RESEARCH PAPER

Pharmacognostical Study and Evaluation of Antioxidant Activities of Gardenia jasminoides

Anjali Chaudhary[1] and Shahbaz Khan[2*]
[1] *Research Scholar, Faculty of Pharmacy, IFTM University, Moradabad, 244102 U.P., India*
[2] *Pharmacy Academy, Faculty of Pharmacy, IFTM University, Moradabad, 244102 U.P., India*
[*] ***Corresponding Author:*** *Dr. Shahbaz Khan*
Pharmacy Academy, Faculty of Pharmacy, IFTM University, Moradabad, 244102 UP, India
shahbazkhanmaju123@gmail.com

Received: 28th Feb, 2026; Revised: 6th March 2026; Accepted: 7th April, 2026; Available Online: 20th April, 2026

ABSTRACT
The present study was conducted to evaluate the pharmacognostical characteristics, phytochemical constituents, and antioxidant potential of the leaves of Gardenia jasminoides. Detailed macroscopic and microscopic examinations were carried out for proper identification and standardization of the plant material. Quantitative microscopy revealed significant diagnostic parameters such as palisade ratio, stomatal index, vein-islet number, and vein termination number. Chemo-microscopic studies confirmed the presence of starch grains, proteins, lignin, fats, and calcium oxalate crystals. Physicochemical evaluation showed acceptable ash values, extractive values, and moisture content, indicating the purity and quality of the crude drug. Successive extraction was performed using petroleum ether, ethyl acetate, ethanol, and water solvents, among which the ethanolic extract showed the highest percentage yield and maximum phytochemical constituents, including alkaloids, flavonoids, steroids, glycosides, and terpenoids. TLC and HPTLC fingerprint profiling confirmed the presence of several bioactive compounds. The antioxidant activity of the extracts was evaluated using DPPH and ABTS radical scavenging assays. The ethanolic extract demonstrated the strongest antioxidant activity with lower IC_{50} values, comparable to ascorbic acid, indicating the presence of potent phenolic and flavonoid compounds. The findings of this study support the pharmacognostic standardization and therapeutic potential of Gardenia jasminoides leaves as a natural antioxidant source.
Keywords: *Gardenia jasminoides, Pharmacognostical evaluation, Antioxidant activity, Phytochemical screening, HPTLC profiling*
How to cite this article: Chaudhary A, Khan S. Pharmacognostical Study and Evaluation of Antioxidant Activities of Gardenia jasminoides. Int J Drug Deliv Technol. 2026;16(52s): 226-237. DOI: 10.25258/ijddt.16.52s.26
Source of support: Nil.
Conflict of interest: None

INTRODUCTION

Medicinal plants have been widely utilized since ancient times for the prevention and treatment of various diseases because of their therapeutic efficacy and minimal side effects. Natural products derived from plants remain an important source of biologically active compounds used in traditional as well as modern medicine. Among these medicinal plants, *Gardenia jasminoides* Ellis, belonging to the family Rubiaceae, is well known for its ornamental, medicinal, and pharmacological importance. The plant is traditionally used in several Asian countries for the treatment of inflammation, fever, liver disorders, infections, and oxidative stress-related diseases. Different parts of the plant contain numerous phytoconstituents such as flavonoids, glycosides, terpenoids, alkaloids, and phenolic compounds that contribute to its therapeutic activities **[1]**.

Pharmacognostic standardization of medicinal plants is an essential step for ensuring the identity, purity, quality, and safety of crude herbal drugs. Detailed macroscopic, microscopic, physicochemical, and phytochemical studies help in the authentication of plant materials and detection of adulteration. In addition, antioxidant studies are important because oxidative stress caused by free radicals is associated with the development of several chronic disorders, including cardiovascular diseases, cancer, diabetes, and neurodegenerative disorders **[2]**.

Therefore, the present study was undertaken to perform a comprehensive pharmacognostical evaluation and phytochemical investigation of *Gardenia jasminoides* leaves along with the assessment of their antioxidant potential using DPPH and ABTS radical scavenging assays. The study aims to establish standard identification parameters and explore the therapeutic significance of the plant as a potential natural antioxidant source **[3]**.

MATERIALS AND METHODS

Identification, collection and authentication of plant material

The leaves of the plant *Gardenia jasminoides* were collected in March 2022 from the Lodhipur region of Uttar Pradesh, District- Moradabad. The plant material was washed and air-dried. Authentication was done by the Scientist in Charge, CSIR – National Institute of Science

**Author for Correspondence: shahbazkhanmaju123@gmail.com*

Communication and Policy Research, New Delhi, India. A voucher specimen (Authentication NO.- NIScPR/RHMD/Consult/2021/3971-72-2) was submitted to the department. The authentication letter were shown in Annex no. 1.

PHARMACOGNOSTICAL STUDY OF GARDENIA JASMINOIDES LEAVES

Macroscopic examination of leaves

The fresh leaves of *Gardenia jasminoides* were visually examined. The organoleptic properties, such as colour, odour and taste of the plant material, were observed and noted. The macroscopic characters of the leaves, which include the type of margin, venation, base, shape, size, apex, midrib, lamina, and presence or absence of petiole, were evaluated based on standard protocol **[4]**.

MICROSCOPIC EXAMINATION OF LEAVES

Quantitative microscopy

- **Palisade ratio:** A piece of leaf (2mm thick) was clarified by boiling with chloral hydrate solution for 5 minutes. It was mounted, and four cells of the epidermis were traced. By focusing down to the palisade layer, sufficient cells were traced off to cover the epidermal cells. The numbers of palisade cells under the four epidermal cells were counted. A range of different parts of the leaf was focused on, traced, and the average was calculated to get the palisade ratio of the leaf **[5]**
- **Stomata number and index**: A piece of leaf (middle part) was clarified by boiling with chloral hydrate solution for 5 minutes. The upper and lower epidermis were peeled separately. The peeled epidermis was placed on a glass slide and mounted with glycerine water. A stage micrometer (3"x 1"dimension and calibrated with a 1 mm scale, subdivided into 0.1 mm) was attached to the stage of the microscope. The prepared slide was placed on the stage, and epidermal cells with at least half of their area lying within the square were noted. The photomicrograph was taken with the aid of a digital microscopic eyepiece attached to a microscope. The number of stomata was counted for four different parts, and the average taken represents the number **[6].**

The stomata index was calculated using the formula below:

Stomata Index= S x 100/E + S

S = number of stomata per unit area

E = number of ordinary epidermal cells (including trichomes) in the same unit area.

