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A COMPARATIVE INVESTIGATION OF SELECTED THREE CULINARY LEAF PLANTS

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Abstract
Plants are essential to the balance of nature and people's lives. Plants are the ultimate source of food and metabolic energy for nearly all animals. Aromatic plants play a valuable and important role in economic, social, cultural and ecological aspects of local communities the world over. The present study dealt with the selected culinary plants from the family Lauraceae and Myrtaceae. These samples were compared for morphological and anatomical characteristics and also carried out phytochemical analysis. In the present study, an attempt has been made to distinguish between commercially available Cinnamon using simple macroscopial and microscopial evaluation. These simple microscopic and macroscopic characters can be used as an effective tool for the identification of true Cinnamon sample which will help to maintain the quality of herbal drugs by avoiding adulteration of fraud Cinnamon.

Key words: Morpho anatomical features, adulteration, phytochemical analysis

Introduction

One of the earliest instances of culinary improvisation by humans has to be wrapping food in leaves and steaming or roasting it. Thought up by some hunter-gatherer ancestor, it is a stroke of ingenuity – simple but brilliant. The leaves make for an impervious casing that protect the food from being exposed to direct heat and prevent dirt or fluids from seeping in. The leaves also trap some steam and seal in the flavours, allowing the food to cook unhurried in mellow heat, steeping in its own juices. The results are fantastic.

Plants are very useful to us and we get most of our food from plants. Everybody needs food to stay alive. Food is one of our basic needs. It gives us energy to work. Most of our food comes from plants . Plants give us many things. We get food from different parts of plants. Food from plants are packed with many nutrients such as vitamins, minerals and antioxidants. Dietary fibre is found only in plants.

Aromatic plants are a particular group of plants that give out fragrance and used their aroma used in perfumery and flavour. A number of aromatic plants and their essential oils are exclusively used also for medicinal purposes in aromatherapy as well as in various systems of medicines. Trees are a major group of plants having much height, woody stem and comparatively long life span than herbs and shrubs. These are showing variation in their presence according to the climate condition as well as their high degree of seed production useful for their maximum dispersal and long term existence in nature and for slow growth rate.

Phytochemicals are naturally occurring, biologically active chemical compounds in plants. They act as a natural defense system for host plants and provide colour, aroma and flavour. Plant cell produces two types of metabolites; primary metabolites involved directly in growth and metabolism (carbohydrate, lipids and protiens), and secondary metabolites considered as end products of primary metabolism and not involved in metabolic activity (alkaloids, phenolics, sterols, steroids, essential oils, lignins and tannins etc).

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Their absence does not cause terrible effects in the plants. Plants are the oldest source of pharmacologically active compounds, and have provided humankind with many medically useful compounds for centuries.

Phytochemicals in freshly harvested plant food may be degraded by processing techniques, including cooking. The main cause of phytochemical loss from cooking is thermal decomposition. Food processing techniques like mechanical Processing can also free carotenoids and other phytochemicals from the food matrix, increasing dietary intake.

Adulteration is a major problem met during the assessment of identity and quality of many herbal drugs. Difficulty arises when they are in the dry state and especially belonging to leaf parts of the plants, where they are usually found to be broken or powdered. For establishing their correct identity they are needed to be examined thoroughly under the microscope. Leaves of the same genus are found to exhibit number of similar common macroscopic characters and hence most likely to be used as an adulterant for official drug.

An adulteration may also be defined as mixing or substituting the orginal drug material with other spurious, inferior, defective , spoiled, useless other parts of same or different plant or harmful substances or drug which do not confirm with the authenticated official standards.

Wood has been of service to mankind through ages. The most unique feature of wood, unlike other natural materials, as its high degree of structural variability. Even two pieces of wood belonging to the same timber species, may not be exactly alike. Even though the basic wood structure of the species is more or less similar; every fragment of it may show some differences. This attracts a unique fascination and attraction for this material.

