

Pharmacognostic, Phytochemical and Antioxidant Studies of *Gardenia latifolia* Aiton: An Ethnomedicinal Tree Plant

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Received: 11th Feb, 18; Revised 23rd Apr, 18, Accepted: 3rd May, 18; Available Online: 25th May, 18

ABSTRACT

Present study deals with the pharmacognosy, phytochemistry, antioxidant activity of crude drugs obtained from leaf, stem bark and fruit of *Gardenia latifolia* Aiton (Family- Rubiaceae), an ethnomedicinal tree species. Different parts of this plant are used in curing skin diseases, wounds, and in snake bite. Pharmacognostic study revealed that epidermal cells of abaxial and adaxial leaf surfaces are irregular in shape and anticlinal walls of the cells are slightly wavy. Stomata are strictly paracytic type and observed only in the abaxial leaf surface, i.e., hypostomatic type of leaf. Stomatal index is 29.85. Palisade ratio is 3.02. Only non-glandular type of trichomes is observed in both the leaf surfaces. Trichome index is 1.42 and 1.98 in upper and lower surfaces of the leaf, respectively. Vessel elements are of moderate in size, perforation plate simple and obliquely placed. Histochemical localization tests revealed the presence of tannins, proteins, alkaloids, glycosides, lignin, etc. in various tissue zones of the leaf petiole and stem part. Phytochemical screening showed the presence of alkaloids, saponins, tannins, anthaquinones, glycosides, etc. in the methanolic extracts of leaf, bark and fruit of this plant. Physical constants like moisture content, total ash, acid insoluble ash, water soluble ash value have been determined for all those three parts of the plant. Total ash value was high in fruit (10.0%) that was followed by bark (5.6%) and leaf (2.65%). Contents of total phenolics, flavonoids and tannins were found higher in bark than that of leaf and fruit parts of this plant. IC₅₀ values of methanolic extracts of leaf, stem bark and fruit in DPPH scavenging activity study were 145.83 µg/ml, 79.74 µg/ml and 117.93 µg/ml respectively. In ABTS scavenging study bark showed minimum IC₅₀ value (73.87 µg/ml) followed by fruit (109.26 µg/ml) and leaf (186.27 µg/ml). Similar trend was also found in Total Antioxidant Activity assay, where maximum antioxidant potential is measured in case of bark (41.20 mg AAE/g) followed by fruit (31.23 mg AAE/g) and leaf (13.45 mg AAE/g). Present study highlighted that bark part of this medicinal plant is more potent than the leaf and fruit parts in respect of its phytochemical content and antioxidant activity. The study also provides some diagnostic pharmacognostic features by which the crude drugs of it can properly be identified.

Keywords: *Gardenia latifolia* Aiton, pharmacognostic features, phytochemical profile, antioxidant activity.

INTRODUCTION

Medicinal plants have a long-standing history in the practices of traditional medicine, which is based on hundreds of years of belief and observations¹. Medicinal plants used traditionally, are now moving from fringe to mainstream as people are becoming more aware of therapeutic properties of these medicinal plant resources and their products in maintaining health and preventing diseases. According to WHO, about 80% of the rural people worldwide rely mainly on herbal medicines for their primary healthcare². With the goal of the development of novel natural products, scientists from every corner of the World engage themselves in medicinal plant research on the line of pharmacognosy, phytochemistry, pharmacology, biological assay, clinical studies and other related areas of research.

Scientific studies in the field of pharmacognosy have been preceded on various lines covering morpho-anatomical characterization of plant parts used as crude drug, their physico-chemical parameters, phytochemical screening, biological assay and on many other diverse approaches. It

is very important to ensure quality and purity of the herbal medicines in order to maximize their efficacy and minimize the adverse side effects. Traditional prescriptions and practices will not sustain for use in later generations, if the drugs are not standardized. Correct identification and quality assurance of the crude drugs is an essential prerequisite to ensure reproducible quality of herbal medicine³. WHO also emphasizes the need to ensure quality control of medicinal plant products by using modern techniques and suitable standards⁴. Also, improper authentication of herbal drugs, its adulteration, contamination with microorganisms, pesticides and heavy metals, has made standardization of herbal drugs an elementary necessity. So, preparation of the pharmacognostic standards for proper identification of the crude drugs and detection of adulteration is treated as an essential step towards natural product research.

Many of the important medicinal plants have so far been investigated pharmacognostically as well as phytochemically. But a huge number of medicinal plants are still left unattended which require a systematic

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Figure 1a: A portion of the tree



