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Pharmacognostic Investigations of *Impatiens balsamina* Linn.

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Abstract: Herbal medicines are the first type of medical system ever developed for treatment. The increasing studies on medicinal plants influenced the construction of an unique herbal pharmacopoeia and transformed how it was processed and administered. The pharmacognostic study of plants is an important parameter for quick identification and authentication of plants with less time and cost. *Impatiens balsamina* (Balsaminaceae) was commonly called garden balsam or rose balsam, native to India and Myanmar. The studies were carried out to evaluate different parameters, including macroscopy, microscopic characters and physicochemical parameters, by performing standard procedures. The detailed characteristics of leaf, stem and root with intensive quantitative microscopy were performed. In physicochemical analysis, various parameters like ash values, foreign organic matter, extractive values, foaming index and loss on drying were performed. In the current study, pharmacognostic factors for quick plant identification and authentication macroscopic and microscopy were investigated, which will be helpful for further research on plant.

Introduction

The commercial, nutraceutical, food beverages, cosmetic and pharmaceutical industries all employ botanicals as a major source of raw materials. According to the World Health Organization (WHO), traditional medicine is a set of practices, theories and methods that includes drugs derived from plants, animals and minerals as well as physical and spiritual therapies, it can be utilized separately or in combination to treat, identify and prevent ailments, as well as to promote wellbeing (Kabra et al., 2019; Bhinge et al., 2022; Raghuvanshi et al., 2022).

Impatiens balsamina (IB) belongs to the Balsaminaceae family. It is an annual herb with a height ranging from 60-100cm. It is native to Western Asia, China, India, Sri Lanka, Myanmar and Malaysia. Traditionally, the plant is used as an emetic, diuretic and cathartic. It is useful in treating joint pain and used against warts. Crushed leaves are used against skin inflammation and a combination of salt with castor oil is used to treat whitlow around fingers and torn nails of fingers and legs. The decoction obtained from roots is used to treat irregular menses. The roots are well known to treat skin inflammation and torn nails. The seed is used as an expectorant and shows anticancer activity. Seeds in powdered form are given to women during labour pain to get strength. The flowers are mucilaginous in nature and used as atonic. They have a cooling effect when applied to burns. Snake bites can be treated by juice of flowers. Flower extracts showed antibiotic activity against fungi

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and viruses (Duke et al., 1985; Chopra et al., 1986; Meenu et al., 2015; Qianet al., 2023).

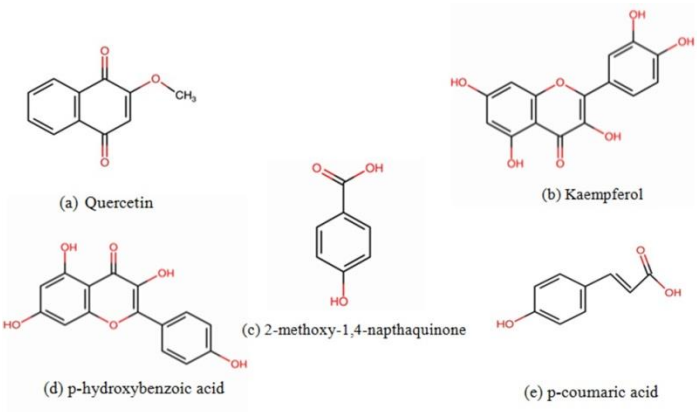

Figure 1. Some major chemical constituents present in *Impatiens balsamina* L.