- **Vein-islet number:** A piece of leaf was cleared by boiling in chloral hydrate solution. With the aid of a stage micrometer, one square millimeter was drawn. The cleared leaf was mounted on a glass slide, and a drop of glycerin was added, then covered with a cover slip. The prepared slide was placed on the stage of the microscope. The average number of vein-islets from four squares was found, and an average number of vein-islets was calculated **[6]**.
- **Vein termination number:** The average number of vein terminations present within a square was counted from four different squares to get the value for one square millimeter **[7]**.
- **Transverse section of leaf:** The leaf sample was studied microscopically by taking transverse section (T.S.) via the midrib with a small portion of lamina and thin section, which was double stained with hematoxylin and saffranin. The stained sections were observed under a compound microscope, and photos were taken with the aid of the photographic microscope.
- **Powder Microscopy:** The powder sample was also mounted in different reagents, and cellular diagnostic and diagnostic cell inclusions were observed. Moreover, the presence or absence of the following were observed: epidermal cells (upper and lower), epidermal hairs (type of trichomes and distribution), xylem, phloem, stomata (type and distribution) and collenchyma. A small quantity of the powdered leaves was also cleared, mounted and observed using a binocular compound microscope fitted to a digital microscopic eyepiece **[8]**.

Chemo-microscopic examination

Chemo-microscopic examination was carried out to determine the presence or absence of starch grain, protein, lignin, fats/oil, calcium carbonate and calcium oxalate crystals using standard techniques **[9]**.

Determination of analytical standards

Analytical standards and physicochemical constants of the leaf were determined to evaluate the quality and purity of the drug. The total ash value, water-insoluble ash value, acid-insoluble ash value, sulphated ash value, extractive values and moisture content **[10]**.

Extraction of Plant Material

The leaves of the plant *Gardenia jasminoides* were dried at room temperature, crushed into a coarse powder and passed through sieve No. 18. The Powdered plant was defatted with petroleum ether (40-60%) to remove fatty material and other pigmentation. The defatted dried plant powder was further extracted successively with ethyl acetate, ethanol and water by using a Soxhlet apparatus. The obtained extracts were concentrated to dryness in a rotatory evaporator until all the traces of solvent were removed. The extracts were stored in a refrigerator at a temperature of 2-8°C **[11]**.

The % yield was calculated as $\% \; yield = \frac{Observed\ yield\ (g)}{Total\ weight\ of\ dry\ powder\ (g)} \times 100$

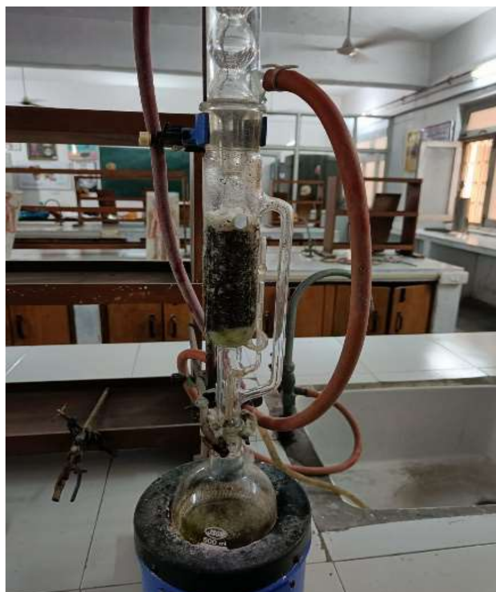

Figure 1: Extraction process of Plant Material

Preliminary Phytochemical (qualitative) screening of the extracts

Preliminary qualitative tests were conducted on crude drug extracts in petroleum ether (60-80ºC), ethyl acetate, ethanolic and aqueous extracts to determine the presence or absence of phytoconstituents such as proteins, carbohydrates, amino acids, steroids, glycosides, saponins, alkaloids, tannins, flavonoids, and phenolic compounds. According to the recommended procedures, the presence or absence of several phytoconstituents was identified and recorded **[12-13].**

Thin Layer Chromatography

A development solvent should not react chemically with the substance in the mixture under examination. Carcinogenic solvents (benzene, etc.) should always be avoided. Commonly used solvents are petroleum ether, carbon tetra chloride, pyridine, diethyl ether, chloroform, methanol, ethyl acetate, acetone, etc. were used for Petroleum ether, ethyl acetate, ethanolic and aqueous extract of *Gardenia jasminoides* **[14]**. After visualizing the spots, the distance travelled by the unknown substance and solvent front was measured and put in the following formula to calculate the R_f values:

$$\mathbf{Rf} = \frac{\text{Distance travelled by the spots}}{\text{Distance travelled by solvent from origin front}}$$

High-Performance Thin Layer Chromatography (HPTLC) Profiling

HPTLC fingerprint profiling studies of petroleum ether extract, ethyl acetate extract, ethanolic extract and aqueous extract were carried out separately, following standard methodology **[15].**

Antioxidant scavenging assay

The antioxidant activity of the ethanolic leaves extracts of *Gardenia jasminoides* were evaluated using DPPH and ABTS assay methods.

Determination of antioxidant activity of *Gardenia jasminoides* leaves by DPPH assay.

The free radical scavenging activity of different leaf extracts of the *Gardenia jasminoides* plant was measured by 1, 1- diphenyl-2-picryl hydrazyl (DPPH) **[16]**. Briefly, 0.1 mM solution of DPPH was prepared by dissolving 0.004 g of DPPH crystalline solid in 100 mL of analytical grade methanol and stored at 4 °C. A 4 mg of the plant extract was dissolved in 10 mL of methanol to prepare 400 µg/mL stock solutions and then serial dilution with methanol was performed to prepare the required concentrated solutions (5, 10,15, 20, 25, 30 µg/mL). 2 mL of plant extract solution from each concentration was taken in a test tube and then, 3 mL of DPPH solution was added in each test tube. After 30 min incubation in the dark, the absorbance at 517 nm was recorded using a UV–Vis Spectrophotometer. The reference standard compound being used is ascorbic acid. A stock solution of 800 µg/mL was prepared by dissolving 2 mg of ascorbic acid in 2.5 mL of distilled water **[17]**.

Then, serial dilution with different concentrated solutions was prepared (5, 10,15, 20, 25, 30 µg/mL). MeOH were used as the blank for respective extracts. A mixture of 3 mL of 0.1 mM DPPH and 100 µL of EtOH for ethanol extract was used as a control. All determinations were performed in triplicate. The percentage of inhibition was plotted against the concentration from which IC_{50} values were calculated.

$$\text{DPPH \% Inhibition} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where A control is the mixture of methanol/ethanol/ water and DPPH solution, and A sample is the mixture of sample extract and DPPH solution.

Determination of antioxidant activity of *Gardenia jasminoides* leaves by ABTS assay.

The ABTS assay was carried out using the procedure used in the previous study. ABTS radical cation was prepared by mixing 7 mM ABTS stock solution with 2.45 mM potassium persulfate in equal quantities **[18]**. Briefly, 7 mM ABTS solution was prepared by dissolving 0.360 g of ABTS salt in 100 mL of distilled water **[19]**. A 2.45 mM potassium persulfate was prepared by dissolving 0.066 g of salt in 100 mL of distilled water. Then, the ABTS cation radical solution was prepared by gently mixing 10 mL of 7 mM ABTS solution and 10 mL of 2.45 mM of potassium persulfate solution. The mixture was left in the dark at room temperature for 12 hours until the reaction was completed and the absorbance was stable.