Figure 1b: A fruit



Figure 1c: A flowering twig

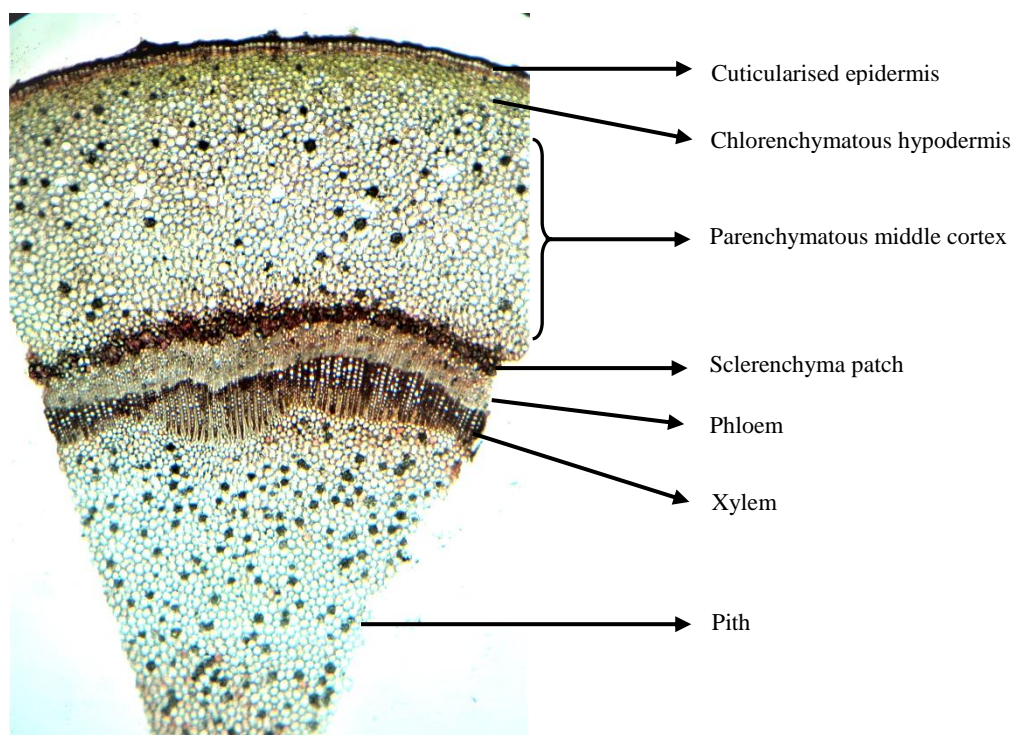


Figure 2: T.S. of stem

scientific study to evaluate their pharmacognostic standards, phytochemical profiles and various biological activities. The biological activity study of medicinal plants is necessary not only for gaining novel natural products from the medicinal flora, but also for validation of the ethnomedicinal claims of those therapeutically potent plants while curing different health conditions. A wide range of biological activity studies like anti-inflammatory, anticancer, antioxidant, etc., are being carried out to identify the active compound or compounds from the medicinal plants and to standardize the effective drugs

from those bioactive phyto-molecules. It has been established that oxidative stress is among the major causative factors in induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immune suppression, neurodegenerative diseases and others^{5,6}. Reactive oxygen species (ROS) exert oxidative damaging effects by reacting with nearly every molecules found in living cells including protein, lipid, amino acids and DNA, if excess ROS are not scavenged by the antioxidant system^{6,7,8}. The most practical way to fight degenerative

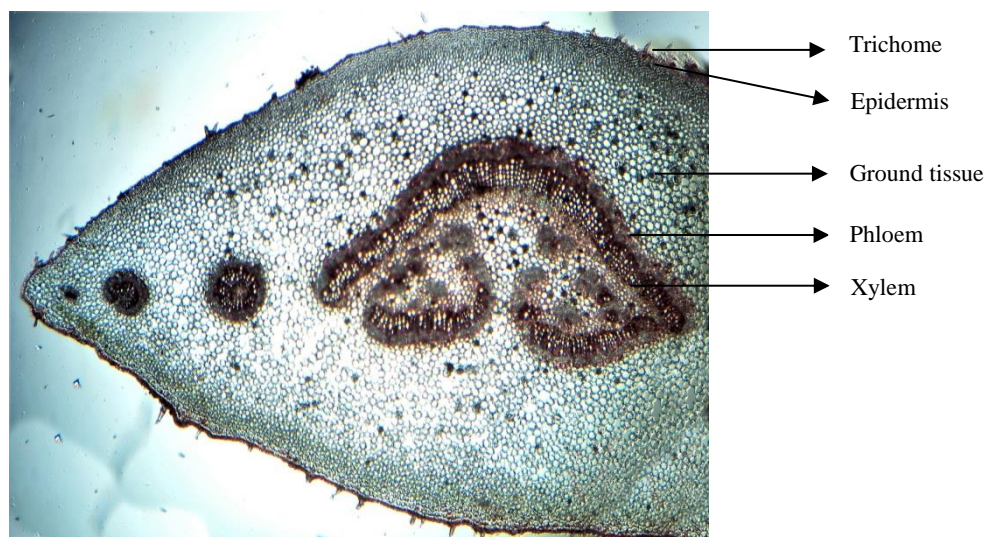


Figure 3: T.S. of petiole

diseases is to increase antioxidant activity in our body and that could be achieved by consumption of vegetables, fruits, cereals and other foods with good content of antioxidant substances^{5,6}. There is a widespread search for exogenous antioxidants from natural sources perhaps, due to the fact that they are less expensive, easily available and believed to have lesser side effects when compared to their synthetic counterparts^{6,7}. A number of phytochemical groups from plant sources are known for their antioxidant potential and among them the phenolics, flavonoids, tannins, etc. have been established as potent antioxidants showing very good ability to inhibit the free radicals.

Gardenia latifolia, commonly known as Indian boxwood or Ceylon boxwood, is a small tree with dense foliage. The different parts of this plant are reported to be used in treatment of a wide range of ailments such as snake bite, skin diseases, stomach pains, inflammatory pain, caries, haemorrhage in humans and ephemeral fever in live stocks. Due to its broad spectrum healing potential, this medicinal tree exhibits itself as a very good research material for various scientific studies. Although very few scientific studies have so far been carried out on *G. latifolia*, where qualitative phytochemical screening of different solvent extracts of the plant parts (leaf and stem bark) and quantification of tannins of bark part were performed^{9,10,11}. In one study, preliminary pharmacognostic standardization of the bark only has been done¹². But no detailed anato-pharmacognostic, photochemical and biological activity studies of the leaf, stem bark and fruit of the plant *G. latifolia* have been undertaken earlier. Although leaf, stem bark and fruit of this medicinal plant are equally important in curing a wide range of diseases and ailments. In this context, present study has been undertaken to evaluate the pharmacognostic, phytochemical and antioxidant properties of these three medicinally important parts of the plant *Gardenia latifolia* Ait. (Rubiaceae).

MATERIAL AND METHODS

Material

Scientific name: *Gardenia latifolia* Ait.

Synonym: *Gardenia calyculata* Roxb.

Vernacular names: Sanskrit - Parpataki; Bengali - Varkura; Hindi- Papra, Paphar, Ban pindalu; Marathi- Ghogari, Papur, Pandru; Telugu- Pedda bikki, Peddakaringuva; Tamil- Kumbay, Perungambil; Kannar- Kalkambi, Adavibikke; Oriya- Kota ranga, Jantia, Damkurdu.

English name: Boxwood gardenia.

Local name: Dom bhurro, Papra, Papro.

Parts used: Stem bark, leaf, root, fruit, gum and resinous sap.

Botanical characters

Small deciduous tree, up to 5m high; branches woody, terete, stout. Leaves opposite, stipulate, subsessile to petiolate; petioles 2-6mm long, flattened, glabrous; stipules 0.5-1.5 × 0.5-1.5 cm, inflated, connate, truncate or slightly toothed above, membranous; lamina ovate, orbicular, entire, apex broadly acuminate, coriaceous, glabrous above, pubescent below specially on veins. Inflorescence terminal, solitary or 2-together. Flowers white to yellowish, pedicellate, fragrant; pedicels 0.6-0.8 cm long, smooth, glabrous. Fruits globose, 3-5 × 2-3.5 cm, woody, with stout beak (Fig.- 1).