The plant contains sterols, phenols, fatty acids, flavonoids, naphthoquinones, coumarins, terpenoids and ester derivatives of naphthalene, nitrogen-containing substances and polysaccharides other substances. The main and most representative component of *Impatiens balsamina* is 2-methoxy-1,4-naphthoquinone, one of the naphthoquinones (Lawsone) and many more viz., Quercetin, kaempferol, p-hydroxybenzoic acid, p-coumaric acid, flavone glycoside, phenolic compounds, oleanane-type triterpenoidal glycosides, saponin and some bioactive compounds were reported given in Figure 1 (Lim et al., 2007; Lei et al., 2010; Li et al., 2013; Kang et al., 2013; Kim et al., 2015; Li et al., 2017; Szewczyket al., 2018; Lee et al., 2020; Hariyanto et al., 2018; Pires et al., 2021; Thiraviya and Anuradha, 2021; Qian et al., 2023). *Impatiens balsamina* is reported to have several pharmacological activities including antitumor, anti-fungal, anti-hepatic fibrosis, antidiabetic, antinociceptive activity, antibacterial, allergy-preventive effects, antioxidant, antimicrobial, wound healing, antiarthritic activity, antipruritic, antidermatitic effect, anthelmintic activity, antihistamine effects, antinociceptive activity, anti-inflammatory as well as toxicological studies (John et al., 1948; Fukumoto, 1995; Ding, 2008, Iwoka et al., 2010; Jain, 2011, Oku et al., 2011; Imam et al., 2012; Kang et al., 2013; Debashree et al., 2013; Onget al., 2015; Li et al., 2015; Manikandan et al., 2016; Hariyanto et al., 2016; Qian et al., 2017; Hariyanto et al., 2018; Yasodha et al., 2021; Deguet al., 2021; Qian et al., 2023).

It is crucial to understand crude drug pharmacognostic properties exactly and in-depth because they have significant exploitation, utilisation value. First step in this direction is a detailed pharmacognostic evaluation of herb, which provides information on the herb‘s visual appearance, microscopy and physical characteristics. Here, we have selected *Impatiens balsamina* and performed a comprehensive examination of its macroscopic, microscopic, physicochemical analysis to support its identification and standardisation of leaf, stem and root.

Methods and Materials

Plant material collection and authentication

Impatiens balsamina plant was collected in December 2022 from Nigdi, Pune, Maharashtra, India. The plant herbarium was prepared at Botanical Survey of India, Pune region, for confirmation, where a voucher specimen of plant was registered with No. BSI/WRC/ Tech./2023/02 given in Figure 2A. Fresh leaves stem and roots were evaluated for macroscopic and microscopic studies. The remaining raw material of the plant was dried under shade for 8 to 10 days. The dried material was made in powder form and then kept in an airtight container till further usage to avoid moisture absorption and contamination.

Macroscopic study

Studying many aspects of plant material, such as its macroscopic size, shape, colour and odour, allowed for a macroscopic examination.

Microscopic study

The transverse sections of leaf, stem and root were cut using a sharp blade and surface preparation prepared. All sections were stained with phloroglucinol: HCL, Sudan red, mount on slides and covered with a cover slip. These slides were observed under a microscope at100x and 400x.

Physicochemical evaluation

The physicochemical evaluation of *Impatiens balsamina*, including foreign organic matter, foaming index, loss on drying, ash and extractive values, were performed using standard methods.

Foreign organic matter

It describes substances that are either not included in herbal medicines or are made up of ingredients that are not originally from plants. 1 gm sample of *Impatiens balsamina* spread on white tile and foreign organic matter was separated and weighed (Anonymous, 1998; Khandelwal., 2008).

Loss on drying

The most common procedure for determining moisture content is to heat the crude drug till it gets a consistent weight at 100^0C from its initial weight. Moisture content of *Impatiens balsamina* plant was determined as per procedure (Khandelwal., 2008; Ankalge et al., 2021).

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Foaming Index

It is carried out in accordance with the established protocol in the WHO guidelines (Anonymous, 1998).

Ash value

A crude drug's quality and purity are assessed using its ash value. Ash value of *Impatiens balsamina* plant was determined as per procedure (Anonymous., 1998; Khandelwal., 2008; Ankalge et al., 2021).

Extractive values

This method calculates the amount of active ingredients that can be extracted from a specific quantity of medicinal plant material using specific solvents. *For example, the extractive value of Impatiens balsamina* plant was determined per procedure (Anonymous., 1998; Khandelwal., 2008; Ankalge et al., 2021).

