slightly cuticularized. Stomata: Amphistomatic, flushed with epidermis **(Fig -4A&B).**

MESOPHYLL: Undifferentiated, wholly consists of spongy parenchymatous tissue, 6- 9 layered, cells polygonal to spherical, oval to oblong, 55-130 (96) µm in diameter. walls thin, contents dense with chloroplasts, often interspersed with raphide sacs and sphaerocrystalliferous idoblasts **(Fig -4A&B).**

GROUND TISSUE: Of Midvein consists of parenchyma and collenchyma tissues. **Collenchyma:** 1-2 layered on the abaxial, and as a group of cells in the hypodermal adaxial ridge, cells oval to spherical, lamellar and angular. 24-44(32) µm in diameter. contents dense, interspersed with raphide sacs **Parenchyma:** 6-9 celled thickness on adaxial and abaxial sides, cells polygonal, oval to spherical, walls thin, with small intercellular spaces, 66-194 (120) µm in diameter, often interspersed by raphide sacs & sphaerocrystalliferous idioblasts, contents dense with chloroplasts including aluerone grains.

VASCULAR TISSUE: At midvein consists of 4 abaxial bundles arranged in an are and one adaxial bundle. Vascular bundles are linear elliptic. Xylem towards the centre and phloem towards the outside, conjoint, collateral, endarch, open, 196 - 328(256) µn vertically long and 46-110 (68) µm laterally wide. Tracheary elements 24-28 in number in 4 bundles, oval to spherical and polygonal, 16-34(24) µm in diameter. Secondary vascular bundles linear to oval in shape, conjoint collateral, endarch, open. Secondary wall thickenings of tracheary elements consist mostly helical and few annualar thickened. Phloem consists of phloem parenchyma, sieve cells and bast fibers, phloem parenchyma compactly arranged without intercellular spaces, contents scanty **(Fig -4A&B).**

Petiole anatomy

T.S. OF PETIOLE: Oval to subspherical in outline flat on adaxial with small ridges and furrows all over often with trichome bases over the surface. 2834-3426 (3140) µm in diameter **EPIDERMIS:** Is 1-layered. cells mostly barrel shaped, few tabular oval to spherical 16-34 (26) µm long 16-28 (20) µm wide isodiametric ones 12-24 (18) µm in diameter **GROUND TISSUE:** Hypodermis in ridged areas possess a group of collenchyma cells 11-26 (18) µm in diameter angular contents scanty cortex is extensive with parenchyma, cells polygonal, oval to spherical, smaller near the periphery and larger inside 43-108 (83) µm in diameter, walls thin. intercellular spaces few contents dense in some with inulin crystals and few cells with raphide sacs

VASCULAR TISSUE: Consists of 11-22 small linear vascular bundles, in a ring and one central bundle towards adaxial side The smallest vascular bundle 108-144 (130) um vertically long. 38-54 (45) µm laterally wide largest vascular bundle 378-421 (394) µm vertically long and 32-54 (43) µm laterally wide Vascular bundles conjoint, collateral, endarch, consists of tracheary elements arranged in radial rows, cells polygonal, lignified, 18-28 (24) µm in diameter. Phloem is scanty, mostly towards outside and a small amount inside as internal phloem. Tracheary elements in L.S. mostly consist of helical and scalariform thickenings **(Fig-4C).**

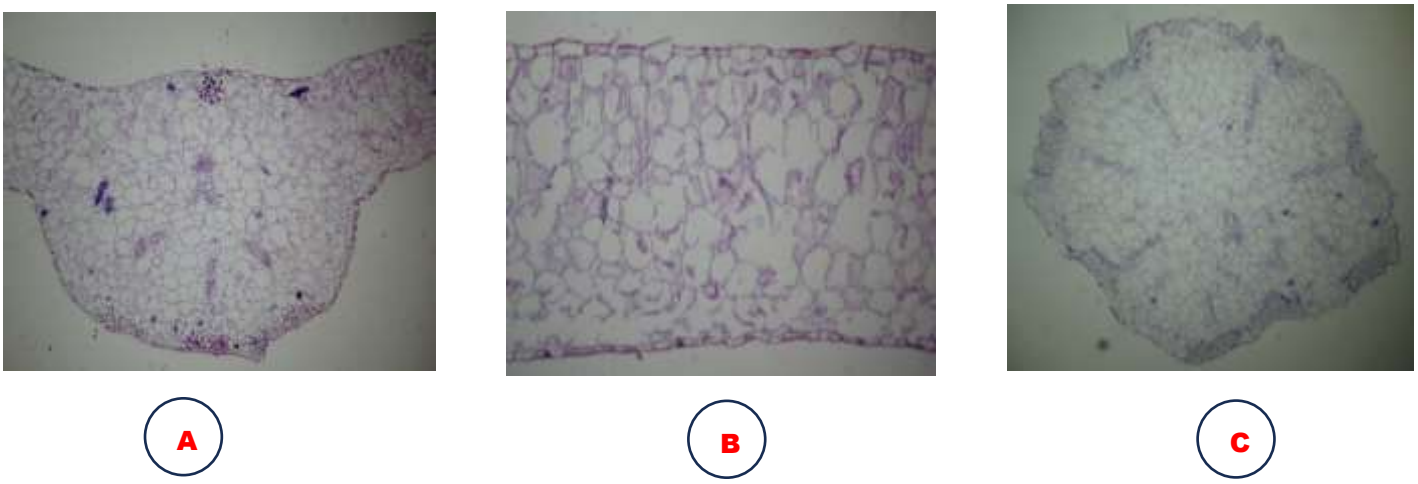

Fig4A. T.S. of leaf midvein X 74; 4B. T.S. of leaf lamina X 104; 4C. T.S. of Petiole

Powder Microscopy analysis of *C. setosum*.

Powder consists of numerous, isolated raphides; pieces of epidermis with straight to curved sides with stomata; pieces of tracheary tissue with helical thickenings; pieces of costal cells, arranged in radial rows; pieces of ground parenchymatous tissue; unidentified fragments several **(Fig 5A-D).**

Fig 5A. Leaf epidermis X 65; 5B. Raphides X 54; 5C. Tracheary elements with helical thickenings X 80; 5D. Costal cells X 100.