Flowering and fruiting season: April to December.

Habitat: Terrestrial, found chiefly on hills and in dry deciduous forests.

Distribution: Native to India but found in tropical and sub-tropical regions of Asia, Africa and Madagascar. It is commonly grown in dry deciduous forest belts from 400-900m altitude throughout the India.

Medicinal importance

Leaves- Pounded young leaves are used to cure snake bites¹³.

Bark- Used in skin diseases^{13,14}. Pounded bark in water also used to cure stomach troubles, heartburn and constipation¹⁵. Stem bark (100-12g) extract with 10-12 pepper and garlic is given twice a day to get relief from ephemeral fever¹⁶. The crushed stem bark is boiled in water and applied to treat caries¹⁷.

Gum- Used to cure cutaneous diseases¹⁵.

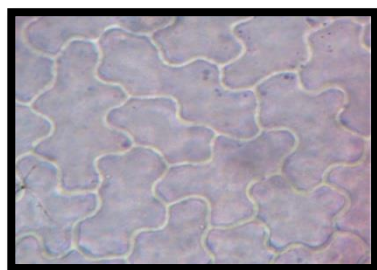


Figure 4: Epidermal cells of the upper surface of leaf



Figure 5: A portion of lower epidermis with a stoma



Figure 6: A non-glandular trichome



Figure 7: A vessel element



Figure 8: A portion of tracheid



Figure 9: A fibre

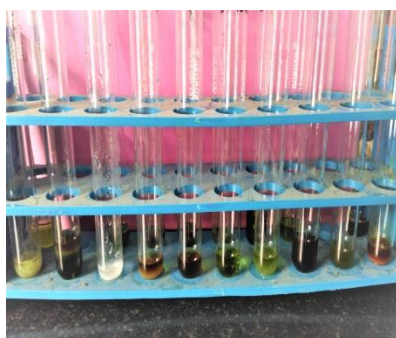


Figure 10: Chemical colour reaction test for ethanolic leaf extract



Figure 11: Chemical colour reaction test for ethanolic stem bark extract



Figure 12: Chemical colour reaction test for ethanolic fruit extract

Table 1: Organoleptic features of different parts of the investigated plant.

| Organoleptic features | Leaf | Stem bark | Fruit |
|-----------------------|------------------|-----------------|--------------------------|
| Colour | Dark olive green | Yellowish brown | Brown |
| Odour | Odourless | Odourless | Odourless |
| Taste | Acrid | Acrid | Slightly acrid and salty |
| Texture | Powdery | Fibrous | Fibrous |

Resinous sap- The sap extracted from the stem tips applied on sores of hand and feet in rainy season¹⁵.

Root- Root part is taken to treat heavy bleeding during menstrual cycle¹⁵.

Fruit- Fruit paste given to cure amoebiasis¹⁵.

Seed- One seed is taken with a leaf of piper for regular menstruation¹⁸.

Veterinary uses- Young leaves are applied to the wounds of cattle; leaf paste mixed with turmeric applied in boils, blisters, ulcers and wounds; fruits along with leaves of

Jasminum auriculatum, stem bark of *Helicteres isora* pounded and the extract given orally for tympany. Stem bark is used as fish poison¹⁵.

Tribal uses- Root is used as galactagogue. Stem barks are used to remove kidney stone, to treat mumps and rinderpest also. Leaves are used for healthy development of fetus. Leaf gall is used to prevent pox. Latex is applied against piles, eczema and muscular pain¹³.

Methods



Figure 13: Spot test for leaf powder under Visible light



Figure 14: Spot test for leaf powder under UV light

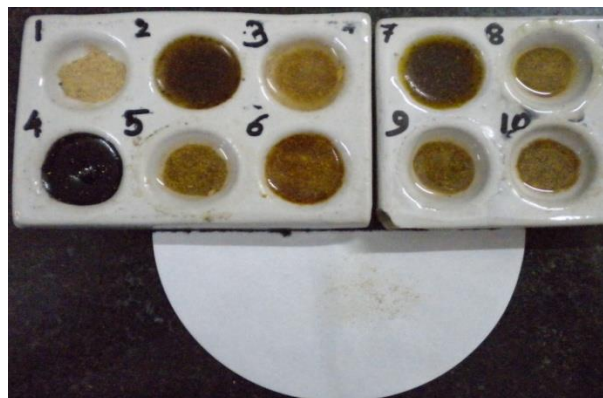


Figure 15: Spot test for stem bark powder under Visible light

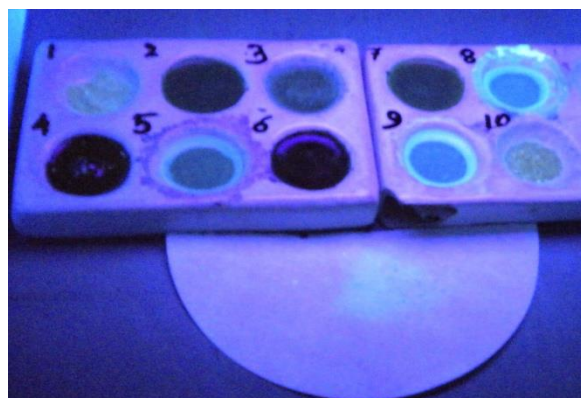


Figure 16: Spot test for stem bark powder under UV light



Figure 17: Spot test for fruit powder under Visible light



Figure 18: Spot test for fruit powder under UV light

Table 2: Moisture content and ash value of different parts of the investigated plant.

| Powdered plant sample | Moisture content (%) | Total ash (%) | Water soluble ash (%) | Acid insoluble ash (%) |
|-----------------------|----------------------|---------------|-----------------------|------------------------|
| Leaf powder | 19.41 | 2.65 | 3.21 | 2.4 |
| Stem bark powder | 10.21 | 5.6 | 4.69 | 1.2 |
| Fruit powder | 16.33 | 10.0 | 5.42 | 1.02 |

The fresh, well grown and matured different parts of the plant were collected from the road side of Santiniketan, Birbhum, West Bengal, India in the month of April, 2016. The plant species has been identified and authenticated with the help of different standard floras. The collected leaves, stem bark and fruits were washed thoroughly under tap water, shade dried, ground them into powder and finally the powdered plant samples are kept in airtight containers separately for future use. The fresh plant

materials were used for the study of macro and micro-morphological, anatomical and xylem maceration studies. The dried plant powders were used for physicochemical, phytochemical and antioxidant activity studies.

