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LEAF VENATION PATTERN IN MEDICINAL AND AROMATIC PLANTS

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ABSTRACT

The study of leaf venation pattern helps us to identify some evolutionary clues and the taxonomic studies. The venation patterns in the leaves differentiate the species from each other and helps in identification of new plant species. In herbal trade, leaf based herbal materials are need to be identified due to its adulteration and misidentification. In this study, 15 different medicinal and aromatic plant species were collected from Guntur region of Andhra Pradesh (India) and the leaf venation characteristics were studied. The leaves are analyzed for macroscopic and microscopic characteristics such as leaf size, shape, margin, type of leaf, major and minor venation pattern. In all the evaluated plant species pinnate type of venation was observed except in *Mangifera indica, Rosa damascena* (reticulate) and *Centella asiatica* (palmate). The minor venation pattern is unicostate in all plant species except *Centella asiatica* which is a multicostate. Compound type of leaf was observed in *Azadirachta indica*, *Rosa damascena* and *Jasminum officinale* and the remaining plant species have simple type of leaf. The macro and microscopic studies of leaves help in identification and differentiation of plant species and the venation pattern would significantly contribute to enrich the taxonomic attributes of the plants studied.

Key words: Adulteration, Aromatic plants, Botanical identification, Herbal trade, Leaf venation pattern, Medicinal plants, Taxonomic studies

INTRODUCTION

In recent years, the medicinal and aromatic plant species are attracting the attention of more and more researchers due to their commercial potential [1]. Over centuries, the people around the world have used medicinal and aromatic plants to fight against various illnesses and maintained the overall health [2,3]. Every part of the medicinal and aromatic plant including seeds, roots, stem, leaves, bark, flowers and fruits are said to have medicinal value [1]. Among these, leaves are most frequently used for the treatment of various diseases followed by other parts of the plant [4]. Traditionally, the morphological features such as size and shape of the leaf are used to differentiate various plant species [5]. Leaves vary among each other by their size, shape and other characteristics like the blade margin and the venation patterns of the leaf lamina [6]. The architecture of the leaves shows a great variance in the angiosperms [7]. The veins in the leaves act as a support to the leaf lamina helping the leaf to maintain its three-dimensional structure and also the transportation of water from roots to leaves [8]. Leaf shapes vary accordingly and include oval, linear, elliptic, cordate, reniform and so on [9]. Veins contribute to the taxonomic character of the leaf through which one can differentiate different species of plants [6].

Phenotypic characters of the plants are important to be studied as it helps us to differentiate among the plant species by their physical appearance such as texture, color, size, shape and venation pattern [10,11]. The study of leaf venation and its pattern provides information related to photosynthetic performance and it has several applications in plant technology, agriculture and paleobiology [12]. Leaves possesses characteristic aroma, flavor and pungent taste due to the presence of aromatic compounds [13]. Leaves are used as herbal teas, beverages, spices, churnas and powders [14]. In economical point of view, the leaves are adulterated with other plant leaves which upon consumption results into adverse effects [15]. Commercially, the medicinal and aromatic plants leaves are the most used parts followed by stem, roots and flowers [16]. Despite the wide use and commercialization of medicinal and aromatic plants, the botanical quality of the samples is not always genuine or pure due to adulteration and plant misidentification [15]. These often results in poor quality of the sample, substituted sample and adulterated sample that affect the effectiveness and safety of the product [17]. The morphological and organoleptic evaluation of the raw material of medicinal herbs and spices are essential in view of herbal adulteration [18]. Identifying the spurious plant, authentication of the authentic plant is required in herbal raw material trade [19]. The morphological features evaluating the leaf based herbal raw materials are leaf size, shape, petiole, venation, leaf base and leaf apex [20,21].

A few studies are reported related to the venation pattern of medicinal and aromatic plants compared with other plant species [22-24]. Foliar venation helps

in the study of various species to specifically identify or describe a particular species collected [25]. In the present study, the morphological features such as leaf size, shape, petiole and leaf venation pattern in fifteen different plant leaves belonging to eleven taxa of medicinal and aromatic plants category are reported. The findings of venation pattern may help to identify an adulterated herbal material and also helps in the authentication of the authentic plant material in herbal trade.