Organoleptic characters

Colour - Light brown; **Touch** -Coarse; **Odour** - Slightly Pungent; **Taste** - Sour.

Quantitative microscopy

The various parameters evaluated for leaf surface constants were observed like stomatal number (upper and lower), stomatal index (upper and lower), E.C.F, (upper and lower), Spongy parenchyma, palisade ratio, vascular bundles tracheary elements. The results are shown in **Table-2**

Table-2: Quantitative Microscopic Data Analysis

S.no	Parameters	Results
1.	Epidermal cell frequency (E.C.F adaxial)	1615 per sq. mm.
2.	Stomatal index (S.I. adaxial)	9.65
3.	Stomatal frequency (S.F. adaxial)	182 per sq.mm.,
4.	Epidermal cell frequency (E.C.F abaxial)	2634 per sq.mm.
5.	Stomatal index (S.I. abaxial)	16.5
6.	Stomatal frequency (S.F. abaxial)	492 per sq.mm
7.	Thickness of lamina	150-202 (168) µm.
8.	Thickness of midvein	1630-1980 (1788) µm.
9.	Palisade cells	Undifferentiated
10.	Spongy parenchyma	55-130 (96) µm in diameter.
11.	Collenchyma	24-44(32) µm in diameter
12.	Parenchyma	66-194 (120) µm in diameter.
13.	No. of Vascular bundles	4
14.	Vascular bundles	196 - 328(256) µm vertically long & 46-110 (68) µm laterally wide.
15.	No. of Tracheary elements	24-28 in number.
16.	Tracheary elements	16-34(24) µm in diameter
17.	Crystals	Raphide sacs & sphaerocrystalliferous
	Petiole anatomy	
18.	Petiole	2834-3426 (3140) µm in diameter
19.	Collenchyma	11-26 (18) µm in diameter
20	No. of Vascular bundles	11-22 small linear vascular bundles
21.	Smallest vascular bundle	108-144 (130) um vertically long. 38-54 (45) µm laterally wide
22.	Largest vascular bundle	378-421 (394) µm vertically long and 32-54 (43)
23.	Tracheary elements	18-28 (24) µm in diameter
	Crystals	Inulin crystals and few cells with raphide sacs

Qualitative Analysis of Phytochemicals

Although phytochemicals are mostly liable for the production of secondary metabolites for their function of plant defense, these chemical compounds also possess therapeutic actions that can ease certain aspects of human health. They are the subject of a considerable amount of research as a consequence of their existence. To identify the secondary metabolites in the *C. setosum* leaf extracts, a phytochemical analysis was conducted. The extracts were examined for alkaloids, flavonoids, saponins, steroids, terpenoids, tannins, glycosides, coumarins, phytosterols, quinones, anthraquinones, cardiac glycosides, leuco anthocyanins, fixed oils, and resins in the presence of petroleum ether, chloroform, ethyl acetate, and methanol.

The qualitative investigation revealed that the *C. setosum* plant's leaf could be classified into multiple phytochemical categories. From the results provided **(Table- 3 and Fig- 6),** we can compare the solvents employed in the extraction process.

According to the findings of the phytochemical investigation, the methanol extract was the only one that included alkaloids, glycosides, and saponins. In every extract, with the exception of the petroleum ether extract, flavonoids and coumarins were found to be. It was discovered that the chloroform and methanol extracts contained both steroids and terpenoids. It was only the chloroform and ethyl acetate extracts that were found to include phenols and tannins, but phytosterols were found in all of the extracts with the exception of the chloroform extract. In every extract, with the exception of the ethyl acetate extract, cardiac glycosides were discovered. The chloroform extract was the only one that included quinones and anthraquinones, but the ethyl acetate extract was the only one by which resins were discovered. Both the petroleum ether and chloroform extracts tested positive for the presence of fixed oils. Last but not least, none of the extracts contained any leuco anthocyanins **(Table- 3).**

The following solvents were used to create the crude extracts, yielding varying percentages: petroleum ether (5.2% yield), chloroform (18.16% yield), ethyl acetate (36.2% yield), and methanol (48.5% yield).

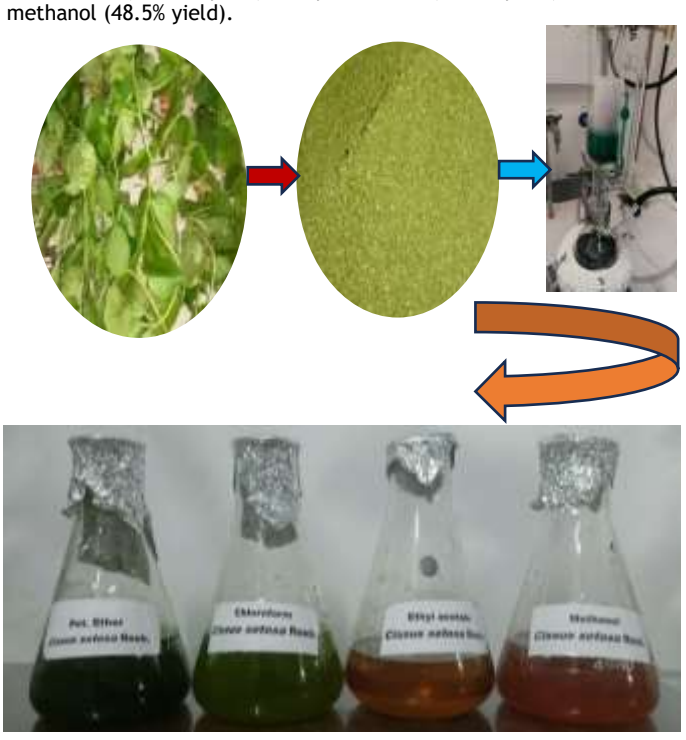

Fig:6. Phytochemical extractions using various solvent combinations

Table :3. Phytochemical analysis of *C. setosum*

S.No	Phyto. Name	Pet. ether	Chloroform	Ethyl acetate	Methanol
1.	Alkaloids	-	-	-	+++
2.	Flavonoids	-	++	+++	+++
3.	Saponins	-	-	-	+++
4.	Steroids & Terpenoids	-	++	-	+++
5.	Phenols	-	++	+++	-
6.	Tannins	-	++	+++	-
7.	Glycosides	-	-	-	+++
8.	Coumarins	-	++	+++	+++
9.	Phytosterols	++	-	+++	+++
10.	Cardiac Glycosides	++	++	-	+++
11.	Quinones	-	++	-	-
12	Resins	-	-	+++	-
13.	Leuco anthocyanins	-	-	-	-
14.	Anthraquinones	-	++	-	-
15.	Fixed oils	+	++	-	-