Study of foliar micromorphology: Leaf samples were cleared following the Bokhari's method¹⁹. The cleared leaf samples were then mounted on the slide with a drop of 10% glycerine and 1% aqueous safranin and observed under compound light microscope.

Table 3: Extractive value of different parts of the investigated plant.

| Plant parts | Extractive value (%) | | | |
|-------------|----------------------|---------------|------------|--------|
| | Ethanol | Ethyl acetate | Chloroform | Hexane |
| Leaf | 1.9 | 0.9 | 0.9 | 0.5 |
| Stem | 7 | 3 | 1 | 0.7 |
| Fruit | 3.1 | 0.9 | 1 | 1 |

Vegetative anatomy (stem and petiole)- For this study, free hand sections of the stem and petiole of the selected plant were made, stained suitably following safranin-light green staining schedule²⁰ and studied under Compound Light microscope (ZEISS, AXIOSTAR plus, 176045). Photographs of the suitable sections were taken with the help of photographic system attached with the said microscope.

Xylem elements study: The stem pieces (1 cm) were macerated following the standard method²¹. Boiled stem samples were then washed in distilled water for several times and observed under compound light microscope for xylem elements study.

Organoleptic study: This study of powdered crude drugs was done with the help of sensory organs following the standard methods²¹ which includes external morphology, colour, odour, taste, etc. of the crude drug.

Physicochemical evaluation

Physicochemical parameters like moisture content, ash value (total ash, acid insoluble ash, water soluble ash and sulphated ash) and extractive value of the powdered plant samples were determined as per guidelines of Indian Pharmacopoeia and WHO^{22,23,24}.

Moisture content study- About 5 gm of plant samples were weighed and dried for few days. Then the sample was incubated at 80°- 90°C temperature for one hour. Final weight of the sample was taken and calculated the percentage of moisture content²⁴.

Fluorescence analysis- For this study, the powdered plant samples were treated with different chemical reagents and observed the change in colour of the treated plant powders when seen under visible and UV light (365 nm)^{1,25,26}.

Histochemical study: Transverse sections of the stem and leaf petiole were kept in several glass slides. Then one to two drops of specific reagents (Wagner's, Dragendorff's, Mayer's, Lugol's, Millon's, 1% lead acetate, Phloroglucinol, Ferric chloride, etc.) were added to the sections and kept for few minutes to allow the specific reaction between reagents and phytochemicals present in the cells. Sections were then observed under the compound light microscope to detect different phytochemical groups localized in different tissue zones in the respective sections^{1,25,26}.

Extraction: Each dried plant part of 10 gm was ground and extracted with a particular solvent of 100ml for 48 hours in a continuous shaking at room temperature. The extract was filtered and then it was dried by using a rotary evaporator under vacuum at a temperature of 45°C.

Determination of extractive value: 10 gm of powdered sample of each plant part was extracted successively in a 100ml conical flask with the solvents ethanol, ethyl

acetate, chloroform and hexane separately. The respective solvent extracts were then allowed to dry at room temperature. After drying, weight of each solvent extract was noted and extractive value was determined by the following formula²⁵.

$$\text{Extractive value (\%)} = \frac{\text{Weight of the residue obtained}}{\text{Weight of the plant material taken}} \times 100$$

Phytochemical study

Preliminary phytochemical screening- Ethanol, ethyl acetate, chloroform and hexane extracts of leaf, stem bark and fruit powders were used for different chemical colour reaction tests with the help of different reagents to detect different phytochemical groups present in the powdered samples following standard methods^{27,28}.

Estimation of total phenolic content- Total phenolic content was estimated by standard method²⁹. Plant sample of 0.5 g was homogenized in 5 ml of 80% ethanol. Homogenates was centrifuged at 10,000 rpm for 20 min. Supernatant was collected and then dried. Residue was dissolved in 5 ml of distilled water. 0.5 ml of aliquot, distilled water and folin- ciocalteau reagent were mixed in a test tube. After 3 minutes, 20% sodium carbonate was added to the test tube and mixed it thoroughly. Test tubes were placed on boiling water bath for 1 min and cooled it at room temperature. Then absorbance was measured at 650 nm wave length against a blank.

Estimation of total flavonoid content- It was estimated employing the aluminium chloride method³⁰. Stock solution of each plant part extract was prepared by dissolving 100 mg of extract in 5ml methanol and the volume was made 10ml with methanol. Then 0.5ml of sample extract was taken in a test tube, subsequently 1.5ml methanol, 0.1ml of 10% aluminium chloride solution, 0.1ml of 1M potassium acetate solution and 2.8 ml distilled water were added to the test tube and mixed it thoroughly. Absorbance was taken at 415 nm against the suitable blank using Shimadzu UV-1800 double beam spectrophotometer.

Estimation of total tannin content- Method of Afify et al., (2012) with slight modification was employed³¹. The powdered plant sample of 500mg and 75ml distilled water were taken in a conical flask. It was then boiled for 30 minutes. After cooling, the boiled plant sample was centrifuged at 2000 rpm for 20 minutes. The residue was discarded and the volume of supernatant was adjusted to 100 ml with distilled water. Then the extract was used for the estimation of the tannins. One mL of the plant extract was taken in a volumetric flask containing 75mL distilled water. Then 5ml of Folin-Denis reagent and 10ml of sodium carbonate solution were added to the flask and volume adjusted to 100 ml with distilled water. Content in the flasks was thoroughly mixed, kept 30 minutes and absorbance was measured at 700nm on Shimadzu UV-1800 double beam spectrophotometer. A blank was prepared with distilled water instead of the sample. Tannins were estimated and calculated with the help of standard curve of gallic acid (0.1mg/mL) and expressed as mg of GAE/g.