MATERIALS AND METHODS

Collection of Plant material

A total of fifteen different medicinal and aromatic plants leaves are collected from different habitats. The selected plants are *Mytragyna speciosa* (Korth) Havil., *Mangifera indica* L., *Coleus blumei (Synonym:Coleus Scutellarioides)* (L.) Benth, *Azadirachta indica* A. Juss., *Rosa damascene* Mill., *Jasminum officinale* L., *Allamanda cathartica* L., *Tecoma gaudichaudi* (Synonym: *Tecoma stans* (L.) Juss. ex Kunth), *Nerium oleander* L., *Ocimum tenuiflorum* L., *Pentalinon luteum* (L.) B.F.Hansen & Wunderlin, *Andrographis paniculata* (Burm.f.) Nees, *Centella asiatica* (L.) Urban, *Ruellia tuberosa* L. and *Hibiscus longifolius* (Willd.). The leaves are collected in the month of January-February from Guntur region of Andhra Pradesh, India. The microscopic studies are done using Stereo zooming microscope with CMOS camera (Lawrence and Mayo) and Trinocular microscope with CMOS camera (Lynx model 1803).

Chemicals and Reagents

The chemicals, solvents and reagents used in the present work are of A R grade (Merck, Qualigens, Loba and SLR purchased from local supplier.

Leaf clearing and Venation

The fresh leaves are collected from different medicinal and aromatic plant species and the major venation patterns are traced out. In addition to this, leaf clearing studies are performed to observe the minor venation pattern clearly. These leaves are placed in a beaker and are washed thoroughly under running tap water to remove the debris and the dust on the leaves. In this method, the reagents phenol and trichloro acetic acid are used in equal proportions (1:1). The crystals formed in the mixture are melted at 60°C in hot air oven. Then, the leaves are immersed in this solution and incubated at 60°C for an hour. Leaving in this solution digests the parenchymatous tissues of the leaves which makes the leaves transparent and the veins remain intact such that the venation pattern and the epidermis can be seen clearly. This treatment helps us to get a proper view of the venation patterns in the leaf including secondary and tertiary venations, and also provides a proper glance of the epidermal layer of the leaf. The solution is poured out and the leaves are washed with water carefully to remove the traces of phenol and tri chloroacetic acid. Further, the leaves are stained with 1% ink pad ink of blue color by diluting it in water and left aside for 10-15 minutes. Excess stain has been removed by washing with water. Next, 50% ethyl alcohol solution added to the leaves and kept for 10-15 minutes. After this treatment, the leaves are washed under running tap water, treated with lactophenol for 5 minutes and

the excess stain is removed by pressing with filter paper. The leaves are observed under stereo zoom microscope to get the microphotographs at desired magnification [26].

RESULTS AND DISCUSSION

During the present investigation on the study of leaf architecture, major and minor venation patterns for fifteen varieties of medicinal and aromatic plants (Figure 1) are analyzed. The results of this analysis showed the important anatomical features of the medicinal and aromatic plants. Medicinal and aromatic plants have long been used by people for treating various diseases in traditional health practice [19]. In recent years, the herbal trading is exponentially increased globally, herbs and spices have become prone to adulteration by deliberate or unintentional means [27]. The deliberate adulteration is economically motivated to get more profit, whereas unintentional adulteration is attributed to improper harvesting, processing of raw material and substituting with close or related plant species [27]. The morphological and organoleptic evaluation of herbal raw materials is essential for identifying and evaluating the authenticated plant species from adulterants [21].