(Low +, Medium ++, High +++, Absent -)

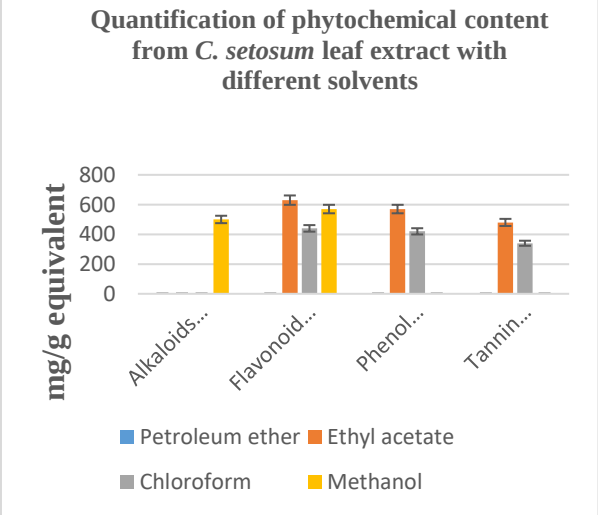

Fig:7. Quantification of Phytochemicals

The phytochemical composition of *C. setosum* leaf extract was evaluated using various solvents to understand the distribution and concentration of different bioactive compounds. In the analysis of alkaloids, methanol-based leaf extract exhibited the highest concentration, measuring 500 mg/g Atropine equivalent. Conversely, no alkaloids were detected in extracts obtained from other solvents. Flavonoid quantification revealed varying concentrations across the solvents. The ethyl acetate extract demonstrated the highest flavonoid content at 630 mg/g Quercetin equivalent, followed by methanol with 570 mg/g, and chloroform with 440 mg/g. However, petroleum ether extract showed no detectable flavonoids. Total phenol and tannin content were also assessed across different solvents. Ethyl acetate extract showcased the highest phenol (570 mg/g) and tannin (480 mg/g) levels, both equivalent to Gallic acid. Chloroform extract followed with phenol and tannin concentrations of 420 mg/g and 340 mg/g, respectively. In contrast, petroleum ether and methanol extracts yielded no phenol or tannin. These findings highlight the solvent-dependent extraction efficiency of various phytochemicals from *C. setosum* leaves.The observed variations underscore the importance of solvent selection in phytochemical extraction and highlight the potential of *C. setosum* as a valuable source of bioactive compounds for various applications **(Fig-7).**

Profile of HPTLC finger printing of *Cyphostemma setosum*

The profile of chromatographic separation of leaf methanol extract scanned at 254 nm, reveals six spots **(Fig.8)** out of which spot 5 possess maximum composition with R_f at 0.92. While, densitogram scanned at 366 nm, revealed 6 spots with 2 spot showing maximum composition at R_f 0.19 respectively **(Fig.10).** It is evident from the data that these are characteristic for the studied drug, which will help in identification and authentication of the drug. This can be considered as valuable standards in pharmacopoeia. At 254 nm, six spots appear at R_f 0.09, 0.16, 0.73,0.81, 0.92 and 0.98 (All brown) **(Fig. 9)** with various concentrations while at 366 nm, 6 spots appear at R_f, 0.13 (blue),0.19 (yellow), 0.37(blue), 0.50(blue), 0.79(blue) and 0.95(blue) **(Fig.11).** This is a vital finger print parameter to ensure the reliability and reproducibility of drug.

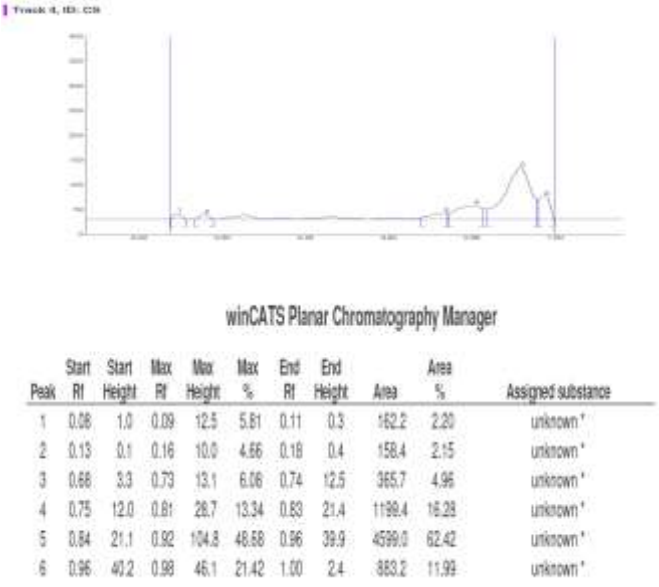

Fig:8. HPTLC densitogram of methanolic extract of *C. setosum* scanned at 254 nm by using chloroform: methanol (8: 2 v/v).

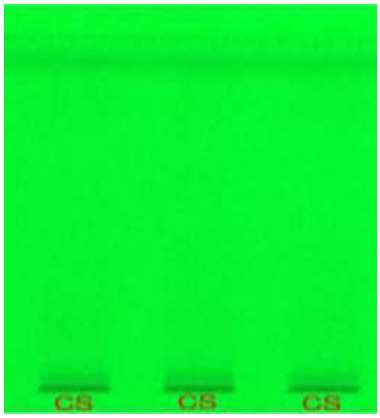

Fig:9. HPTLC image of *C. setosum* scanned at 254 nm by using chloroform: methanol (8: 2 v/v).