Table 4: Microchemical colour reaction tests of different solvent extracts of the investigated plant.

| Chemical groups | Tests | Colour change | Solvent extracts of different parts of the plant | | | | | | | | | | | |
|-----------------|---------------------------------|-------------------------------------|--|--------|----|----|-----------|--------|----|----|-------|----|----|----|
| | | | Leaf | | | | Stem bark | | | | Fruit | | | |
| | | | Et | E A | Ch | Hx | Et | E A | Ch | Hx | Et | EA | Ch | Hx |
| Alkaloids | Mayer's reagent | White/Cream ppt. | + | + | - | - | + | + | - | - | + | + | - | - |
| | Wegner's reagent | Orange brown ppt. | + | + | - | - | + | + | + | - | + | - | - | - |
| | Dragendrof's reagent | Orange brown ppt. | + | - | - | - | ++ | ++ | - | - | + | - | - | - |
| Reducing sugars | Fehling's reagent | Brick red ppt. | + | + | - | - | - | - | - | - | ++ | - | - | - |
| | Benedict's reagent | Brick red ppt. | - | - | - | - | - | - | - | - | ++ | + | - | + |
| Steroids | Salkowski test | Reddish-blue and green fluorescence | + | - | - | - | + | ++ | - | + | ++ | - | - | + |
| Anthraquinones | Bontrager's test | Pink colour | - | - | - | - | + | + | - | - | - | - | - | - |
| Proteins | Lugol's reagent | Faint yellow colour | - | - | - | - | - | - | - | - | ++ | + | - | - |
| | Millon's reagent | White ppt. | + | + | - | - | - | - | - | - | + | - | + | - |
| Saponins | 1% Lead acetate solution | White ppt. | - | + | - | + | - | + | - | + | + | + | + | - |
| Amino acids | Ninhydrin reagent | Purple colour | + | + | + | - | - | - | - | - | - | - | - | - |
| Lignin | Phloroglucinol + HCl | Red | ++ | + | - | - | ++ | + | - | - | ++ | - | + | |
| Tannins | 10% NH ₄ OH solution | Yellow | - | + | - | - | ++ | - | - | - | - | + | - | - |
| | 10% lead acetate solution | White | - | - | - | - | ++ | - | - | - | - | - | - | - |
| | 5% FeCl ₃ solution | Blackish-green colour | + | - | - | - | ++ | - | - | - | + | - | - | - |
| Flavonoids | Shinoda test | Magenta colour | ++ | + | + | + | +++ | + | + | + | +++ | + | + | + |
| | 10% NaOH solution | Yellow colour | ++ | + | - | - | +++ | - | - | - | +++ | + | - | - |

+ = Present ; - = Absent

Antioxidant activity study

ABTS radical scavenging activity- ABTS radical scavenging activity was determined following the standard method³². The stock solutions of 7.4mM ABTS·+ and 2.6 mM potassium persulfate were prepared. The working solution was then prepared by mixing these two stock solutions in equal quantities and allowing them to react for 12 hr at room temperature in the dark condition. After that the solution was diluted by mixing 1ml ABTS and 60ml methanol to obtain an absorbance of 1.1 ± 0.02 units at 734 nm using the spectrophotometer of the mixture. Each plant

sample extract of 150 µl was allowed to react with 2850 µl of the ABTS solution for 2 hrs in a dark condition. Then absorbance was taken at 734nm using the UV-VIS spectrophotometer (UV1800). The standard curve was prepared with ascorbic acid. Results were expressed in % of scavenging activity. The experiment was carried out in triplicate. The IC₅₀ value was determined from the % inhibition vs. concentration of different plant extracts and ascorbic acid by comparing the absorbance values of control (A₀) and test compounds (A_t). Radical scavenging activity was determined by the following formula -

Table 5: UV fluorescence nature of the powdered samples of the investigated plant.

| Materials and treatment | Leaf powder | | Stem bark powder | | Fruit powder | |
|---|---------------------|-------------------------|---------------------|-------------------------|---------------------|-------------------------|
| | Under visible light | Under UV light (366 nm) | Under visible light | Under UV light (366 nm) | Under visible light | Under UV light (366 nm) |
| Powder as such | Light bronze green | Grass green | Light buff | Pale cream | Light brown | Deep bronze green |
| Paper stretches with powder | Light bronze green | Grass green | Light buff | Pale cream | Light brown | Deep bronze green |
| Treated with 1N NaOH | Night | Night | Middle brown | Night | Dark brown | Royal blue |
| Treated with 1N HCl | Grass green | Azure blue | Middle buff | French blue | Middle brown | Oxford blue |
| Treated with 80% H ₂ SO ₄ | Night | Night | Night | Royal blue | Dark brown | Royal blue |
| Treated with Antimony trichloride | Night | Camouflage red | Middle buff | Rail blue | Middle brown | Aircraft blue |
| Treated with 50% HNO ₃ | Venetian red | Dark brown | Golden brown | Royal blue | Middle brown | Royal blue |
| Treated with 5% KOH | Night | Night | Dark brown | Oxford blue | Dark brown | Royal blue |
| Treated with Methanol | Olive green | Crimson | Middle buff | Cobalt blue | Middle brown | Arctic blue |
| Ethanol | Olive green | Dark crimson | Middle buff | Cobalt blue | Dark brown | Light violet |
| Treated with Acetone | Olive green | Terracota red | Middle buff | Light straw | Dark brown | Dark crimson |

% Radical scavenging activity = $(A_0 - A_t/A_0) \times 100$

DPPH radical scavenging activity- DPPH radical scavenging activity was determined following the standard method³². The stock solution was prepared by dissolving 24 mg DPPH in 100ml methanol. The working solution was obtained by mixing 10ml stock solution with 45ml methanol to obtain an absorbance of 1.1 ± 0.02 units at 515 nm using the UV-VIS spectrophotometer (UV1800). Plant extracts of 150µl volume then allowed to react with 2850µl of DPPH solution for 24 h in the dark. Then the absorbance was taken at 515nm. The standard curve was prepared with ascorbic acid. Results were expressed in % of scavenging activity. The experiment was carried out in triplicate. The IC₅₀ value was determined from the % inhibition vs. concentration of different plant extracts and ascorbic acid by comparing the absorbance values of control (A₀) and test compounds (A_t). Radical scavenging activity was determined by the following formula –

% Radical scavenging activity = $(A_0 - A_t/A_0) \times 100$

Total antioxidant activity- The total antioxidant activity of the extracts was evaluated by the phospho-molybdenum method³³. A 0.3 ml of plant extract was mixed with 3 ml of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in the test tubes. Then test tubes containing the reaction solution were incubated at 95°C for 90 min. After incubation, the absorbance of the test solution was measured at 695 nm using a UV-VIS spectrophotometer (UV1800) against blank after cooling at room temperature. The total antioxidant activity is expressed as the number of

milligram equivalent of ascorbic acid per gram sample. The calibration curve was prepared by mixing ascorbic (200, 150, 100, 50, 25 and 10 µg/ml) with methanol.