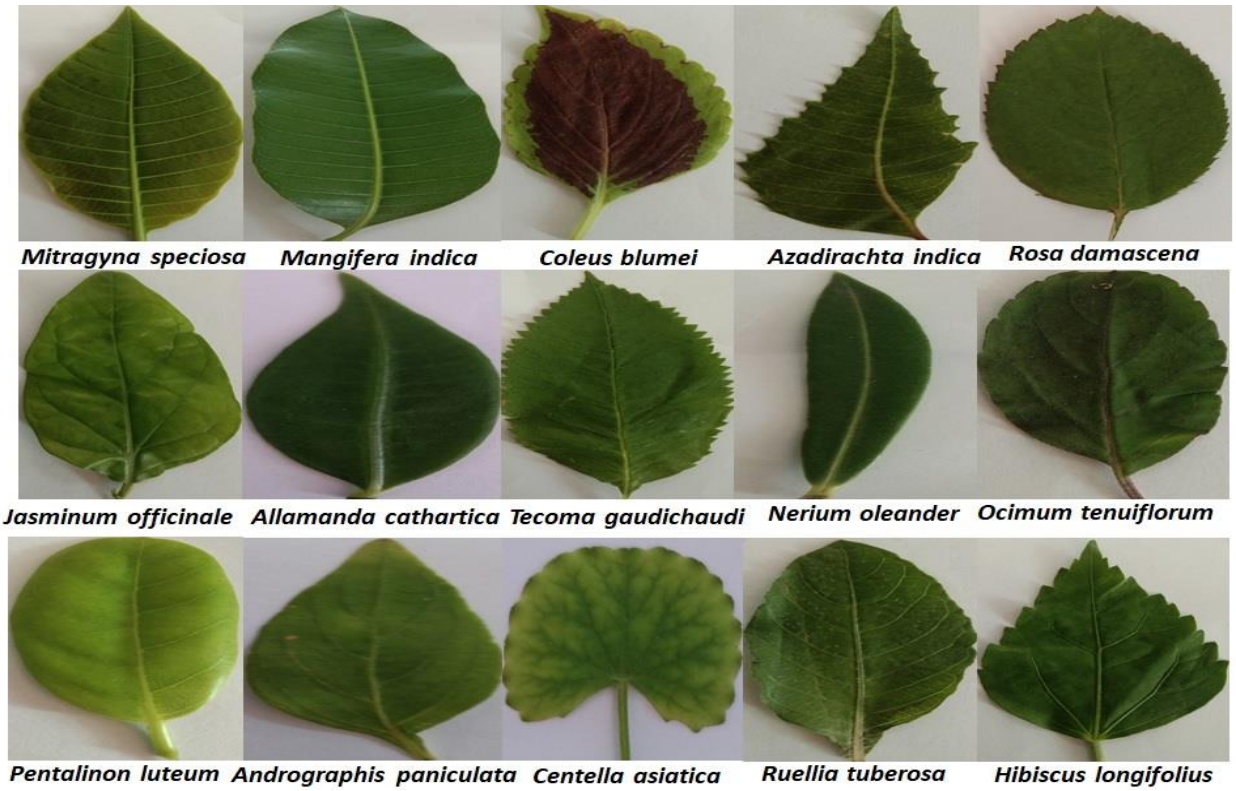

Figure 1. Leaves of medicinal and aromatic plants selected for leaf venation studies.

The qualitative features of leaf architecture of selected plants are listed in the Table 1. The plants scientific name, common name, family, order, phyllotaxy and type of the

leaf details are listed. The selected plant species belong to the families namely Rubiaceae, Anacardiaceae, Lamiaceae, Meliaceae, Rosaceae, Oleaceae, Apiaceae, Bignoniaceae, Apocyanaceae, Acanthaceae and Malvaceae. The leaf type for all plant species is foliage and the phyllotaxy of the plant leaves was alternate and opposite. For the plant species *Mitragyna speciosa, Mangifera indica, Azadirachta indica, Rosa damascena, Centella asiatica* and *Hibiscus longifolius*. The plants such as *Azadirachta indica, Rosa damascena* and *Jasminum officinale* have compound type of leaf whereas remaining plant species have simple type of leaves.