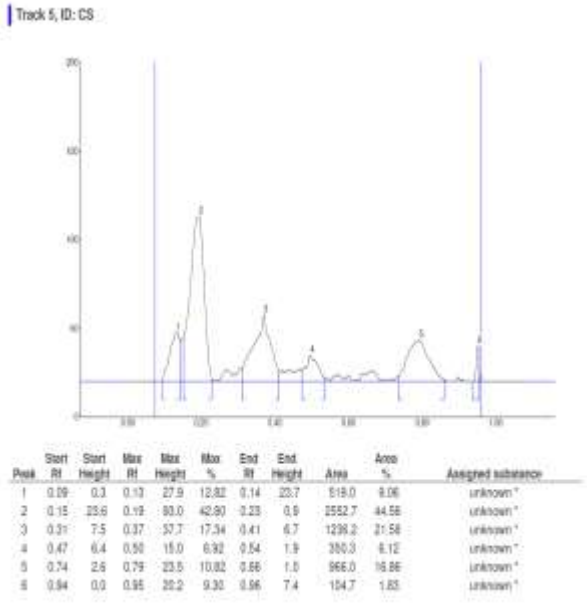

Fig:10. HPTLC densitogram of methanolic extract of *C. setosum* scanned at 366 nm by using chloroform: methanol (8: 2 v/v).

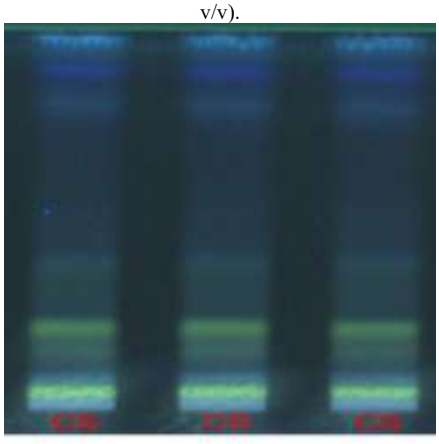

Fig :11. HPTLC image of *C. setosum* scanned at 366 nm by using chloroform: methanol (8: 2 v/v).

Fourier Transform Infrared Spectroscopy (FTIR)

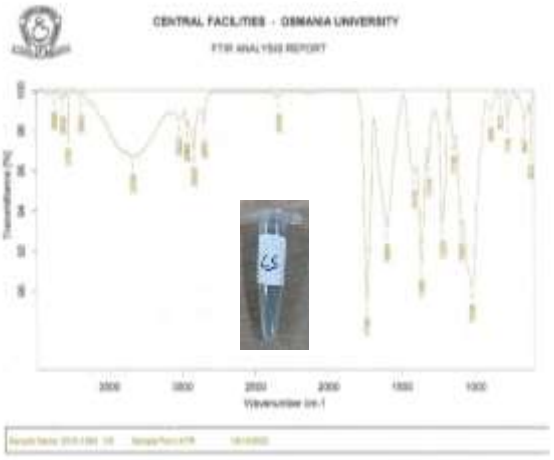

Fig-12. FT-IR Spectral analysis of *C.setosum*

Table:4 . FTIR analysis on Leaf extract of ***C. setosum***

Sr no.	Peek	Bonds	Bond strength	Bond vibrations	Functional groups
1.	3338	O-H (s)	Strong	Stretching	Hydroxyl
2.	3023	C-H (s)	Medium	Stretching	Alkene
3.	2968	C-H	Medium	Stretching	Alkane
4.	2920	C-H	Medium	Stretching	Alkane
5.	2851	C-H(s)	Medium	Bending	Alkane
6.	2342	C≡N	Weak	Sharp	Nitrile
7.	1740	C=O	Strong	Stretching	Aldehyde
8.	1604	C=C	Medium	Stretching	Aromatic
9.	1419	O-H	Medium	Bending	Alcohol
10.	1369	CH_2	Medium	Bending	Methyl
11.	1235	C-N	Medium	Stretching	Amine
12.	1026	C-O(s)	Strong	Stretching	Carbonyl

DISCUSSION

Through the development of analytical technology, access is provided to resources within the plant kingdom that have not been utilized up to this point, which enables the discovery of new natural medicines. It is possible that these resources could serve as a substitute for the synthetic substances that are now utilized by the medical community.

Cyphostemma setosum (Roxb.) Alston.is an herbaceous plant known for its folkloric treatment of ailments and belongs to the family Vitaceae (Cooke, 1903; Annon, 2008; Kirtikar &Basu ,2012). The macerated leaves are used as a poultice to promote suppuration and to aid in the extraction of the guinea worm (Behl, 1996), Antidiabetic potential (Jayachitra *et al.*, 2016). The toasted and oiled leaves are applied on tumors to bring them to suppuration (Viswanath *et al.*, 2018), invitro antioxidant and in vivo antiulcer activity (Chinnamaruthu *et al.*, 2013; Jayachitra, *et al.*, 2018).

The review of literature reveals lack of information in *C. setosum* However, information on pharmacognostic studies on Cissus in general and *C. quadrangularis* in particular is available (Austin *et al.*, 2004, Anon., 2011).

LEAF EPIDERMIS

EPIDERMAL CELL COMPLEX: Information on leaf epidermis in surface is scanty with epidermal cells in surface reported as polygonal with straight sides in *C. quadrangularis* (Austin *et al.*, 2004). Presently the epidermal cells are 5-8 sided. polygonal isodiametric and anisodiametric with thick sides, straight to curved on either sides. Epidermal cells in frequency are lesser 1615 per sq.mm., on adaxial while more in number 2634 per sq.mm., on abaxial

STOMATAL COMPLEX: Stomata have been reported as anomocytic in *C. quadrangularis* (Austin *et al.,* 2004, Anon., 2011) but in the species presently studied the stomata are mostly tetracytic, few anomocytic on either surface. Further, the subsidiaries are mostly of f-type, few a-type and rarely c-type. In frequency stomata are lesser 182 per sq.mm., on adaxial and 492 per sq.mm., on abaxial while the Stomatal index is 9.65 on adaxial and 16.5 on abaxial

TRICHOME COMPLEX: The trichomes have been reported as stalked, deciduous, pearl gland in *Cissus* (Metcalfe & Chalk. 1950), However, in the species presently studied, multiseriate capitate hair, occur all over on either sides conspicuously

LEAF ANATOMY: In T.S. leaf at midvein in *C. quadrangularis* have been reported as a small protuberance in the adaxial side and broader in the abaxial side, while keeled on adaxial and convexly rounded on abaxial side (Anon., 2011). In *C. setosum* presently studied, the leaf is slightly ridged as a cone towards adaxial side and prominently ribbed on the abaxial side. The midvein is 1630-1980 (1788) µm thick and the lamina 150-202 (168) µm thick