RESULTS

Foliar micromorphology: General descriptions of the epidermal cells, stomata and trichomes along with their measurements are given below.

Epidermis- Cells are irregular in shape in both upper and lower surfaces of the leaf. Cell walls are wavy in outline on both the lower surfaces. Size of the epidermal cells on upper surface is $52.96 \pm 4.24 \mu\text{m} \times 25.82 \pm 3.13 \mu\text{m}$ and it is $49.81 \pm 1.97 \mu\text{m} \times 24.95 \pm 4.20 \mu\text{m}$ on the lower leaf surface. Frequency of the epidermal cells is 731.30/mm² on the upper surface and it is 804.66/mm² on the lower surface. Palisade ratio is 3.02 (Fig. 4 and 5).

Stomatal complex- Leaves are hypostomatic type; stomata are present only on the lower epidermal surface. Stomata are strictly of paracytic type. Size of the stomata is $34.76 \mu\text{m} \times 19.72 \mu\text{m}$. Stomatal frequency is 183.75/mm². Stomatal index is 29.85 (Fig.- 5).

Trichomes- Trichomes are non-glandular, unicellular, horn shaped with pointed tips and present on both the surfaces of the leaf. Size of the trichomes of adaxial surface is $101.34 \mu\text{m} \times 17.76 \mu\text{m}$ and it is $99.23 \mu\text{m} \times 16.42 \mu\text{m}$ in case of abaxial surface. Frequency of trichomes is 1.42 /mm² and 1.92 /mm² for adaxial and abaxial surfaces, respectively (Fig. 6).

Vegetative anatomy

Table 6: Phytochemical profiles of the different parts of the investigated plant.

| Plant parts | Total phenolics (mg of GAE /g) | Total flavonoids (mg of CE/g) | Total tannins (mg of GAE /g) |
|-------------|--------------------------------|-------------------------------|------------------------------|
| Leaf | 71.23±1.21 | 11.24±1.01 | 5.23±1.79 |
| Stem bark | 126.20±2.34 | 18.56±3.21 | 38.98±0.85 |
| Fruit | 98.21±1.77 | 9.59±3.25 | 29.68±1.92 |

Table 7: Antioxidant potential of the ethanolic extracts of the investigated plant.

| Plant part | IC ₅₀ value of DPPH radical scavenging activity± SEM (µg/ml) | IC ₅₀ value of ABTS radical scavenging activity± SEM (µg/ml) | Total antioxidant capacity (mg AAE/g) ± SEM |
|------------|---|---|---|
| Leaf | 145.83±1.87 | 186.27±1.01 | 13.45±2.11 |
| Stem bark | 79.74±2.46 | 73.87±3.14 | 41.20±4.29 |
| Fruit | 117.93±2.44 | 109.26±0.84 | 31.23±0.47 |

Stem anatomy- Cross-section of the stem is almost circular in outline. The epidermis is uniseriate, cuticularised and many non-glandular unicellular, horn shaped trichomes are present on it. Cortex is made of- a) 6 to 8 cell layers of thick Collenchymatous hypodermis, and b) 40-45 layers of parenchymatous zone. Sclerenchymatous patches are present just above the phloem layer. Vascular bundle is collateral, conjoint and open type with phloem and xylem. At the centre of the stem massive, parenchymatous pith is present (Fig. 2).

Petiole anatomy- In T.S. outline of the petiole is plano-convex, eye shaped. Here epidermis is uniseriate with many trichomes. Beneath the epidermis, 2-3 layered hypodermis is present; followed by 40-43 layers of ground tissue. Vascular bundle is 7 in number; central one is largest and 'horse shoe' shaped. There are 3 vascular bundles on either side of the central bundle and they are circular in outline. The size of these vascular bundles is gradually decreased towards the end of the wing (Fig-3).

Xylem element study: General description along with measurements of the xylem elements of stem has been presented below.

Vessel elements- Perforation plates of the vessel elements are simple and are transverse or obliquely placed. Pits on the side wall of the elements are simple and arranged in horizontal rows. Tail absent. Size of the vessel element is 152.56 µm × 34.26 µm and frequency is 25.63/mm² (Fig-7).

Tracheids- They are very long and with spiral side wall thickening. Diameter of the tracheid is 25.61µm and frequency is 29.59/mm² (Fig -8).

Fibres- Fibres are typically libriform type with pointed ends. Pits present. Size of the fibre is 215.6µm × 14.83µm and frequency is 53.85/mm² (Fig -9).

Histochemical study: Histochemical study has been carried out to detect various phytochemicals groups localized in different tissue zones of the stem. Different phytochemical groups like tannins, proteins, alkaloids, lignin, saponins, etc. have found localized in different tissue zones of the stem. It has also been observed that vascular bundles and cortical zone are the main active sites for synthesis of different phytochemical groups.

Organoleptic features of the powdered plant samples: The colour, odour, taste and texture of the three parts of the investigated plant have been presented in the table below.

Moisture content and Ash value: Moisture content and ash values of the fruit, leaf and stem bark powder drugs are given in tabular form (Table-2). Moisture contents of leaf, stem bark and fruit powder were 19.41%, 10.21% and 16.33%, respectively. Ash values of powdered leaf, stem bark and fruit were 2.65%, 5.6% and 10.0%, respectively. In leaf, values of acid insoluble ash and water soluble ash were 2.4% and 3.21%, respectively. Values of acid insoluble ash and water soluble ash in stem bark were 1.2% and 4.69%, respectively. The percentage of acid insoluble ash and water soluble ash in fruit was 1.02% and 5.42%, respectively.

Extractive value: Percentage yield of individual solvent extracts of the plant parts (extractive values) varies according to the nature of the solvent. It is found that extractive value of ethanolic extract was highest among the four solvent extracts for all three parts of this plant. In three different parts of the investigated plant, the extractive values for polar solvents (i.e. Ethanol and ethyl acetate) were much higher than that of non polar solvents (i.e. Chloroform, hexane).