The radical cation formed is further diluted in the ratio (1:1) with ethanol to adjust the absorbance value to 0.700 at 734 nm using a UV–Vis Spectrophotometer. A 5 µL of *Gardenia jasminoides* leaf extract at concentrations (5, 10,15, 20, 25, 30 µg/mL) was mixed with 4000 µL of ABTS solution and allowed to stand in the dark for 2 h at room temperature. The absorbance was determined at 734 nm using a UV–Vis Spectrophotometer. Ethanol was used as the blank for ethanol extract, respectively. A mixture of 10 mL of (7 mM ABTS, 2.45 mM K2S2O8) and 20 mL of ethanol for ethanol extract was used as a control **[20]**.

The reactivity of the various concentrations of each solvent extract was compared to that of ascorbic acid. All the measurements were carried out at least three times. The percentage scavenging of ABTS + radical was calculated for different concentrations (5 to 30 µg/mL) of extract and standard using the following equation:

$$\text{ABTS \% Scavenging} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Where $A_{control}$ is the absorbance of mixtures of 10 mL of (7 mM ABTS, 2.45 mM K2S2O8) with blank solvents and A_{sample} is the absorbance of the mixture of sample extract/ standard and ABTS.

Statistical Analysis

All the data were presented in terms of average ± SD. The mean differences between the different classes were calculated with the help of one-way ANOVA (MS-Excel 2019) and considered significant if $p < 0.05$.

RESULT AND DISCUSSION

Macroscopic Characteristics of *Gardenia jasminoides*

The macroscopic or gross morphological features of *G. jasminoides* are essential for its proper identification and authentication in traditional medicine and pharmacognosy. The plant exhibits distinct characteristics in its leaves, flowers, fruits, stems, and roots, which are described below in detail.

Table 1: Plant parts and their characteristic features

Plant Part	Characteristic Features
Leaves	Opposite or in whorls of three; elliptic to oblong or ovate; 5–15 cm long, 3–6 cm wide; glossy dark green upper surface; thick and leathery texture; prominent midrib.
Flowers	Solitary and terminal or axillary; funnel-shaped; 5–9 white petals turning creamy yellow with age; 5–10 cm in diameter; strongly aromatic; blooms in late spring to summer.
Fruits	Berry-like, ovoid or oblong; 2–4 cm long; green when immature, turning yellow to orange on ripening; ridged or warty surface; persistent calyx lobes; contains yellow-orange pulp with numerous seeds.
Stem	Woody and erect; light brown or greyish when mature; green in young shoots; smooth to slightly rough; visible nodes and internodes.
Roots	Taproot system, yellowish-brown; woody texture; aromatic odour when fresh.

RESULTS OF MICROSCOPIC EXAMINATION OF LEAVES

The leaves of *Gardenia jasminoides* were subjected to detailed microscopic analysis to establish diagnostic characters that support species identification and pharmacognostic standardisation. Both quantitative microscopy and anatomical observations were performed. The results obtained from these evaluations are described below.

Quantitative Microscopy

Quantitative microscopic analysis was carried out to determine specific anatomical parameters that are valuable for species identification, authentication of crude drug material, and pharmacognostic standardization. The evaluated parameters included palisade ratio, stomatal number, stomatal index, vein-islet number, and vein-termination number, which serve as constant and reproducible diagnostic features irrespective of environmental variations. The results are presented below.

Table 2: Summary of Quantitative Microscopy Findings

Parameter	Observed Value	Significance
Palisade Ratio	5.8	Indicates greater photosynthetic efficiency
Stomatal Number	32	Supports the stomatal distribution study
Epidermal Cells	68	Used for SI calculation
Stomatal Index	32%	Confirms amphistomatic leaf type
Vein-Islet Number	12/mm²	Crucial for the identification of a powdered sample

Vein-Termination Number	10/mm²	Assists in species differentiation

Transverse Section of Leaf

A transverse section (T.S.) of the lamina, including the midrib, presented a clear picture of internal tissue organization. The dorsiventral nature of the leaf was normal. The upper investment was formed of compact rectangular cells, one-layered and bearing an indistinct non-striated cuticle. Underneath the upper epidermis, two palisade parenchyma layers of tightly packed columnar cells with numerous chloroplasts, indicative of active photosynthesis, could be observed. The spongy parenchyma beneath the palisade layers was 4–5-layered with loosely arranged, rounded cells having large intercellular spaces. For it is these spaces that facilitate the exchange of gases, which are so important for respiration and photosynthesis.

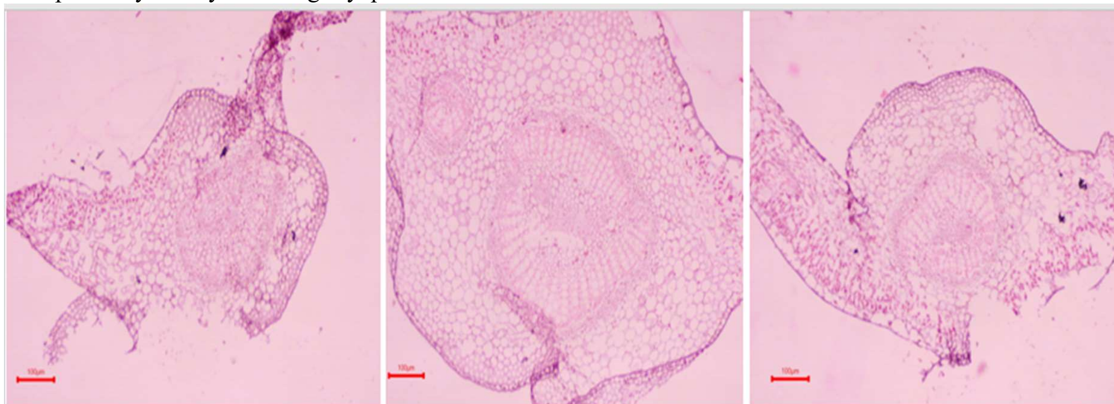

Figure 5.7: TS of *Gardenia jasminoides* leaves

The midrib area had a well-developed vascular bundle in collenchymatous ground tissue. The vascular bundle was collateral with xylem to the adaxial (upper) side and phloem on the abaxial (lower) side. Lignified xylem vessels and sieve tubes in the phloem were recognised. Collenchyma tissues were detected on the upper and lower parts of the midrib, contributing to structural support. Rare trichomes were observed and limited to the lower epidermis

. They were found as unicellular, non-branched elements. Stomata were also denser on the abaxial side, and of the anomocytic type, the surrounding cells being irregularly arranged.

Powder Microscopy

Examination under a microscope of the powdered drug provided identification characteristics. Pieces of the epidermis with curved anticlinal walls were noted. The powder displayed the lignified spiral and scalariform xylems, sieve elements of phloem, and parenchymatous cells. Simple and sometimes compound starch grains were abundant. Prismatic crystals of calcium oxalate were conspicuous, and they have taxonomic value. Also visible were sporadic remains of trichomes and aberrant stomata, which supported the numbers collected from intact leaf regions. It has been particularly useful for the identification of raw drug samples and the detection of adulteration powdered samples.