The epidermis has been reported as 1- layered made of rectangular to barrel shaped (Austin *et al.,* 2004) which is presently confirmed. The ground tissue at midvein has been reported to possess collenchyma and parenchyma tissues and centrally located vascular tissue in *C. quadrangularis* which is presently confirmed in the species studied. Further, the collenchyma has been reported as 2-6 layered on abaxial and adaxial sides in *C. quadrangularis* (Austin *et al.,* 2004) a group of collenchyma cells in the keel of *C. quadrangularis* (Anon., 2011) while presently in the species studied, collenchyma occurs as group of cells in adaxial and 1-2 layered on the abaxial side Further, the collenchyma cells are lamellar and angular The ground tissue parenchyma in the midvein is reported as cells which are large, thin walled with intercellular spaces, frequently interspersed with mucilage cells in *C. quadrangularis* (Austin *et al.,* 2004). The ground tissue of parenchymatous tissue made of thin-walled cells containing chloroplasts near the periphery in *C. quadrangularis* (Anon., 2011c). But in the species presently studied the parenchyma is quite extensive, made of 16-20 layered vertically and 20-26 layered laterally, often interspersed with raphide sacs and sphaerocrystalliferous idioblasts along with chloroplasts and aleurone grains.

The mesophyll has been described as homogenous or undifferentiated in *C. quadrangularis* (Austin *et al.,* 2004, Anon., 2011c) and also *C. setosum* similarly reported by Varghese, K, V., B., & M, V. (2022), which is presently confirmed in the species studied. Further, the mesophyll is often interspersed with sphaerocrystalliferous idioblasts as also reported earlier in *C. quadrangularis* (Austin *et al.,* 2004). However. large sized mucilage cells as observed in mesophyll region in *C. quadrangularis* 1s absent in species presently studied. The vascular tissue has been reported to possess a ring of 4-6 vascular bundles without bundle sheaths (Anon., 2011) while vascular bundle has been reported as single in the apical region 3-4 in the middle region 4-6 in the basal region. Further collenchymatous cap is reported over each bundle, vascular bundles are open, collateral and endarch (Austin *et al.,* 2004). In contrast in the species presently studied the midvein consists of 4-abaxial bundles in an arch and one adaxial bundle. The vascular bundles are open, conjoint, collateral and endarch and confirms the earlier studies (Austin *et al,* 2004). Tracheary elements in L.S. show mostly helical thickenings and few annular thickenings.

PETIOLE ANATOMY: Review of earlier literature reveals that, there is no information on the petiole anatomy of *C. setosum*. However, occurrence of raphides and druses were reported earlier in *Cissus hastata* and *Cissus rotundifolia* (Najmaddin *et al.*, 2013, Cody and Horner, 1983) which is presently confirmed in the *C. setosum*

In transaction, petiole is oval to sub spherical in outline, flat on adaxial with small ridges and furrows all over Epidermis is 1-layered with cells mostly barrel shaped, few tabular, oval to spherical. Ground tissue is heterogenous with collenchyma and parenchyma tissues. Hypodermis in ridged areas possesses a group of collenchyma cells which are angular with scanty contents. Cortex is extensive with parenchyma, consisting of cells which are polygonal, oval to spherical and dense contents dense in some with inulin crystals as also reported earlier in *C. hastata* (Najmaddin *et al,* 2013) and in *C. rotundifolia* (Cody and Horner, 1983).

Vascular tissue consists of 11-22 small linear v bundles in a ring and one central bundle towards adaxial side. The vascular bundles are conjoint, collateral, endarch. consists of tracheary elements arranged in radial rows. Phloem is scanty, mostly towards exterior and a small amount of phloem inside as internal phloem. The tracheary elements in L.S. mostly show helical & scalariform thickenings.

C. setosum produces major phytochemicals such as, alkaloids, tannins, saponins, carotenoids, terpenoids and around 26 bioactive components were reported (Hemayet *et al.,2012,* Gopalakrishnan *et al.,* 2014), The phytochemical tests revealed the presence of alkaloids, tannins, flavonoids, carbohydrates, proteins, gums and quinones (Varghese, 2022). The current study employed the Soxhlet extraction method to perform both a qualitative and quantitative phytochemical analysis. Phytochemicals found to be present included alkaloids, flavonoids, saponins, terpenoids, steroids, quinones, anthraquinones, cardiac glycosides, leuco anthocyanins, fixed oils, and resins. A range of solvents were used to create the crude extracts.

The petroleum ether solvent yielded the lowest percentage yield (5.2% yield), whereas the ethyl acetate solvent provided the highest percentage yield (48.5% yield).

The methanol extract of the *C. setosum* leaves an examination of the fingerprints was carried out using high-performance thin-layer chromatography (HPTLC). At a wavelength of 254 nm, there were a total of six spots that were identified, with spot two (R_f 0.92) producing the highest concentration of 62.42 percent, as indicated by the data. There were additional six spots that were discovered at a wavelength of 366 nm, with spot two (R_f 0.19) having a maximum concentration of 44.56 percent. These marks have the potential to serve as biomarkers and fingerprint parameters for the purpose of identifying plants.

CONCLUSION

In conclusion, the extensive array of analyses conducted on *Cyphostemma setosum*, including pharmacognostic evaluation, qualitative and quantitative phytochemical analyses, as well as HPTLC and FTIR studies, collectively highlight the immense medicinal significance of this plant. The thorough examination of its morphological, microscopic, and physicochemical characteristics not only ensures its proper identification but also lays the groundwork for its standardization in medicinal preparations, ensuring consistency and efficacy.

The qualitative analysis revealed the presence of a diverse range of bioactive compounds such as alkaloids, flavonoids, phenols, tannins, and terpenoids, all of which are known for their various pharmacological activities. Furthermore, the quantitative assessment provided valuable insights into the relative abundance of these compounds, offering crucial information for dosage determination and formulation development.

The application of advanced analytical techniques like HPTLC allowed for the precise separation and identification of individual chemical constituents within *Cyphostemma setosum*, while FTIR spectroscopy provided deeper insights into their molecular structures and functional groups. This comprehensive chemical profiling not only confirms the plant's rich phytochemical composition but also sheds light on the potential synergistic interactions among its bioactive components, which may contribute to its therapeutic efficacy.