Identifying wood species accurately is very important in ethnobotanical research on wood usage. Among the several methods proposed until now, the microscopic method is the most accurate and widely used owing to the tremendous efforts by the wood anatomists and the corresponding identification keys proposed by the IAWA (The International association by the wood anatomists) community. This method allows us to mostly identify it up to the genus level.

Materials and Methods

Materials

Three different culinary leaf plants *Cinnamomum malabatrum*, *Cinnamomum zeylanicum* belonging to Family Lauraceae and *Pimenta dioica* belonging to Family Myrtaceae were selected for the present investigation. The aim of study was to evaluate morpho-anatomical and phytochemical variations.

Selection of materials

All the three plants were collected from same locality. The selection of plants was based on the local awareness and usage of plant parts for different purposes.

Methods

1. Morphological parameters

Morphological parameters of leaf and stem of the three selected plants were observed. The characters observed were habit, height of the plant, phyllotaxy, morphology of leaves, nature of bark, colour, taste and odour.

2. Anatomical characters

The anatomical characters of the leaves of all three selected plants were observed clearly. The characters taken into consideration were transverse section of leaf through midrib and analysing the upper and lower stomatal variations in the leaves. With the help of a thin razor, thin sections of the material were taken, stained with safranin and observed under microscope for the comparison of the internal structure.

3. Testing cinnamon adulteration

Two commercial samples of cinnamon were purchased from spice markets. The methods

used to detect adulteration in this study were macroscopic and microscopic evaluation.

3.1. Macroscopial evaluation
Different macroscopic parameters like appearance, colour, surface characters, taste, odour and thickness were observed and analysed.

3.2. Macroscopial evaluation
Fine hand sections of bark of cinnamon sample were taken using standard procedures and were stained with aqueous safranin and mounted in glycerine and also conducted maceration. Microphotographs of sections were taken.

4. Phytochemical studies
Phytochemical screening of extracts of the three culinary leaves were carried out by the standard methods in order to identify the diverse secondary metabolites present in the leaf extracts of three plants. Qualitative assay of Phenols, Terpenoids, Flavanoids and Saponins were conducted.

4.1. Test for Phenols
Sodium hydroxide test: Five milligram of each leaf extract was dissolved in 0.5 ml. 20% sulphuric acid solutions. Followed by addition of few drops of aqueous sodium hydroxide solution, it turns blue which indicates the presence of phenols.

4.2. Test for Terpenoid
Freshly prepared leaf extract was mixed with 2 ml. of chloroform and concentrated H2SO4 (3 ml.) was carefully added to form a layer.

4.3. Test for Flavanoids
One millilitre of NaOH was added to 3 ml. of each leaf extracts and observed for yellow colouration.

4.4. Test for Saponins (Foam Test)
0.5 mg. of leaf extract was diluted with 20 ml. distilled water and shaken well in a graduated cylinder for 15 minutes. The formation of foam to a length of 1 cm. indicated the presence of saponins and steroids.

Results and Discussion
Morphological parameters
Three different culinary leaf plants *Cinnamomum malabatrum*, *Cinnamomum zeylanicum* belonging to Family Lauraceae and *Pimenta dioica* belonging to Family Myrtaceae had shown differences in their morphology. The characters studied and the differences observed were represented in Table 1.

The characters observed were habit, height of the plant, colour, taste, odour and morphology of leaves. The anatomical characters of the leaves and stem of all the three samples were observed clearly. The characters taken into consideration were transverse section of leaf through midrib, stem anatomy and stomata. In recent years, anatomical characters have been used in taxonomy (Agbagwa and Ndukwu, 2004; Kharazian, 2007; Eminagaoglu *et al.*, 2012; Ozcan *et al.*, 2014).

The *Cinnamomum malabatrum* was moderate evergreen tree, bark smooth or slightly cracked, light brown, leaves were opposite or sub opposite, elliptic to oblong, glabrous, pink, when young three nerved from close above the base almost to the apex, flowers long, pale yellowish, fruits ellipsoid.