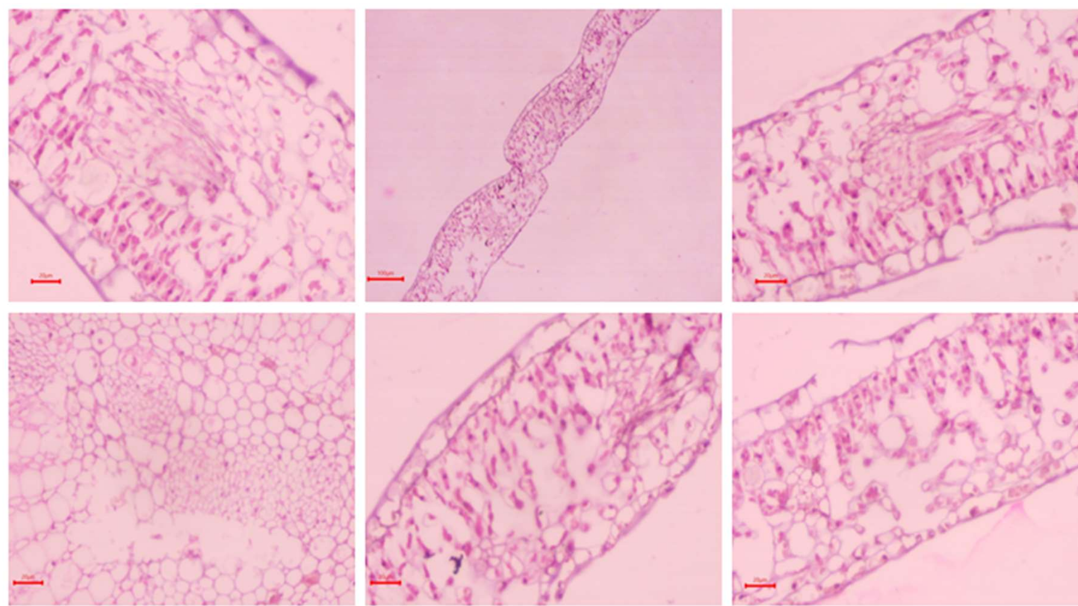

Figure 5.8: Powder microscopy of *Gardenia jasminoides* leaves

Results of Chemo-microscopic Examination

The powder of the leaf of *Gardenia jasminoides* was found to contain chemo-microscopically several intracellular inclusions and structural biomolecules. These findings confirm the pharmacognostic identity of the plant, and they constitute essential standards for its authentication as well as for detecting adulterants. Microchemical tests were executed with specific reagents and recorded under a compound microscope.

Table 5.3: Result of Chemo-microscopic Examination

S. No.	Constituent Tested	Test Performed	Observation	Inference
1	Starch Grains	Iodine Solution	Blue-black coloration	Present
2	Proteins	Millon's Reagent	Reddish-brown coloration	Present
3	Lignin	Phloroglucinol + Conc. HCl	Pink-red colouration in vascular tissue	Present
4	Fats/Oils	Sudan III	Orange-red coloration	Present
5	Calcium Carbonate	Acetic Acid	No effervescence	Absent
6	Calcium Oxalate Crystals	Dil. HCl + Microscopic Observation	Prismatic and druse crystals are seen	Present

Chemo-microscopic characters of *Gardenia jasminoides* also substantiate its pharmacognostical parameters. The starch, proteins, lignin, fats, and oxalate crystals showed the biochemical complexity, as well as the constituents in which they are present, indicating that the plant is a structurally complex one. Lack of calcium carbonate also aids in distinguishing from contaminants. These data attest to its authenticity and indicate that it could be used for medicinal or industrial purposes.

RESULTS OF ANALYTICAL STANDARDS

Physicochemical parameters of *Gardenia jasminoides* leaf powder were determined employing pharmacopoeial procedures, which are very important in determining the authenticity, purity, identity, and quality of crude herbal drugs. These criteria provide the basis of official standards, prevent counterfeit and assure uniformity for each batch, and may also support traditional or contemporary therapeutic uses. The major analytical standard results are shown in Table 3, accompanied by an explanation of each parameter.

Table 5.4: Evaluation parameters and their observed value

Parameter	Observed Value (% w/w)
Total Ash Value	8.65%
Acid-Insoluble Ash	1.12%
Water-Insoluble Ash	2.25%
Sulphated Ash	9.18%
Alcohol-Soluble Extractive Value	14.50%
Water-Soluble Extractive Value	18.75%
Moisture Content (Loss on Drying)	5.68%

The obtained values fall within the acceptable pharmacopoeial range, reaffirming the quality, cleanliness, and suitability of *Gardenia jasminoides* leaf powder for herbal formulation development. These parameters are extensively used in pharmacognostic standardization, regulatory frameworks, and research-based formulation studies.

Extraction and Yield Analysis of *Gardenia jasminoides* Leaves

The powder from 100 g of Gardenia *jasminoides* leaves was extracted sequentially with solvents, starting with petroleum ether and increasing polarity to ethyl acetate, ethanol, and then water. Percent extractives varied with the extraction procedures, which reflects the solubility of phytoconstituents in different solvents.

Table 5.5: Extraction and Yield Analysis of *Gardenia jasminoides* Leaves

Solvent Used	Amount of Extract (gm)	Percentage Yield (%)
Petroleum Ether	1.22	0.24
Ethyl Acetate	1.95	0.39
Ethanol	18.75	3.75
Water (Aqueous Extract)	0.88	0.17

Preliminary phytochemical screening

Phytochemical screening of various secondary metabolites present in leaf extracts of *Gardenia jasminoides* (petroleum ether, ethyl acetate, ethanol, and aqueous was done. Phytochemical Screening of Ethanolic extract comprised the presence of different types of secondary metabolites, including alkaloids, flavonoids, steroids, terpenoids, glycosides, and reducing sugar, as presented in Table 5. EE contained the maximum number of secondary metabolites and, hence, we proceeded with the study from EE.

Table 5.6: Preliminary phytochemical constituents present in different extracts

Sr. No.	Chemical Test	Result of PEE	Result of EAE	Result of EE	Result of AE

1.	Alkaloids	-	-	++	-
2.	Flavonoids	-	-	++	+
3.	Tannin	-	-	-	-
4.	Steroids	-	+	++	-
5.	Volatile oil	-	-	-	-
6.	Glycoside	-	-	+	+
7.	Amino acids	-	-	-	-
8.	Fat and oils	+	+	-	-
9.	Reducing sugar	-	-	+	+
10.	Terpenoids	-	-	++	+

Slightly present (+), Present (++), Negative (-);

PEE: Petroleum ether extract, EAE: Ethyl acetate extract, EE: Ethanolic extract, AE: Aqueous extract

Thin Layer Chromatography (TLC) Analysis of *Gardenia jasminoides* Extracts

Phytochemical investigation. The various extracts of *Gardenia jasminoides* leaves were screened for the presence of phytoconstituents by TLC analysis. Solutions for each extract were prepared using different solvent systems according to their polarity. The petroleum ether extract exhibited fewer nonpolar compounds (consistent with a low yield). The ethanolic extracts depicted several phytochemical spots, which were indicative of their bioactive contents. Fewer and more polar compounds were observed with ethyl acetate and Aqueous extract.