Overall, the findings of these studies underscore the promising medicinal potential of *Cyphostemma setosum*. Its diverse array of bioactive compounds, coupled with the elucidation of their chemical profiles, suggests a wide range of therapeutic

applications, including but not limited to antioxidant, anti-inflammatory, antimicrobial, and anticancer activities. Consequently, further research and development efforts are warranted to explore and harness the full therapeutic capabilities of this botanical resource, paving the way for its integration into modern healthcare practices and pharmaceutical formulations.

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REFERENCES

- Gajula, P., Palakurthy, K., & Kusuma, S. (2022). Pharmacognostic studies on leaves of *Gymnanthemum amygdalinum* with special reference to a new addition to the flora of south India. *Indian Drugs*, *59*(6).
- Sharma PV. (2013). Dravyaguna-vigyana, Chaukhambha Bharati academy Varanasi.;1:142
- Pratap GP, Husain MK, Kazmi MH, Sudarshsanam G, Prasad GP. (2018). Ethno-medico documentation of medicinal plants in Madanapalle mandal of Chittoor District, Andhra Pradesh. Indian J Ayurveda Res.;1:11-8.
- Pratap GP, Prasad GP, Sudarshanam G.(2009a). Ethno Medical Studies In Talakona Forest Range of Chittor District, Andhra Pradesh. Anc Sci Life. a;28(3):42-9. PMID 22557321. PMCID PMC3336320.
- Pratap GP, Prasad GP, Sudarsanam G.(2009b). Ethno medical studies in Kailasagirikona forest range of Chittoor district, Andhra Pradesh. Anc Sci Life. Oct;29(2):40-5. PMID 22557350. PMCID PMC3336314.
- Sekhar J, Pratap GP, Sudarsanam G, Prasad GP.(2011). Data on herbal remedies from the tribes of Chittoor District, Andhra Pradesh in India. Life Sci Leaf.;17:621-30.
- Pratap GP, Sudarsanam G, Prasad GP.(2011). Ethn Inf Treat Snake Bites Kadapa Dist Andhra Pradesh. 12:368-75.
- Hardy K .(2021). Paleomedicine and the evolutionary context of medicinal plant use. Rev Bras Farmacogn 31:1–15. https://doi.org/10.1007/s43450-020-00107-4.
- Alamgir ANM .(2018). Molecular pharmacognosy. A new borderline discipline between molecular biology and pharmacognosy. In: Rainsford KD (ed) Therapeutic use of medicinal plants and their extracts: Volume 2. Phytochemistry and bioactive compounds. Progress in Drug Research, vol 74. Springer, Cham, pp. 665–720. https://doi.org/10.1007/978-3-319-92387-1_8
- Yang J, Jia M, Guo J .(2019). Functional genome of medicinal plants. In: Huang L-Q (ed) Molecular pharmacognosy. Springer, Singapore, pp 191–234. https://doi.org/10.1007/978-981-32-9034-1_7.
- Allard PM, Bisson J, Azzollini A, Pauli GF, Cordell GA, Wolfender JL .(2018). Pharmacognosy in the digital era: shifting to contextualized metabolomics. Curr Opin Biotechnol 54:57–64. https://doi.org/10.1016/j.copbio.2018.02.010
- Gesto-Borroto R, Medina-Jiménez K, Lorence A, Villarreal ML .(2021). Application of DNA barcoding for quality control of herbal drugs and their phytopharmaceuticals. Rev Bras Farmacogn 31:127–141. https://doi.org/10.1007/s43450-021-00128-7.
- Yadav, M. (2017). Herbal drugs and phytoconstituents useful for the management of diabetes. International Journal of Green Pharmacy *(*IJGP), 11(01).
- Mahady GB. (2001). Global harmonization of herbal health claims. J Nutr. ;131:1120S–1123S.
- Pullaiah T.(2006). Encyclopedia of world medicinal plants, 2nd. Vol. Regency Publication, New Delhi. p. 569.
- Sujatha, G. and Mariya Selvam, S. (2015). Ethnomedicinal survey of flora used by Chettiapatti inhabitants, Manapparai. Asian Journal of Biochemical and Pharmaceutical Research 4(5): 16-31.
- Nandagopalan, V., S.P. Anand, A. Lakshmi Prabha, U. Selvakumar and A. Doss .(2011). An ethnobotanical study in the Pudukkottai District, South India. Asian Journal of Experimental Biological Science 2(3): 412-421.
- Durairaj, R. and P. Annamalai, (2013). Studies on medicinal plants of Koradacheri village, Kodavasal Taluk, Thiruvarur district, Tamil Nadu, India. International Journal of Pharmacy 4(10): 99-107.
- Vaidyanathan, D., M.S. Salai Senthilkumar and M. Ghouse Basha (2013). Studies on ethnomedicinal plants used by Malayali tribals in Kolli hills of Eastern Ghats, Tamil Nadu, India. Asian Journal of Plant Science and Research 3(6): 29-45.
- Datta, S.C., (2009). Systematic Botany, 4th Ed. New Age International (P) Limited, Publishers, New Delhi.
- Shanmugam, S., K. Rajendran and K. Suresh (2012). Traditional uses of medicinal plants among the rural people in Sivagangai district of Tamil Nadu, Southern India. Asian Pacific Journal of Tropical Biomedicine 2(1): S429-S434.
- Salai Senthilkumar, M.S., D. Vaidyanathan, N. Sisubalan and M. Ghouse Basha (2014). Medicinal plants using traditional healers and Malayali tribes in Jawadhu hills of Eastern Ghats, Tamil Nadu, India. Advances in Applied Science Research 5(2): 292-304.
- Govindarajan, Nartunai & Reddy, P.M.K. & Rajendran, N.N. & Shewade, Deepak. (2003). Antinociceptive effect of the extract of Cyphostemma setosum (Roxb.) Alston as tested by formalin assay. 40. 96-98.
- Nallella Sreeramulu, Sateesh Suthari, A Ragan and Vatsavaya S Raju (2013). Ethno-botanico-medicine for common human ailments in Nalgonda and Warangal districts of Telangana, Andhra Pradesh, India. Annals of Plant Sciences, 2013, 02 (07), 220-229.
- Prabhakar, G., Kamalakar, P., & Shailaja, K. (2015). Indigenous Knowledge on Medicinal Plants Prevalent in Cure Chronic Disorders in Chityal Nalgonda District Telangana India. Int. J. Sci. Res, 4, 773-777.
- Chinnamaruthu J, Marimuthu J, Krishnamoorthy K, Subramania MP. (2013). Estimation of total phenolics, flavonoids and tannin contents and evaluation of invitro antioxidant properties of *Cissus setosa* Roxb. Int J Curr Pharm Res.; 5:63-7.
- Jayachitra C, Jamuna S, Ali MA, Paulsamy S, Al-Hemaid FM. (2018). Evaluation of traditional medicinal plant, *Cissus setosa* Roxb. (Vitaceae)for anti-ulcer property. Saudi J BiolSci.;25:293-7.
- Nafuka SN, Mumbengegwi DR. (2013). Phytochemical analysis and in vitro anti-plasmodial activity of selected ethnomedicinal plants used to treat malaria associated symptoms in Northern Namibia. Int Sci Technol J Namibia. ;**2**: 78–93.
- Chute, G.S. and Tiwari, V.J., (1999). Indigenous ethnomedicinal plants used by tribal people of Bhandara and Gadchiroli districts of Maharashtra state. Indian J. Nat. Prod. 15(1), 3-8.
- Murthy, E.N., Reddy, C.S., Reddy, K.N. and Raju V.S., (2007). Plants used in ethno veterinary practices by Koyas of Pakhal wildlife sanctuary, Andhra Pradesh, India. Ethnobotanical Leaflets, 11: 1-5.
- Raman, N. (2006). Phytochemical Techniques. New India publishing Agency, NewDelhi.
- Behl PN, Captain RM, Bedi BMS, Gupta S. (1966). Skin-irritant and Sensitizing Plants Found in India, New Delhi. PN Behl, Irwin Hospital;
- Jayachitra, C., Jamuna, S. and Paulsamy, S.(2016). In vitro antidiabetic potential of traditional medicinal climber, *Cyphostemma setosum* (roxb.) Alston.International Journal of Recent Advances in Multidisciplinary ResearchVol. 03, Issue 07, pp.1627-1629, July, 2016.