Results:

The Pharmacognostic evaluation of plant *Impatiens balsamina* was done. Macroscopic evaluation of the leaf revealed that it is green, while the stem is slightly yellowish green, and where roots are light yellow, as shown in Figure 2. The leaves are arranged alternately. The length is 2–9.5 cm and 0.5–2.8 cm in width. It has a dentate margin.

Figure 2. Parts of *Impatiens balsamina* Linn. A: Flower, B: Fruit, C: Seeds, D: Leaf, E: Whole plant, F: Stem and G: Roots

The stem is simple or branched and grows up to 60-65cm in height, where roots reach up to 9cm. Detailed microscopic evaluation of plant parts like leaf, stem and root were performed. Transverse section of leaf was shown presence of normocytic stomata with two kidney-shaped guard cells surrounded by 5 subsidiary cells, epidermal cells with wavy wall sphaera-raphide calcium oxalate crystals, unicellular glandular trichomes as well as non-lignified fibres arising from the epidermis given in Figure 3.

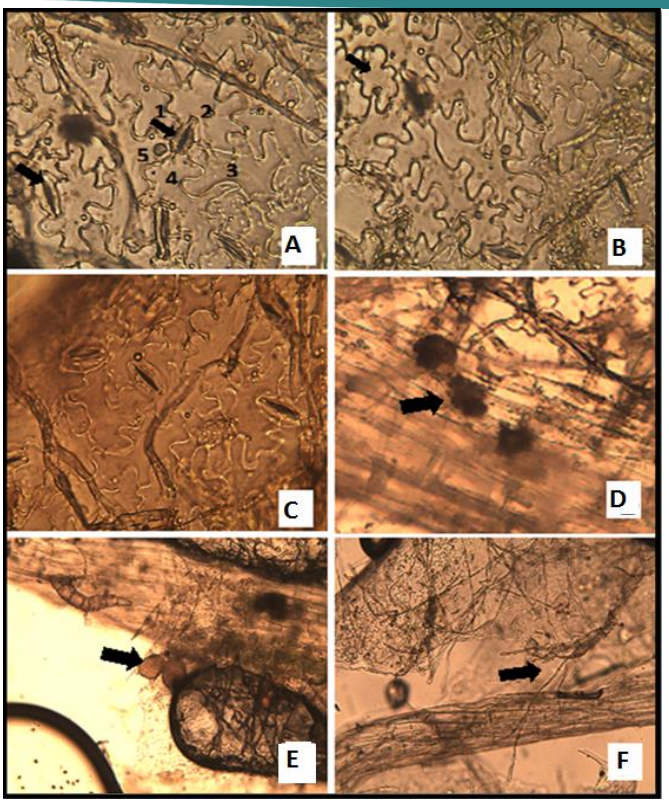

Figure 3. Surface view of the leaf epidermis A: anomocytic stomata with two kidney shaped guard cell surrounded by 5 subsidiary cells (450x); B: epidermal cell with wavy wall (450x); C: stomata with 4-5 subsidiary cells (450x); D: epidermis containing sphaera-raphide calcium oxalate crystals (450x); E: unicellular glandular trichomes (100x); F: non-lignified fibres arising from the epidermis (100x)