Table 5.7: TLC: *Gardenia jasminoides* extract R_f value

Extract	Solvent System	Number of Spots Observed	Rf Values	Nature of Spots (Under UV/After Derivatization)
Petroleum Ether Extract	Hexane: Ethyl acetate (8:2)	2	0.52, 0.67	Blue under UV, orange after iodine vapors
Ethyl Acetate Extract	Toluene: Ethyl acetate (7:3)	3	0.30, 0.55, 0.78	Green, fluorescent under UV
Ethanolic Extract	Chloroform: Methanol (9:1)	6	0.15, 0.20, 0.34, 0.40, 0.65, 0.82	Yellow and blue fluorescent under UV light
Aqueous Extract	Butanol: Acetic acid: Water (4:1:5)	1	0.20	Light blue under UV, no significant color after spraying

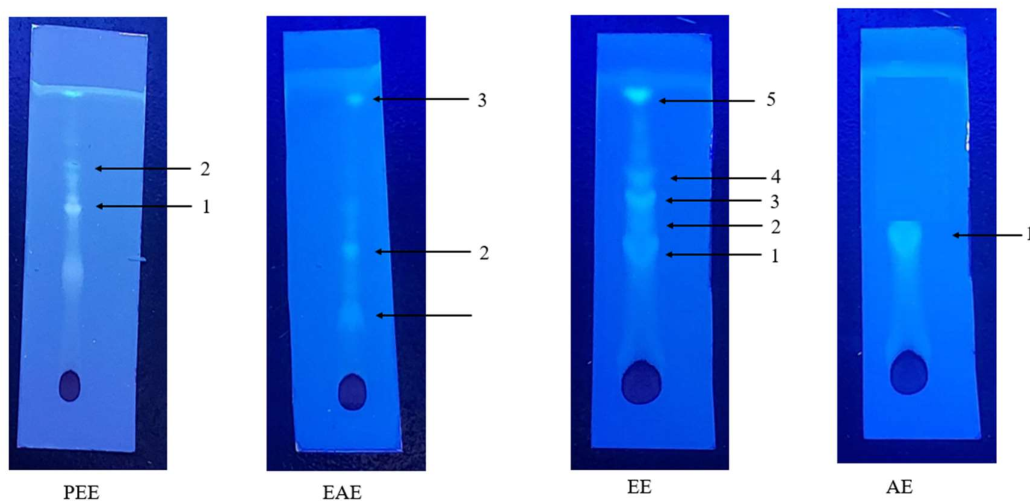

Figure 5.10: TLC Profile of *Gardenia jasminoides* extracts

HIGH-PERFORMANCE THIN LAYER CHROMATOGRAPHY

Fingerprint Study of the Root Bark Extracts (PEE, EAE, EE, and AE) of *Gardenia jasminoides*

After treating the leaves of *Gardenia jasminoides* with different solvents, viz., petroleum ether, ethyl acetate,

ethanol, and aqueous, a detailed phytochemical profile was determined using high-performance thin-layer chromatography (HPTLC). HPTLC provides a better resolution, better sharpness in band separation, and more accurate Rf values than conventional TLC. All of the extracts were spotted onto pre-coated silica gel 60 F254 plates and developed with solvent systems adapted to the polarity of the extracts. Following the development, the plates were first observed under UV light (254 nm and 366 nm), and then further derivatized with iodine vapor, ninhydrin, or anisaldehyde-sulfuric acid reagent for better spot visualization.

The petroleum ether extract showed two distinct bands with an Rf value of 0.65. Under UV light at 366 nm, these bands appeared blue, while iodine vapor exposure resulted in an orange-brown coloration, suggesting the presence of non-polar compounds like sterols or terpenoids.

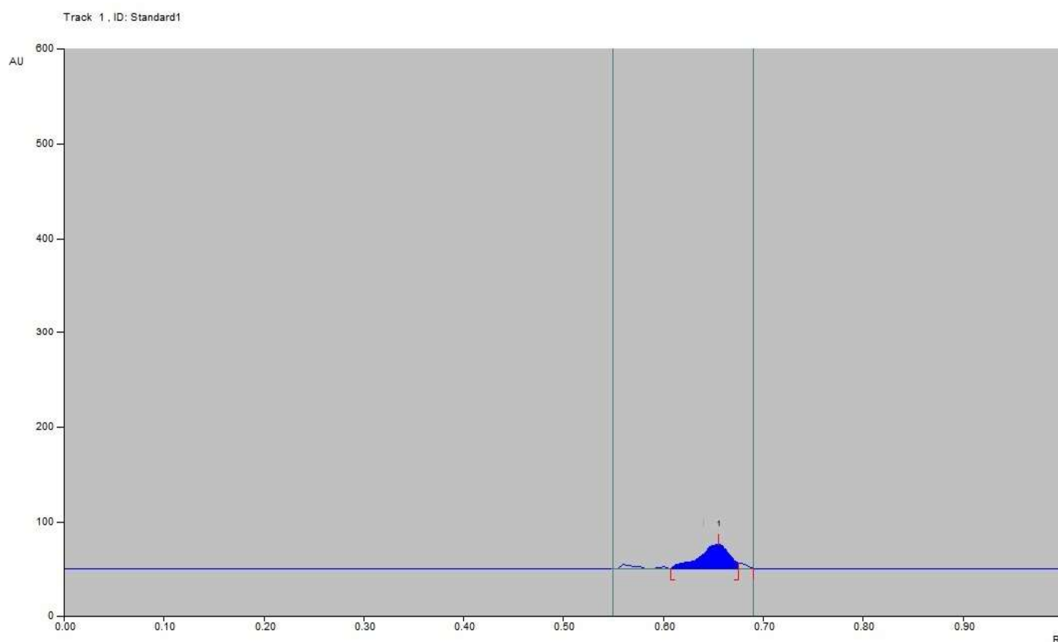

Figure 5.11: HPTLC densitometric chromatogram of PEE scanned at 254 nm

The ethyl acetate extract displayed three clear spots with Rf values at 0.58, 0.62, and 0.69. Under UV visualization, spots showed bright green fluorescence, indicating the presence of flavonoids and semi-polar secondary metabolites. Post-derivatization revealed faint yellow coloration, further supporting the presence of phenolic compounds.