- Viswanath K., Binoy Varghese Cherianand M. Vijey Aanandhi. (2018). *Vitis setosa*: An ethnobotanical review. Drug Invention Today.; 10:1050-1052.
- Chinnamaruthu J, Marimuthu J, Krishnamoorthy K, Subramania MP. (2013). Estimation of total phenolics, flavonoids and tannin contents and evaluation of invitro antioxidant properties of *Cissus setosa* Roxb. Int J Curr Pharm Res.; 5: 63-7.
- Jayachitra C, Jamuna S, Ali MA, Paulsamy S, Al-Hemaid FM. (2018). Evaluation of traditional medicinal plant, *Cissus setosa* Roxb (Vitaceae)for anti-ulcer property. SaudiJ Biol Sci.; 25: 293-7.
- Varghese, K, V., B., & M, V. (2022). Pharmacognostical, preliminary phytochemical and in vitro antioxidant evaluation of the aerial parts of *Cyphostemma setosum* (roxb.). Indian Drugs. https://doi.org/10.53879/id.58.12.12439.
- Hemayet H, Islam HS, Kanti DS, Shibam M, Arpona H, Arif A. (2012). Evaluation of phytochemical screening and anti-nociceptive properties of teeth an Olic extract of *Vitis setosa* leaves. Glob J Pharm Res. 2012; 1: 637-7.2.
- Gopalakrishnan R, Manikandan P, Bhuvaneswari R.(2014). Anti-microbial potential and bioactive constituents from aerial parts of *Vitis setosa* . Wall. JM ed Plant Res.; 8: 454-60.
- Fisher DD, (1968). Protein staining of ribboned epon section for light Microscopy. Histochem. 16:81-96
- Gahan PB, (1984). Plant Histochemistry and Cytochemistry: An Introduction Academic press, Florida, U.S.A.
- Anonymous, (2015). Quality Standards of Indian Medicinal Plants, Medicinal Plants Division, Indian Council of Medicinal Research, New Delhi. vol, 13, 329-330.
- Gajula, P., Palakurthy, K. and Kusuma, S., (2022). Pharmacognostic Studies on Leaves of *Gymnanthemum amygdalinum* with Special Reference to A New Addition to The Flora of South India. Indian Drugs, 59(6).
- Kamalakar, P., & Shailaja, G. P. K. (2014). Phytochemical screening and TLC profiling of seeds of *Crotalaria verrucosa* Linn. International Journal of Scientific Research, *3*(9), 25-30.
- Kokate CK, (1999). Practical Pharmacognosy. Vallabh Prakashan Publication, New Delhi, India. 4th Edn.
- Ramakrishnan S, Prasannan KG and Rajan R, (1994). Textbook of medicinal biochemistry orient Longman, New Delhi, India.
- Ruthmann AC, (1970). Methods in cell research, Cornell University Press, New York, U.S.A.
- Sasikumar R and Balasubramanian P, Govindaraj P and Krishnaveni T,(2014). Preliminary Studies on Phytochemicals and antimicrobial activity of solvent Extract of Coriandrum sativum L. roots, Journal of Pharmacognosy and Phytochemistry. 2, 6: 74-78.
- Trease GE, and Evans WC, (1982). Pharmacognosy. Baillene Tindal, London. P -149.
- Wagner XS, Bladt Z Gain and Suie EM, 1996. Plant drug analysis. Springer Verlag, Berlin, Germany. 360.
- Yasuma A and Ichikawa, (1953). Ninhydrin-schiff and alloxan- Schiff staining. A new histochemical staining method for proteins J.Lab clin Med.41: 296-299.
- Kumar A, P N, Kumar M, Jose A, Tomer V, Oz E, Proestos C, Zeng M, Elobeid T, K S, Oz F. (2023). Major Phytochemicals: Recent Advances in Health Benefits and Extraction Method. Molecules. Jan 16;28(2):887. doi: 10.3390/molecules28020887. PMID: 36677944; PMCID: PMC9862941.
- Singh, I. S., & Kwalyambumu, D. S. (2024). Phytochemical analysis and antioxidant potential of Pseudolachnostylis maprouneifolia. Journal of Phytology, 16, 49–63. https://doi.org/10.25081/jp.2024.v16.8762.
- Adil, M., Filimban, F.Z., Ambrin (2024). Phytochemical screening, HPLC analysis, antimicrobial and antioxidant effect of Euphorbia parviflora L. (Euphorbiaceae Juss.). Sci Rep 14, 5627 .https://doi.org/10.1038/s41598-024-55905
- Tan, P.V. (2018). The Determination of Total Alkaloid, Polyphenol, Flavonoid and Saponin Contents of Pogang gan (Curcuma sp.). International Journal of Biology.
- Kalita Pallab, Barman Tapan K., Pal Tapas K., Kalita Ramen.(2013). Estimation of total flavonoids content (tfc) and anti-oxidant activities of methanolic whole plant extract of *Biophytum sensitivum* linn. Journal of Drug Delivery & Therapeutics; 2013, 3(4), 33-37.
- Shubhangi A. Patil, Pratibha S. Salve, Rohan S. Phatak, Niranjan D. Chivate. (2023). Quantitative Estimation of Total Phenolic, Total Flavonoid content and Assessment of In-Vitro Antioxidant Capacity of Psidium guajava L. Leaves Extracts. Research Journal of Pharmacy and Technology; 16(3):1028-2. doi: 10.52711/0974-360X.2023.00172.
- Ahmed, W., Azmat, R., Mehmood, A., Qayyum, A., Ahmed, R., Khan, S.U., Liaquat, M., Naz, S. and Ahmad, S. (2021). The analysis of new higher operative bioactive compounds and chemical functional group from herbal plants through UF-HPLC-DAD and Fourier transform infrared spectroscopy methods and their biological activity with antioxidant potential process as future green chemical assay. Arabian Journal of Chemistry, 14(2), p.102935.
- Bhargava, A., Shrivastava, P. & Tilwari, A. (2021). HPTLC analysis of Fumaria parviflora (Lam.) methanolic extract of whole plant. Futur J Pharm Sci 7, 1 https://doi.org/10.1186/s43094-020-00150-x.
- Ananya M, Nikhita Mirekar, Sana Iddalagi, Narayanachar, & Vijay Danapur. (2024). HPTLC Fingerprint Profile and Standardization of Plantago ovata Forssk. International Journal of Pharmaceutical and Bio Medical Science, 4(1), 37–43. https://doi.org/10.47191/ijpbms/v4-i1-06.
- Cook, T. (1903). The flora of the Presidency of Bombay, vol.1.254.
- Anon., (2008).Reviews on Indian Medicinal Plants, ICMR, New Delhi, vol.6, 408.
- Kirtikar, KR and Basu B.D.2012. Indian Medicinal plants, Bishen Singh Mahendra Pal Singh, Dehradun.
- Johansen D.A (1990).Plant microtechnique, McGraw Hill. Book Co. New York, pp.523.
- Pandya DJ, Desai TR, Nadpara NP, Mehta HA, Modi AM(2010). Pharmacognostic study and establishment of quality parameters of leaves of Bombax insigne Linn. Int J Pharmacogn Phytochem Res.;2(3):1-5.
- Kokate, CK. (2005). Practical pharmacognosy. New Delhi: Vallabh Prakashan.
- Ali M (2008). Textbook of pharmacognosy. New Delhi: CBSPublishers and Distributors;.Al-snafi A. E. (2016).The Constituents and Pharmacology of *Corchorus aestuans*-A Review. Pharm. Chem. J. 3:208–214.
- McVeigh I. A. (1935). simple method for bleaching leaves. Stain Technol. ;10(1): 33-4.doi: 10.3109/10520293509116004.
- Gokhale SB, Kokate CK. (2008). Practical pharmacognosy. 12th ed. Pune: Nirali Prakashan.
- Khandelwal KR .(2007). Practical pharmacognosy. 18th ed. Pune: Nirali publication.
- Thitikornpong W, Phadungcharoen T, Sukrong S .(2011).Pharmacognostic evaluations of *Lagerstroemia speciosa* leaves. J Med Plants Res. 5(8):1330-7.
- Kumar D, Kumar K, Kumar S, Kumar T, Kumar A, Prakash O. (2012). Pharmacognostic evaluation of leaf and root bark of Holoptelea integrifolia Roxb. Asian Pac J Trop Biomed. 2(3):169-75. doi: 10.1016/S2221-1691(12)60036-7.
- Khan SA, Ibrar M, Barkatullah. (2016). Pharmacognostic evaluation of the leaf of Rhus succedanea var.