Name	**Common name**	**Family**	**Order**	**Leaf type**	**Phyllotaxy**	**Type of leaf**
Mitragyna speciosa	Kratom	Rubiaceae	Gentianales	Foliage	Alternate	Simple
Mangifera indica	Mango	Anacardiaceae	Sapindales	Foliage	Alternate	Simple
Coleus blumei	Painted nettle	Lamiaceae	Lamiales	Foliage	Opposite	Simple
Azadirachta indica	Neem	Meliaceae	Sapindales	Foliage	Alternate	Compound
Rosa damascena	Damask rose	Rosaceae	Rosales	Foliage	Alternate	Compound
Jasminum officinale	Jasmine	Oleaceae	Lamiales	Foliage	Opposite	Compound
Allamanda cathartica	Golden trumpet	Apiaceae	Apiales	Foliage	Opposite	Simple
Tecoma gaudichaudi	Yellow bells	Bignoniaceae	Lamiales	Foliage	Opposite	Simple
Nerium oleander	Oleander	Apocyanaceae	Gentianales	Foliage	Opposite	Simple
Ocimum tenuiflorum	Holy basil	Lamiaceae	Lamiales	Foliage	Opposite	Simple
Pentalinon luteum	Hammock vipers' tail	Apocyanaceae	Gentianales	Foliage	Opposite	Simple
Andrographis paniculata	Green Chiretta	Acanthaceae	Lamiales	Foliage	Opposite	Simple
Centella asiatica	Gotu kola	Apiaceae	Apiales	Foliage	Alternate	Simple
Ruellia tuberosa	Meadow weed	Acanthaceae	Lamiales	Foliage	Opposite	Simple
Hibiscus longifolius	Hibiscus	Malvaceae	Malvales	Foliage	Alternate	Simple

Table 1 – Qualitative features of leaf architecture of selected medicinal and aromatic plants plant species.

The macro and microscopic analysis of leaves such as size, shape, petiole, phyllotaxy and venation helps us to study the orientation of the veins in the leaves and the leaf lamina for identification and characterization of particular medicinal and aromatic plant species [21, 28]. The organoleptic, macroscopic and microscopic combination help in identification and evaluation of raw materials that provide high degree of confidence [21]. The shape, length, breadth, margin, petiolate, major and minor venation patterns of leaves studied under current investigation are listed in Table 2.

Table 2. Leaf venation pattern in the presently studied medicinal and aromatic plant species

Plant Name	Shape	Length (mm)	Breadth (mm)	Margin	Petiolate	Major venation pattern	Minor venation pattern
Mitragyna speciosa	Ovate	9.5	4.9	Entire	+	Pinnate	Unicostate
Mangifera indica	Elliptical	11.6	2.3	Entire	+	Reticulate	Unicostate
Coleus blumei	Ovate	4.5	2.4	Dentate	+	Pinnate	Unicostate
Azadirachta indica	Lanceolate	5.8	2.5	Dentate	+	Pinnate	Unicostate
Rosa damascena	Ovate	3.1	1.6	Serrate	+	Reticulate	Unicostate
Jasminum officinale	Lanceolate	7.2	3.6	Entire	+	Pinnate	Unicostate
Allamanda cathartica	Lanceolate	6.5	1.6	Entire	+	Pinnate	Unicostate
Tecoma gaudichaudi	Elliptical	9.2	3.5	Serrate	+	Pinnate	Unicostate
Nerium oleander	Lanceolate	11.0	1.9	Entire	+	Pinnate	Unicostate
Ocimum tenuiflorum	Ovate	4.9	2.9	Crenate	+	Pinnate	Unicostate
Pentalinon luteum	Elliptical	7.0	4.9	Entire	+	Pinnate	Unicostate
Andrographis paniculata	Lanceolate	4.1	1.4	Entire	+	Pinnate	Unicostate

Centella asiatica	Reniform	3.4	3.7	Crenate	+	Palmate	Multicostate
Ruellia tuberosa	Elliptical	5.4	2.3	Undulate	+	Pinnate	Unicostate
Hibiscus longifolius	Elliptical	7.0	4.0	Serrate	+	Pinnate	Unicostate