Preliminary phytochemical screening of the powdered plant samples: Phytochemical screening of different solvent extracts of leaf, stem bark and fruit parts of the investigated plant showed presence of different phytochemical groups in varying degrees (Table-4).

Fluorescence analysis: The drug powders of the plant parts treated with different chemical reagents gave characteristic colour when seen under UV light (366 nm) and it was compared with colour observed under ordinary light. In some cases, marked differences in colour change were observed when different solvent treated powdered drugs seen under UV light (366 nm) the name of the colours have been given with the help of British Standard Colour Chart (<http://www.britishstandardcolour.com>) (Table - 5) (Fig.- 13-18).

Total phenolic content: Phenolics are one of the major groups of antioxidant compounds reported to be involved in free radical scavenging activity and also responsible for curing a wide range of ailments. Total phenolic contents in leaf, stem bark and fruit were 71.23 mg of GAE/g tissue, 126.20 mg of GAE/g tissue and 98.21 mg of GAE/g tissue, respectively. Highest content of phenolic compounds was observed in stem bark among the three parts investigated (Table- 6).

Total flavonoid content: Flavonoids are very important group of phenolics that show a wide range of therapeutic properties. Total flavonoid contents in leaf, stem bark and fruit were 11.24 mg of CE/g tissue, 18.56 mg of CE/g tissue and 9.59 mg of CE/g tissue, respectively. Flavonoid contents were significantly higher in both the leaf and stem parts than the fruit (Table -6).

Total tannin content: High content of tannins was observed in stem bark part (38.98 mg of GAE /g) which is followed by the tannin contents of fruit (29.68 mg of GAE /g) and leaf (5.23 mg of GAE /g) (Table -6).

DPPH radical scavenging activity: DPPH radical scavenging activity was significantly high for ethanolic extract of stem bark of the investigated species. IC₅₀ value of the stem bark extract was 79.74 µg/ml, for fruit it was 117.93 and for leaf it was 145.83 µg/ml. Stem bark part showed more antioxidant potential in respect of DPPH radical scavenging activity than leaf part of this plant (Table -7).

ABTS radical scavenging activity: ABTS radical scavenging activity was significantly high for ethanolic extract of stem bark part of the investigated species. IC₅₀ values of the stem bark, leaf and fruit extracts were 73.87, 109.26 and 186.27 µg/ml, respectively. Stem bark showed more antioxidant potential in respect of DPPH radical scavenging activity than root part of this plant (Table -7).

Total antioxidant activity: The total antioxidant capacity (TAC) is based on the reduction of valency of Molybdenum from 6 to 5 by the extract and subsequent formation of green phosphate- molybdenum complex at acid pH. It is employed to evaluate the total antioxidant capacity of both water- and fat-soluble antioxidants. Total antioxidant capacity of the ethanolic extract of stem bark part was 41.20 mg AAE/g and for leaf and fruit parts it was 13.45 mg AAE/g and 31.23 mg AAE/g, respectively (Table-7).

DISCUSSION

Present investigation reveals some of the characters obtained from the pharmacognostic, physicochemical and phytochemical studies, are found very distinct and they can be used as marker for the identification of the crude drugs in its fresh as well as dried form obtained from leaf, bark and fruit of the plant *Gardenia latifolia* Aiton. Foliar micromorphology does have immense importance in plant identification and also in authentication of leaf drugs^{34,35}. Here in this study, it has been observed that epidermal cells are irregular in shape in both upper and lower surfaces of the leaf. Cell walls are wavy in outline on both the surfaces. All these features of leaf epidermal cells provide distinctiveness to certain extent which will help in identification of leaf part of this plant. Palisade ratio is 3.02 which is also specific to this medicinal species. Studies of stomata can have a great taxonomic as well as pharmacognostic value in proper identification of different plant taxa including medicinal plants. Stomata are strictly of paracytic type and present only on lower epidermal surface. Stomatal index is 29.85, which is very distinct for this particular taxon. Trichome features are also very important in proper identification of the plants and

considered as one of the valuable taxonomic marker now^{36,37,38,39}. Trichomes here are non-glandular, horn shaped, unicellular with pointed tip and present on both the surfaces of the leaf. Size of the trichomes of adaxial surface is relatively larger (101.34 µm x 17.76 µm) than the trichomes of abaxial surface (99.23 µm x 16.42 µm). In the petiole, number of vascular bundle is 7 and middle bundle is largest one, horse shoe shaped. Number and shape of the vascular bundles highlight here very marked character which may be considered as one of the important tools for proper authentication of leaf drug of this medicinal plant. In Pharmacognosy, the physicochemical characters plays a vital role in setting fingerprint for a crude drug and are successfully employed in detection of adulterants and improper handling of the crude drug^{22,23,24,25,26}. Moisture content of a crude drug is an important parameter in respect of its shelf life because insufficient drying favours the growth of molds and microorganisms which ultimately spoil the biomass and active principles of the crude drugs. So, moisture content is directly related to maintain the stability and quality of crude drugs. In this study, a noticeable difference was observed between moisture contents of leaf (19.41%), stem bark (10.21%) and fruit parts (16.33%) of this plant. Among the physical constants, ash value is considered as an important tool in appraisal of purity and identity of a crude drug and ash value also highlights the inorganic matter present in the crude drug^{22,23}. In this study, it is also noticed that total ash content is greater in fruit (10%) than the stem bark (5.6%) and leaf (2.65%) which indicates that fruit part of this medicinal plant consists of more amounts of inorganic minerals like carbonate, oxalate, phosphate including silica and siliceous earthy matters. Values for various parameters of ash observed in all the three parts of this plant are different and distinct from one another which can be used as identifying marker in authentication of the crude drugs obtained from the leaf, bark and fruit of the plant *G. latifolia* and also for quality control of those crude drugs. In pharmacognostic evaluation of the crude drugs, extractive value is considered as one of the diagnostic features and is used in proper identification of the crude drugs. It also determines the amount of active constituents extracted by the particular solvent from certain amount of the crude drug³⁹. Extractive value helps to indicate the nature of chemical constituents present in the drug and also useful in estimation of specific constituents soluble in a particular solvent. Values of the extractable matters vary according to the polarity of solvent and purity of the crude drug. Here in this study, ethanol was found to be the best extractive solvent among the four solvents used as it extracted out highest yield of the chemical constituents from all the three parts of this plant investigated. Stem bark extracted in ethanol showed maximum extractive value (7%) which revealed the presence of greater amount alcohol soluble phyto-constituents such as alkaloids, flavonoid, phenolic, terpenoids and steroids in the stem bark than other two parts like leaf (1.9%) and fruit (3.1%) investigated. The other solvents like, ethyl acetate, chloroform and hexane showed very low extractive values which indicate that comparatively lesser number and