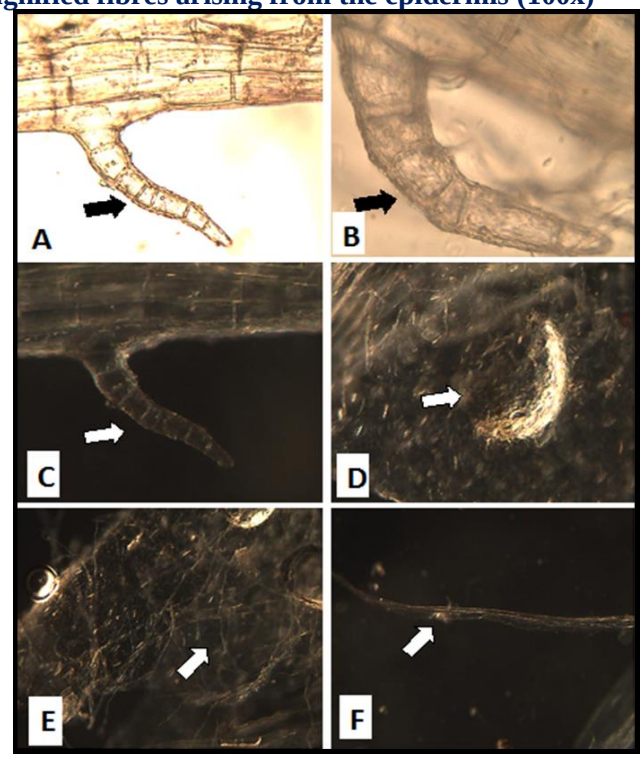

Figure 4. Epidermis of *Impatiens balsamina* leaf A: covering multicellular trichome (100x); B: covering multicellular trichome (450x); C: covering multicellular trichome dark field (100x); D: unicellular glandular trichomes dark field (450x); E: epidermis contain acicular calcium oxalate crystals dark field (100x); F: non-lignified fibres arising from the epidermis dark field (100x)

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Epidermis of *Impatiens balsamina* leaf, when observed under dark field, presence of covering multicellular trichome, epidermis containing acicular calcium oxalate crystals and non-lignified fibres arising from the epidermis were seen given in Figure 4.

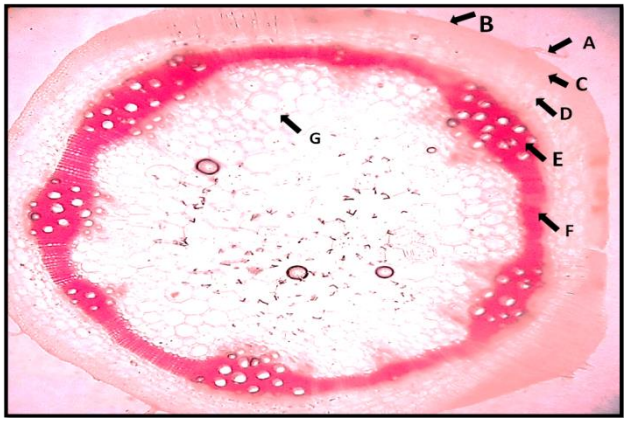

Figure 5. Transverse section of *Impatiens balsamina* stem stained with Phloroglucinol: HCl (1:1) (100x) A: unicellular trichome; B: Unicellular layer of circularised epidermis; C: Palisade-like cells, D: Cortex E: lignified vascular bundles with xylem vessels and compactly arranged phloem; F: lignified inter fascicular cambium; G: lignified Pith cells

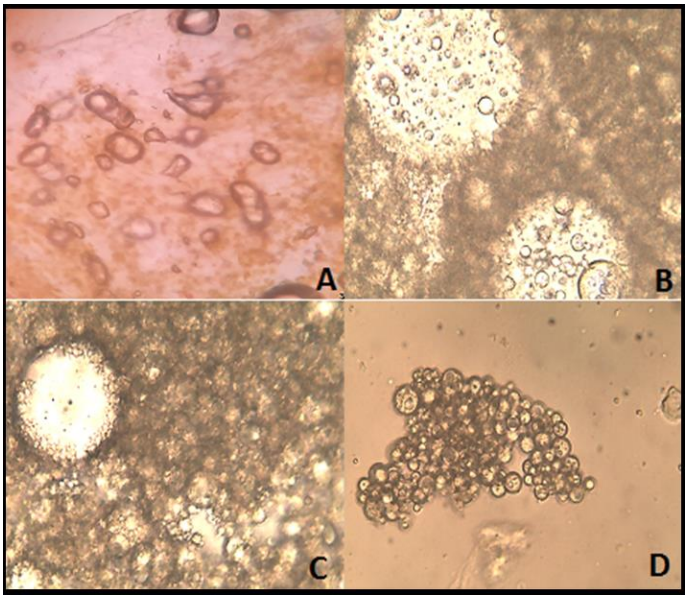

Figure 6. Transverse section of *Impatiens balsamina* stem A: Calcium oxalate crystals (400x); B: Xylem vessels (400x); C: Xylem vessels and xylem parenchyma (100x); D: Pith cells (100x)