The main shapes of the leaves are ovate (*Mitragyna speciosa, Coleus blumei, Rosa damascena,* and *Ocimum tenuifolium*), elliptical (*Mangifera indica, Tecoma gaudichaudi*, *Pentalinon luteum, Ruellia tuberosa* and *Hibiscus longifolius*), lanceolate (*Azadirachta indica, Jasminum officinale, Nerium oleander* and *Andrographis paniculata*) and reniform (*Centella asiatica*). The length of the selected leaves ranges from 3.1mm -11.6 mm and breadth ranges from 1.4mm to 4.9 mm which are represented in the Table 2 for each leaf. All the plant species are petiolate and the margins varied from one plant to another plant. The plants such as *Mitragyna speciosa, Mangifera indica, Jasminum officinale, Allamanda cathartica, Nerium oleander, Pentalinon luteum* and *Andrographis paniculata* leaves have entire margin. The plants *Coleus blumei, Azadirachta indica* have dentate type of margins. Serrate type of margin is observed in plants such as *Rosa damascena, Tecoma gaudichaudi, Hibiscus longifolius.* Crenate type of margin is observed in *Ocimum tenuifolium* and *Centella asiatica. Ruellia tuberosa* has undulate type of wavy margin.

The design and venation pattern of leaf is very important for plant photosynthesis performance in agriculture and plant technology [29]. Leaf veins are essential for structure of a leaf to maintain its three-dimensional structure, orientation and mechanical support to leaves [30]. The leaf veins are responsible for transport of water, nutrients and biological products by photo assimilation process due to the presence of xylem and phloem [6, 31]. Leaves have many features for taxonomic significance and herbal trade [32]. Out of all features, leaf venation has potential significance and the venation pattern vary strongly across the major plant lineages but reticulate venation evolved frequently [6]. Typically, three orders of venation have been observed which includes first order veins (major veins) run from petiole to the leaf apex, second order veins branching at intervals and the third order veins branching in between the leaf lamina [33]. The venation pattern is mainly pinnate, reticulate and palmate (Figure 2). Pinnate type of venation has been observed in 12 species of plants such as *Mitragyna speciosa, Coleus blumei, Azadirachta indica, Jasminum officinale, Allamanda cathartica, Tecoma gaudichaudi, Nerium oleander, Ocimum tenuiflorum, Pentalinon luteum, Andrographis paniculata, Ruellia tuberosa* and *Hibiscus longifolius.* Reticulate type of venation is observed in two plant species namely *Mangifera indica* and *Rosa damascena. Centella asiatica* has palmate type of venation. In

the present study, the macro and microscopic analyses of fifteen different medicinal and aromatic plants are performed and different venation patterns have been evaluated microscopically using trinocular and stereo zoom microscopes. The study has significant importance because the presently studied medicinal and aromatic plant varieties provided valuable information on their vein architecture and venation patterns. The results obtained in this study helps in identification, purity and effectiveness of herbal materials developed as a botanical. There are a few studies related to venation pattern in medicinal and aromatic plants [34, 35]. The selected plants have simple and compound type of leaf belongs to foliage category.