Himalaica. J. D. Hooker. Afr J Tradit Complement Altern Med. 13(6):107-20.

- Karuppaiyan R, Nandini K. (2006). Techniques in Physiology, Anatomy, Cytology and Histochemistry of Plants, Publisher- Kerala Agricultural University, Vellanikkara, Thrissur, 1st ed. Vol. 28.
- Cuéllar-Cruz M, Pérez KS, Mendoza ME, Moreno A. Biocr ystals in plants. (2020). A shor t review on biomineralization processes and the role of phototropins into the uptake of calcium. Cr ystals. 10(7):1-23. doi: 10.3390/cryst10070591.
- Mazen AMA, Zhang D, Franceschi VR .(2003). Calcium oxalate formation in Lemma minor: physiological and ultr astr uctur al aspects of high-capacity calcium sequestration. New Phytol. 2003;161(2):435-48. doi:10.1111/j.1469- 8137.2004.00923.x.
- Anon., (2011).The Siddha Pharmacopoeia of India, New Delhi, part- I first edition, vol.II, 64-66, 106-108.
- Austin, A. Kannan R, and Jegadeesan M. (2004). Pharmacognostical studies on Cissus quandragularis L. variant I&II. Ancient Sci.Life, vol.XXIII(4); 33-47.
- Metcalfe CR and Chalk L. (1950). Anatomy of the Dicotyledons, Vol.I 7II, Claredon Press, Oxford.
- Najmaddin, C., Hussin, K. and Maideen, H., (2013). Comparative leaf anatomy of selected species in Vitaceae and Leeaceae. American Journal of Applied Sciences, 10(4): 414-417.
- Cody, A.M. and Horner, T., (1983). Twin raphides in the Vitaceae and Araceae and a model for their growth. Bot. Gaz., 144(3): 318-330.