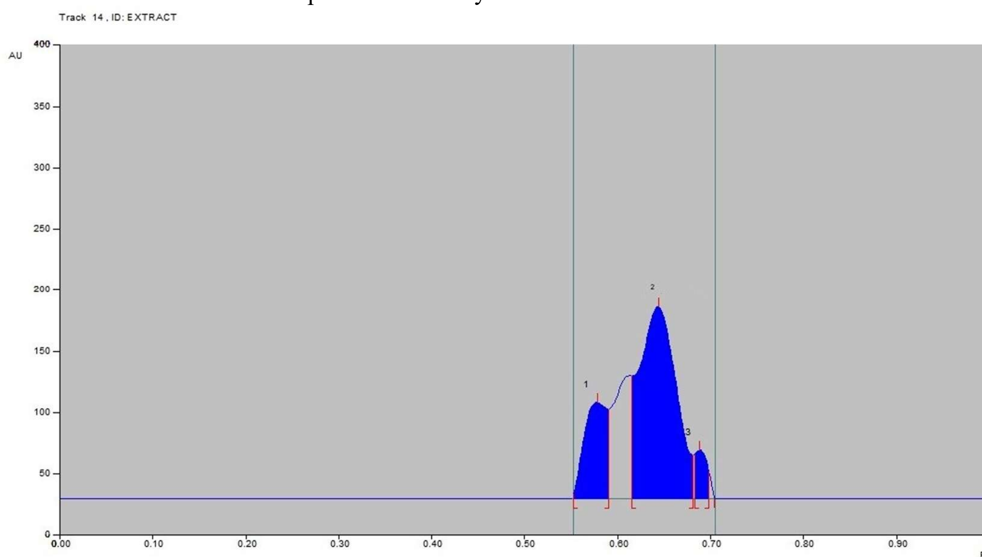

Figure 5.12: HPTLC densitometric chromatogram of EAE scanned at 254 nm

The ethanolic extract exhibited the most complex phytochemical fingerprint, with four spots at Rf values 0.14, 0.24, 0.41, 0.47, 0.61, 0.71, and 0.78. UV visualization showed yellow-green and blue fluorescing bands, while post-spraying with anisaldehyde-sulfuric acid reagent resulted in intense purple bands. These results are indicative of a diverse array of polar phytochemicals such as alkaloids, glycosides, and flavonoids.

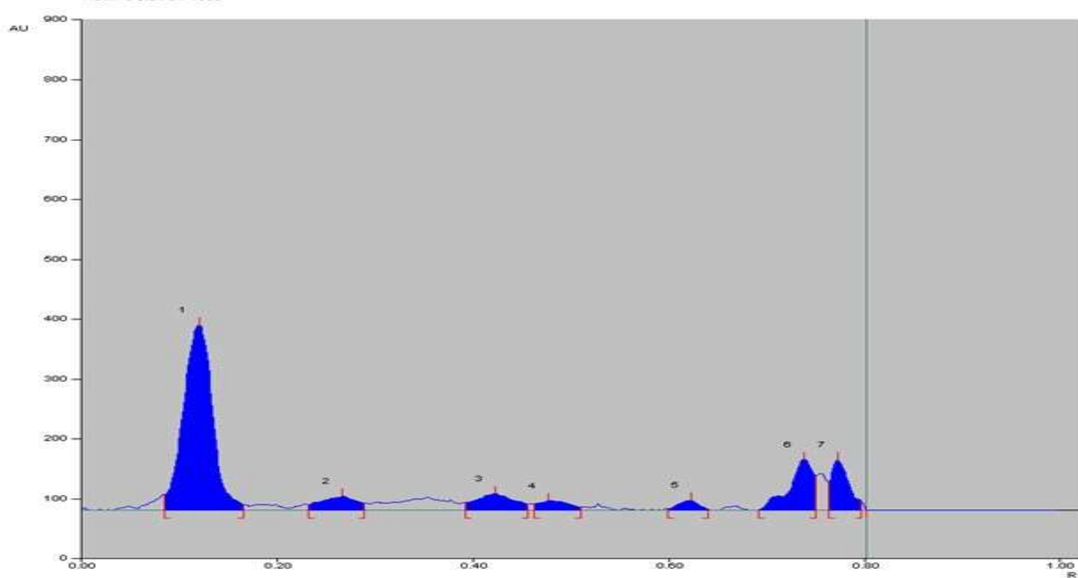

Figure 5.13: HPTLC densitometric chromatogram of EE scanned at 254 nm

The aqueous extract yielded two bands with Rf values of 0.18 and 0.45. The spots were light blue under UV light, and no significant color change was observed after ninhydrin spraying, suggesting the presence of a few highly polar compounds like simple sugars or amino acids.

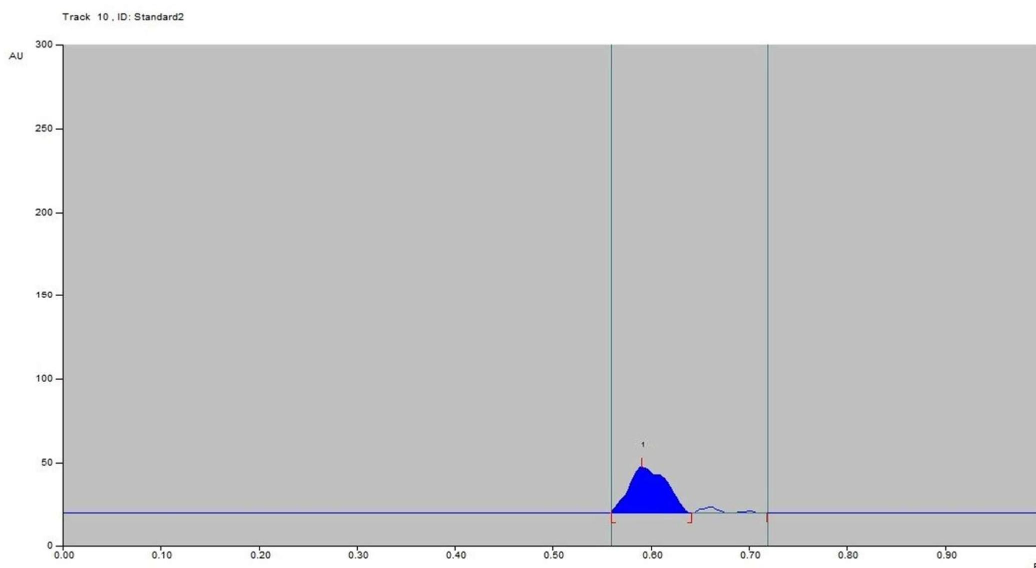

Figure 5.14: HPTLC densitometric chromatogram of AE scanned at 254 nm

Thus, the HPTLC profiles revealed a varied distribution of phytochemicals among the different extracts, with ethanol emerging as the most efficient solvent for the extraction of a wide range of bioactive compounds.