Impatiens balsamina stem transverse section stained with Phloroglucinol: HCl (1:1) and observed under 100x presence of numerous unicellular trichomes were seen in Figure 5. An unicellular layer of circularised epidermis surrounded by cuticle and below that single layer of palisade-like cells were observed under 100x. In the cortex region, 4 to 5 layers of cells were seen in Figure 5 under 100x, and the presence of square and prism-shaped calcium oxalate crystals seen under 400x. Below cortex, lignified vascular bundles with xylem vessels and compactly arranged phloem, lignified inter fascicular cambium is observed in under 100x. In the central part of the transverse section of stem, thin-walled lignified pith cells were seen in Figure 6 under 400x.

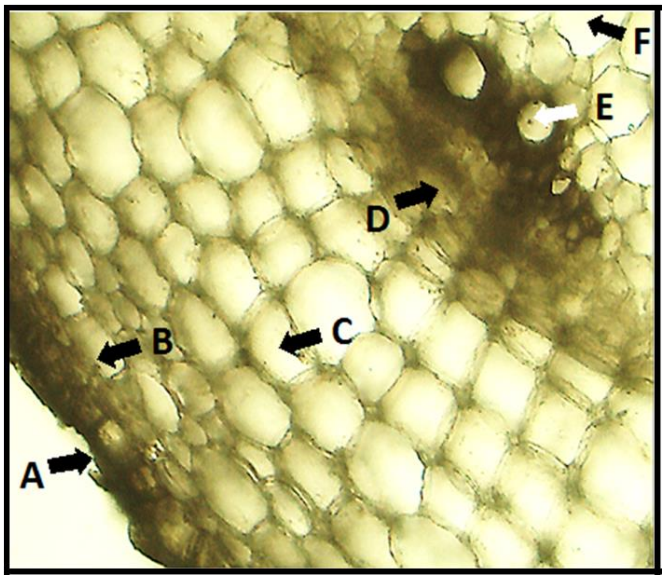

Figure 7. Transverse section of *Impatiens balsamina* root (400x) A: unicellular suberized cork cell layer; B: tangentially elongated indistinct phellogen and phelloderm layers; C: Cortical parenchyma cells, D:compactly arranged phloem; E: lignified xylem vessels; F: Pith cells

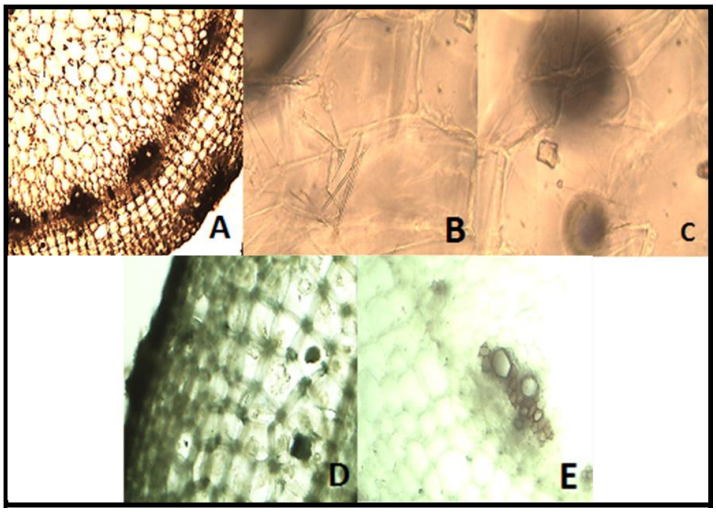

Figure 8. Transverse section of *Impatiens balsamina* root; A: Different layers of the anatomical structures (50x); B: Long acicular calcium oxalate crystals (400x); C: Prismatic calcium oxalate crystals (400x); D: Rosette calcium oxalate crystals (400x); E: lignified xylem vessels