amount of extractable phytochemical groups (phytosterols, fixed oils, fats, waxes, etc.) have been leached out from the three parts of the plant. So, it can be concluded that alcoholic solvent is the best option among the solvents taken here for extraction of phytochemicals and ethanolic extractive values can be used as marker for identification of the crude samples of all the three parts of this medicinal plant.

The fluorescence analysis of the crude drug powder produces characteristic colour changes when the drug samples treated with different chemical reagents are exposed to UV light. This unique colour change is used as a finger print for proper identification of crude drugs when other physical and chemical parameters of the crude drugs felt inadequate²². The same drug powder treated with various chemical reagents appears with different colours when seen under different wavelength of light. Here, methanol, ethanol and acetone treated powdered samples of three plant parts showed characteristic colour changes when illuminated under UV light which are quite distinct from its colour observed under visible light. That marked changes in colours under UV light provide very distinct characters which are specific to the respective crude drugs obtained from the investigated three parts of this plant.

Plants possess numerous phytochemical constituents, many of which are known to be biologically active compounds and are responsible for exhibiting diverse pharmacological activities. Chemical analysis and biological assay are considered as very important aspects in pharmacognostic evaluation of crude drugs obtained from medicinal plants^{40,41,42}. Preliminary phytochemical screening is useful in prediction of the nature of crude drugs and also valuable for detection of phytoconstituents present in it. The important phytochemical groups detected from the leaf, stem bark and fruit of this investigated plant are alkaloids, anthraquinones, phenolics, saponins, tannins, glycosides, etc. Presence of such important phytochemical groups in three different parts of this medicinal tree clearly indicates their therapeutic properties and also validates to some extent the wide range of ethnomedicinal uses of this investigated medicinal plant.

Among all the secondary metabolites found in plants, phenolics are known one of the major therapeutically significant phytochemical groups. Plants have diverse groups of phenolic compounds, such as simple phenolics, phenolic acids, anthocyanins, hydroxycinnamic acid derivatives and flavonoids. All these classes of phenolics have gained extensive attention because of their wide range of physiological functions, including free radical scavenging, anti-mutagenic, anti-carcinogenic and anti-inflammatory effects^{40,41}. It has been reported that the antioxidant activity of phenolic compounds are mainly due to their redox potential, hydrogen donating and singlet oxygen quenching properties⁴³. From this study it is evident that different parts of the plant *G. latifolia*, contain quite good amounts of phenolics [range from 71.23 to 126.20 mg GAE/ g] and flavonoids [range from 9.59 to 18.56 mg CE/ g] which highlight the medicinal properties of this plant used in curing the skin infection, wounds, eczema, etc. It is also well established that phenolics,

flavonoids and tannins are very much effective against various types of inflammation, wounds and body pain^{44,45}. Presence of good amount of these therapeutically active compounds also highlights the prospect of this plant to be an effective anti-inflammatory drug source.

Here antioxidant activities of the ethanol extracts of selected plant parts were assessed using the DPPH, ABTS and TAC assay which are most widely used methods for estimation of antioxidant activity of phytochemicals. Plant extracts rich in phenolics and other antioxidant phytochemicals exhibited significantly low IC₅₀ value. Here in DPPH radical scavenging assay, stem bark extract shows the lowest IC₅₀ value (79.74 µg/ml) among the plant parts investigated (for fruit IC₅₀ value is 117.93 µg/ml and for leaf it is 145.83 µg/ml). In ABTS radical scavenging activity study, IC₅₀ value shows the same trend, that is, stem bark again showed the lowest value (73.87 µg/ml) among the parts studied. Total antioxidant capacity of the ethanolic extract of stem bark part is 41.20 mg AAE/g which indicates the highest value for its total antioxidant activity. The other two parts such as fruit and leaf showed lower value of total antioxidant activity, i.e. 31.23 mg AAE/g and 13.45 mg AAE/g, respectively. Moreover, all the three parts of the investigated plant showed variable IC₅₀ value which is due to unequal distribution of antioxidant molecules such as phenolics, flavonoids, etc. identified in those different parts of this medicinal plant. Based on the results obtained here in this study, it was found that the ethanol extracts of stem bark showed greater antioxidant activity in all the three methods employed here, than the antioxidant activity recorded in the other two parts of this plant. This higher antioxidant activity of stem bark is correlated with its higher contents of phenolics, flavonoids and tannins estimated. The scavenging compounds act in a synergistic manner which enhance the free radical quenching activity by several folds^{42,43}. The compounds of different phenolic groups have the functional groups including hydroxyls that are responsible for their radical scavenging activity. This result confirms the importance of different groups of phenolics as the potential antioxidant agents^{44,45,46}.

Antioxidant activity of different parts of *Gardenia latifolia* is nicely correlated with the total phenolic, total flavonoid and total tannin contents of the leaf, stem bark and fruit parts which illustrate the species as a potent source of antioxidant substances and simultaneously encourages the scientific world to investigate novel antioxidants as well as therapeutically active natural products. Among the leaf, stem bark and fruit parts, it was found that stem bark is more potent in respect of its phytochemical content and antioxidant activity. Further scientific studies of all these three parts especially the stem bark of this medicinal plant are highly recommended to standardize noble antioxidant phytochemicals. Some of the pharmacognostic characters obtained through this study will be used as marker in proper identification of the crude drugs obtained from the leaf, stem bark and fruit of *Gardenia latifolia* and they will also be helpful in detection of its adulterants.

ACKNOWLEDGEMENTS

We are thankful to the Head, Department of Botany, Visva-Bharati, for providing the necessary laboratory facilities. We are also thankful to the UGC for financial assistance sanctioned in the form DRS-SAP Phase II research programme.

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