DETERMINATION OF ANTIOXIDANT ACTIVITY OF GARDENIA JASMINOIDES LEAVES

DPPH Assay

The free radical scavenging activity of *Gardenia jasminoides* leaf extracts (petroleum ether, ethyl acetate, ethanol, and aqueous) was evaluated by the DPPH assay and compared with ascorbic acid as a reference standard. The extracts demonstrated a dose-dependent increase in DPPH radical inhibition with increasing concentrations (5–30 µg/mL).

Table 5.8: Percentage inhibition of DPPH radicals by *Gardenia jasminoides* extracts compared with ascorbic acid

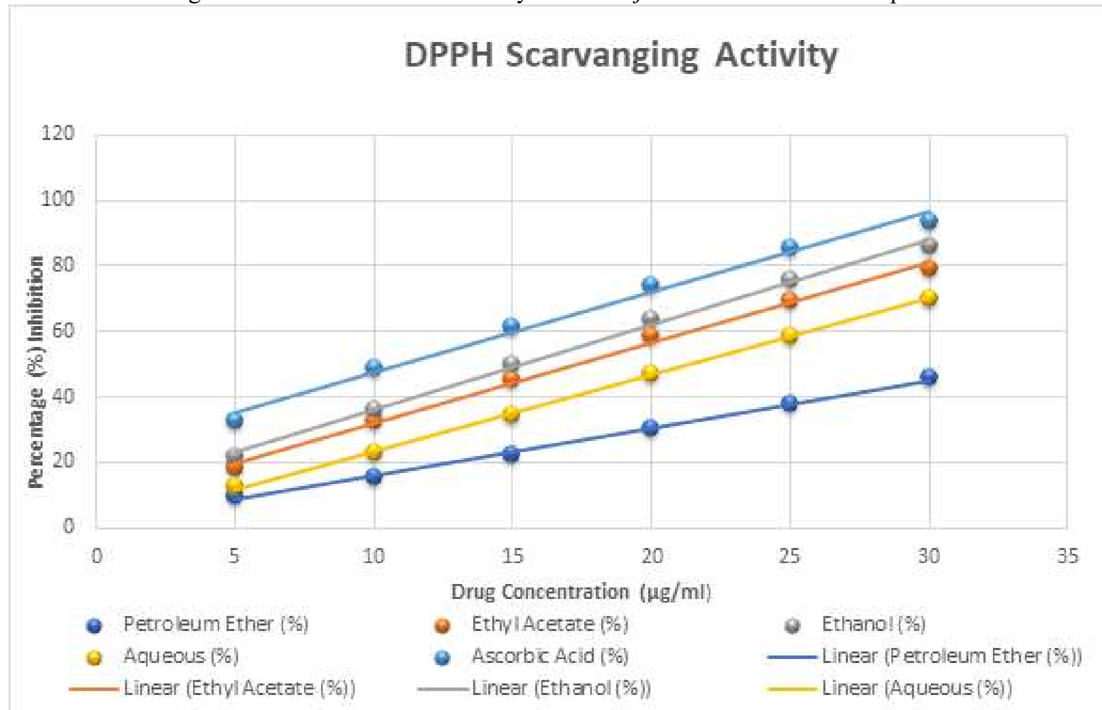

Figure 5.15: DPPH Scavenging Activity

IC_{50} Values

The IC_{50} values (the concentration necessary to scavenge 50% DPPH radicals) were obtained from the inhibition curves. Pharmacological studies have proven that the leaves of Gardenia jasminoides possess antioxidant properties. The activities of the tested extracts were in the order of ethanol extract, which exhibited a superior antioxidant effect ($IC_{50} \approx 15.40$ µg/mL), closely followed by the ethyl acetate fraction ($IC_{50} \approx 17.30$ µg/mL). The antioxidant activity of the aqueous and petroleum ether extracts was found to be considerably less compared methanolic extract. As anticipated, a common denominator, the ordinary ascorbic acid, exhibited significantly higher activity ($IC_{50} \approx 10.99$ µg/mL). These results indicate that the ethanol- and ethyl acetate-soluble fractions are a good source of phenolic and flavonoid derivatives, which may be responsible for their antiradical activity.

Table 5.9: IC_{50} values of the plant extracts and standard compound

Sample	IC_{50} (µg/mL)
Ascorbic Acid (Std)	10.99
Ethanol Extract	15.40
Ethyl Acetate Extract	17.30
Aqueous Extract	21.39
Petroleum Ether Extract	33.50

ABTS Assay

The free radical scavenging activity of *Gardenia jasminoides* leaf extracts (petroleum ether, ethyl acetate, ethanol, and aqueous) was evaluated by the ABTS assay and compared with ascorbic acid. All extracts demonstrated a dose-dependent increase in ABTS radical inhibition with increasing concentrations (5–30 µg/mL).

Table 5.10: Percentage inhibition of ABTS radicals by *Gardenia jasminoides* extracts compared with ascorbic acid

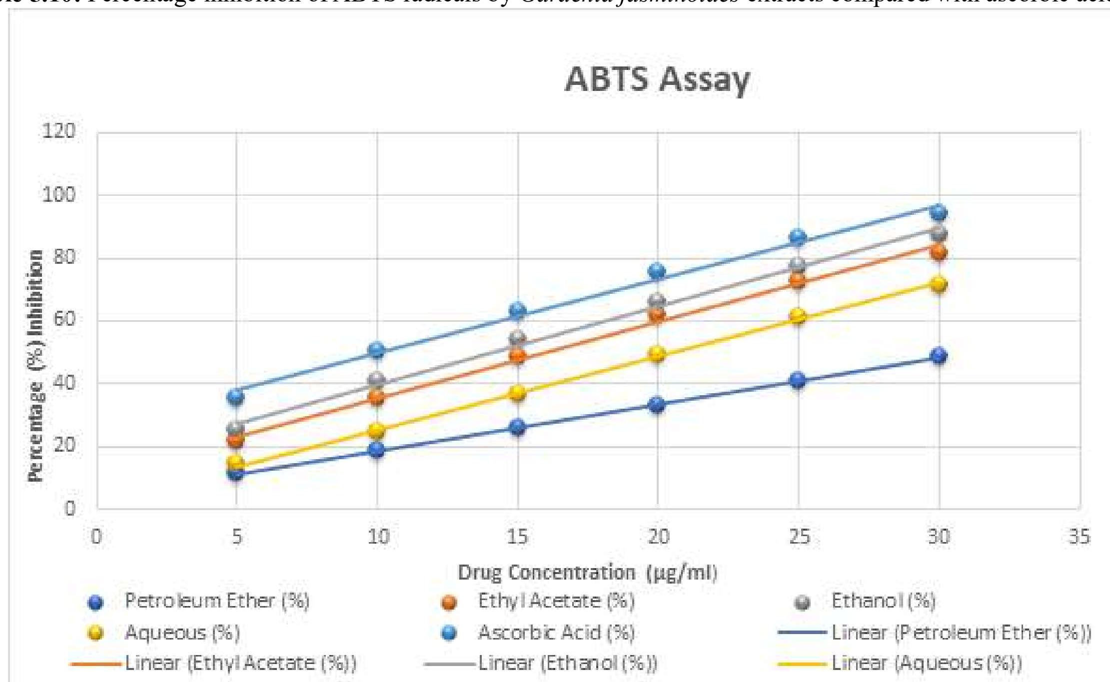

Figure 5.16: ABTS Assay

IC_{50} Values

The ABTS assay confirmed significant antioxidant activity in *Gardenia jasminoides* leaves. Ethanol and ethyl acetate extracts showed the highest scavenging potential, while aqueous and petroleum ether extracts were comparatively less active. The results correlate with the DPPH assay, suggesting high phenolic and flavonoid content in the ethanol extract.