The transverse section of root observed under 400x presence of unicellular suberized 2-3 cork cell layer, followed by tangentially elongated indistinct phellogen and 5-6 parenchyma cells layers in phelloderm containing long acicular, prismatic and rosette shaped calcium oxalate crystals. The presence of a compactly arranged bunch of phloem and lignified xylem vessels were also

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observed. Pith contains parenchymatous cells given in Figure 7 and 8.

Phytochemical screening of aqueous, ethanolic and petroleum ether extracts showed presence of carbohydrates, amino acid, protein, steroids, glycosides, tannins, saponins and flavonoids. Physicochemical evaluation analysis includes foreign organic matter, loss on drying, foaming index, ash and extractive value in Table 1.

between the patches of xylem and phloem in the stem. Moreso, the pith cells in were lignified and numerous calcium oxalate were present in the stem signifying the role of lignin and crystals in maintaining rigidity. Thus, it is clear from the fact that the plant shows secondary growth due to presence of inter fascucular cambium. However, the type of vascular bundle in leaf was of collateral nature wherein the xylem and phloem lie

Table 1. Physicochemical evaluation of crude drug as per WHO guidelines

Powdered drug	Foreign organic matter	LOD (% w/w)	Foaming Index	Percent Total Ash	Extractive value (% w/w)	
					Alcohol soluble	Water soluble
Leaves	Less than 1%	2.25	Less than 100	4	10	14.5
Stems	Less than 1%	3.25	Less than 100	11	11	13.5

Discussion

Owing to the increased discoveries in therapeutic benefits of *Impatiens balsamina* plant, need arises for its authentication not merely by morphological studies but microscopical as well because, mostly drug is use in powdered form. Thus, the current work focuses specifically upon morphological and microscopical investigations of the leaves and stems of *Impatiens balsamina*. Alternate arrangement, presence of reticulate veination and dorsiventral nature of the leaves substantiates its closeness to the dicot class. However, three ridges in its pollen grain make it suitable for eudicot clade (Furness and Rudall, 2004). The normocytic stomata show the presence of two kidney-shaped guard cells surrounded by 5 subsidiary cells and was clearly observed with *Impatiens balsamina* leaves. The sphaeraraphide crystals impart rigidity to the leaves in order to withstand wind blows. Development of calcium oxlate crystals results due to lack of sclerenchyma. Unicellular glandular trichome houses pesticidal components to protect itself from leaf borers. Moreover, numerous non-lignified fibers can be seen originating from its epidermis that helps maintain flexibility to the leaf structure. Multicellular covering trichomes help in protection of the leaf from smooth-skinned insect.

The staining with pholoroglucinol : HCl (1:1) imparts pink-red color to the lignified cells due to formation of stable complex. This pink-red color was clearly observed in stems and leaves indicting the distribution of lignin. The stem vascular bundle were found to be of conjoint, collateral open type as cambium ring was clearly visible adjacent to each other.

Foaming index for both stem and leaf powder was observed to be below 100 indicating absence of considerable quantity saponins. The total ash value indicates both the physiological and non-physiologial inorganic material present in the plant and was found to be lower. Alcohol soluble extractive value was close to 10 for both leaves and stems indicating the presence of lipophilic and amphipolarphyto-constituents. However, the water soluble extractive value yields the polar components of the plant such as sugars, salts and glycosides that were on higher side.

Conclusion

The evaluation parameters studied in present study will be supportive data in the quantitative and qualitative standardisation of *Impatiens balsamina*. The phytochemical studies of different extracts were carried out, and it would need further technical examination to establish its chemical identity using chemical and biological markers for its authentication.

Conflict of Interest

None

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