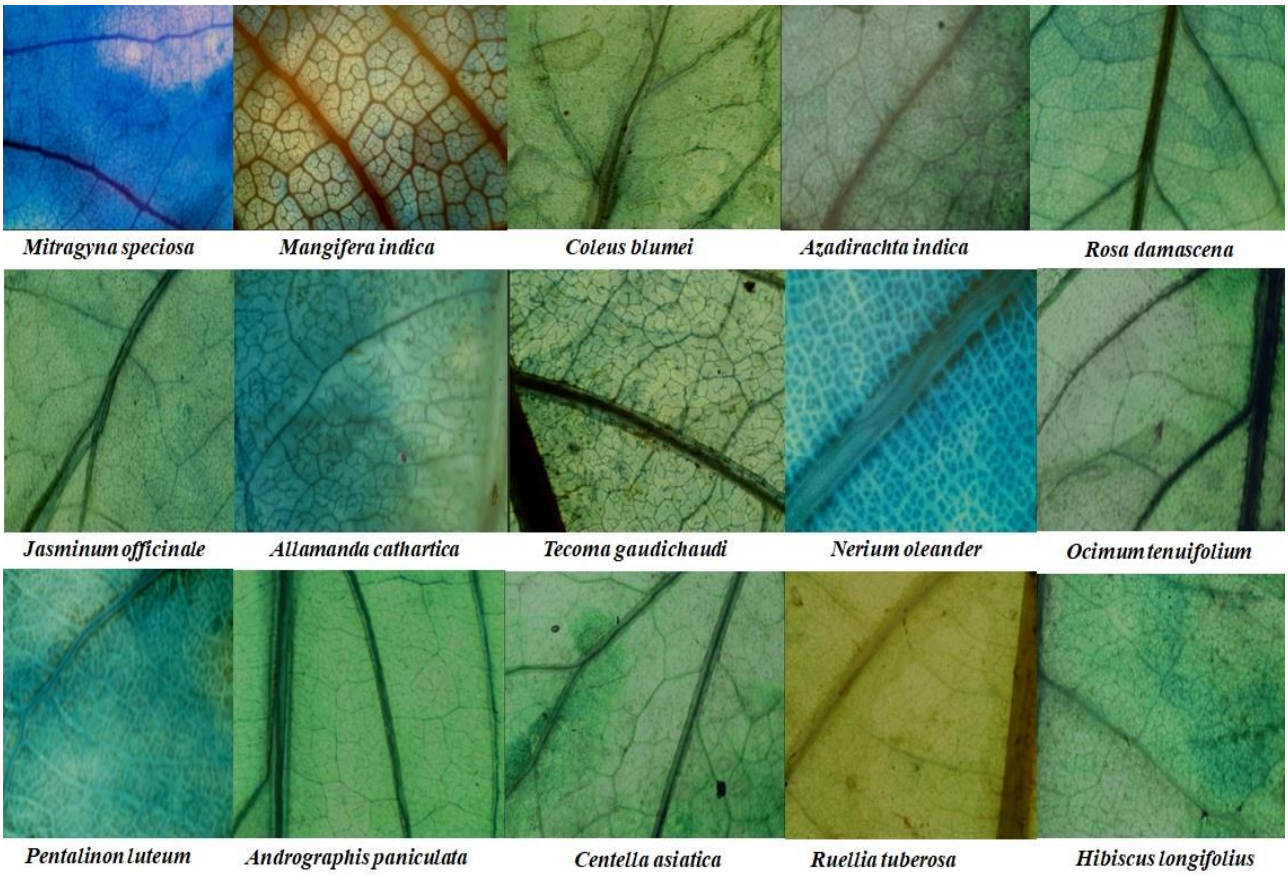

Figure 2. Leaf venation pattern of selected medicinal and aromatic plants.

The plant leaves have bioactive compounds with various biological activities and effective for various diseases [36]. The plants such as *Rosa damascena*, *Jasminum officinale*, *Ocimum tenuiflorum* and *Hibiscus longifolius* produce bioactive compounds with aroma and flavor. They are used in food, pharmaceutical and cosmetic industries [37-40]. The other plants such as *Mitragyna speciosa*, *Coleus blumei*, *Mangifera indica*, *Azadirachta indica*, *Allamanda cathartica*, *Tecoma gaudichaudi*, *Nerium oleander*, *Pentalinon luteum*, *Andrographis paniculata*, *Centella asiatica* and *Ruellia tuberosa* are having

medicinal importance used for various pharmacological activities [41- 50].

Conclusion

The leaf venation pattern contributes as a marker in plant systematics and also helpful for cataloguing medicinal and aromatic plants. The importance of leaf venation as taxonomic entities can be preserved for longer period of time and for systematic evidence also. The results of minor venation patterns obtained in this study are useful for studying various characteristic features, identifying and classifying different medicinal and aromatic plant species. For preserving the plant ecosystem, plant conservation and trading of herbal materials, the scientific data related to classical techniques like morphological, macroscopic, microscopic and organoleptic studies are essential and provides the identification, purity and quality assessment of medicinal and aromatic plants. Proper identification of botanical ingredients in medicinal and aromatic plants is critical for maximizing their efficacy and minimizing the adulteration. Further, the addition of research on medicinal and aromatic plants leaf venation will speed up the discoveries and generates the scientific knowledge in plant biology, agriculture and herbal trade.

Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

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