Table 5.11: IC_{50} values of the plant extracts and the standard compound

Sample	IC_{50} (µg/mL)
Ascorbic Acid (Std)	10.07
Ethanol Extract	14.06
Ethyl Acetate Extract	16.03
Aqueous Extract	20.53
Petroleum Ether Extract	31.08

CONCLUSION

The present investigation successfully established the pharmacognostical, physicochemical, phytochemical, and antioxidant profile of *Gardenia jasminoides* leaves. Macroscopic and microscopic studies provided important diagnostic characteristics useful for the identification and authentication of the plant material. Physicochemical parameters such as ash values, extractive values, and moisture content confirmed the purity and quality of the crude drug. Phytochemical screening revealed the presence of several biologically active secondary metabolites, particularly in the ethanolic extract, including alkaloids, flavonoids, steroids, glycosides, and terpenoids. TLC and HPTLC fingerprint analyses further confirmed the diverse phytochemical composition of the plant extracts.

The antioxidant evaluation demonstrated that the ethanolic extract exhibited significant free radical scavenging activity in both DPPH and ABTS assays, with activity comparable to the standard ascorbic acid. The strong antioxidant potential may be attributed to the presence of phenolic and flavonoid compounds in the extract. Overall, the study validates the traditional medicinal importance of *Gardenia jasminoides* and suggests that its leaves can serve as a promising natural source of antioxidant compounds for future pharmaceutical and therapeutic applications. Further studies on isolation and characterization of active constituents may help in the development of novel herbal formulations and antioxidant agents.

Acknowledgement: Author are thankful to the management of institution and staff of college, department and university.

Conflict of Interest: None

Funding Source: None

REFERENCES

1. Atef NM, Shanab SM, Negm SI, Abbas YA. Evaluation of antimicrobial activity of some plant extracts against antibiotic susceptible and resistant bacterial strains causing wound infection. *Bull Natl Res Cent* [Internet]. 2019 Dec 5;43(144):1–11.
2. Kalaichelvi K, Dhivya SM. Ethno-medicinal knowledge of plants used by Irula tribes of Nellithurai Beat, Karamadai Range, Western Ghats and phytochemical screening of selected Lamiaceae species. *Adv J Pharm Life Sci Res.* 2016;4(2):54–64.
3. Uzor PF, Osadebe PO, Omeje EO, Agbo MO. Bioassay-guided isolation and evaluation of the antidiabetic principles of *Combretum dolichopetalum* root. *Br J Pharm Res.* 2014;4(18):2155–71.
4. Eze PM, Nnanna JC, Okezie U, Buzugbe HS, Abba CC, Chukwunwejim CR, et al. Screening of metabolites from endophytic fungi of some Nigerian medicinal plants for antimicrobial activities. *EuroBiotech J*. 2019 Jan 1;3(1):10–8.
5. Wiegand I, Hilpert K, Hancock REW. Agar and broth dilution methods to determine the minimal inhibitory concentration (MIC) of antimicrobial substances. *Nat Protoc.* 2008;3(2):163–75.
6. Evans WC. *Trease and Evans Pharmacognosy.* 16th ed. London: W.B. Saunders Company Ltd.; 2009. p. 569–70.
7. Khandelwal KR. *Practical Pharmacognosy: Techniques and Experiments.* 19th ed. Pune: Nirali Prakashan; 2008. p. 15–18.
8. Krishnamurty KV. *Methods in Plant Histochemistry.* Madras: Vishwanadhan Pvt. Ltd.; 1988. p. 1–70.
9. Sasmal DS, Kumar P, Papiamitra M, Padmacharan B, Uma RL, Dash SK, et al. Review on genus *Canthium*: Special reference to *Canthium coromandelicum* – an unexplored traditional medicinal plant of the Indian subcontinent. *Am J Phytomed Clin Ther.* 2014;2(6):796–813.
10. Sofowora AE. *Medicinal Plants and Traditional Medicine in Africa.* 3rd ed. Ibadan, Nigeria: Spectrum Books Limited; 2008. p. 79–81.
11. Ajaykumar RS, Rajendra DW. Pharmacognostic study and development of quality parameters of *Hamelia patens* Jacq. stems. *Der Pharm Lett.* 2016;8(8):6–13.
12. Kumar D, Pravin PV, Zulfikar AB, Jeevan D, Yogesh K, Santosh B. Macroscopical and microscopical evaluation of leaves of *Clerodendrum inerme* Gaertn. *Int J Biol Med Res.* 2011;2(1):404–8.
13. Swamy P, Mulla SK. Preliminary pharmacognostical and phytochemical evaluation of *Portulaca quadrifida* Linn. *Int J PharmTech Res.* 2010;2:699–702.
14. Patnia S, Saha AN. Physicochemical, phytochemical and elemental analysis of stem bark and roots of *Berberis asiatica. Adv Appl Sci Res.* 2012;3:3624–8.
15. Mritunjay K, Mondal P, Borah S, Mahato K. Physicochemical evaluation, preliminary phytochemical investigation, fluorescence and TLC analysis of leaves of the plant *Lasia spinosa* (Lour.) Thwaites. *Int J Pharm Pharm Sci.* 2013;5:306–10.
16. Purohit AP, Kokate CK, Gokhale SB. *Pharmacognosy.* 13th ed. Pune: Nirali Prakashan; 2005. p. 256–9.
17. Shwetajain SC, Khatri P, Jain A, Vaidya A. Pharmacognostic and phytochemical investigations of the leaves of *Zizyphus xylopyrus* (Retz.) Willd. *Int J Pharm Pharm Sci.* 2011;3:122–5.
18. Kripa KG, Sangeetha R, Chamundeeswari D. Pharmacognostical and physicochemical evaluation of the plant *Leucas aspera. Asian J Pharm Clin Res.* 2016;9:263–8.
19. Rajesh V, Riju T, Venkatesh S, Babu G. Memory-enhancing activity of *Lawsonia inermis* Linn. leaves against scopolamine-induced memory impairment in Swiss albino mice. *Orient Pharm Exp Med.* 2017;17:127–42.
20. Maqsood S, Singh P, Samoon MH, Balange AK. Effect of dietary chitosan on non-specific immune response and growth of *Cyprinus carpio* challenged with *Aeromonas hydrophila. Int Aqua Res.* 2010;2:77–85.