Cinnamomum zeylanicum trees were 10 – 15 meters (30-50 ft.) tall. The leaves were oval – oblong in shape and 7-18 cm (3-7 inches) long. The flowers which were arranged in panicles had a greenish colour and distinct odour. The fruit was a purple 1 cm drupe containing a single seed.

Pimenta dioica was a small dioecious evergreen tree, 7-10 m tall with a slender trunk (50-100 cm at the base) with many branches 1-2 m above the ground. The bark was pale silver brown smooth and shiny and sheds strip 25-75 cm long. Leaves were born in cluster at the end of branches. Flowers were white and in branching clusters. Berries were green when unripe, turning deep purple to glossy when ripe.

Anatomical Characters

Internal structures of the three culinary leaves had shown variation between them and the observed details are represented in Table 2. Anatomically the plants were compared by observing the transverse section of leaves through midrib and analyzing the stomatal variation in the leaves.

In *Cinnamomum malabatrum*, broadly convex, dorsiventrally shows an arc of well-developed conjoint, collateral, oval shaped meristele. In the centre of the midrib and dorsiventral laminar extensions on the lateral sides pericyclic band encircling the meristele shows continuously running narrow arc of fibres on the upper side and discontinuously running group of fibres in its lower side. Both the epidermis were thick walled, stomata was present on the lower side only, covered with their cuticle and bear few simple glandular trichomes – thick walled parenchymatous cells were present.

Lamina had shown a row of narrow and compactly arranged palisade cells embedded with oval to spherical oil cells followed by few rows of spongy parenchyma embedded with mucilage cells and small vascular bundles sheathed dorsiventrally with sclerenchymatous band reaching up to both epidermis of the lamina.

In *Cinnamomum zeylanicum*, epidermis was single layered and covered by smooth cuticle; cuticle thick on upper side and thin on lower side. Cell walls were sinuous as *Cinnamomum malabatrum*. In *Cinnamomum* zeylanicum, trichomes were microscopic and occured only on the lower surface. Trichome distribution was sparse in *Cinnamomum zeylanicum*. Trichomes were short medium or long. Structurally trichomes were identical. They were unicellular, unbranched and non glandular thick walled and enclosed a narrow lumen in the centre.

Cinnamomum leaves were hypostomatic with stomata confined to the lower surface of leaves. Stomata were anomocytic surrounded by a variable number of cells that were indistinguishable in size or form from the rest of the epidermal cells.

In *Pimenta dioica*, in the leaf margin, the mesophyll cells were gradually replaced by the angular collenchyma to end in approximately 5 layers of sub-epidermal cells. Epidermal cells were covered by a thick cuticle layer. In transverse sections, along the main vein from the periphery to the centre, a thick cuticular epidermis covering the adaxial and abaxial surfaces was present and it was unistratified. The parenchyma cells surround the vascular bundle core. The central vascular bundle was arranged in the form of a continuous bicollateral were in which the xylem appears slightly compressed and was surrounded by the phloem internally and externally.

As in *Cinnamomum malabatrum* and *Cinnamomum zeylanicum, Pimenta dioica* had stomata present only on the lower surface of leaves .There were many anomocytic stomatas present and surrounded by subsidary cells.

Testing Cinnamon adulteration

Both macroscopic and microscopic observations were conducted for checking the adulteration of commercial cinnamon barks . Anatomical and maceration results of the two tested samples were represented in Tables 3 and Table 4.

Two commercial samples of Cinnamomum was collected and observed their macroscopical and microscopical characters. In this study first, simple macroscopial evaluation was performed through naked eye. Macroscopical evaluation of the sample instantaneously tells the difference between them. Microscopial evaluation was done of different regions of the bark of samples. The sections obtained were observed for oil cells, starch grains and pericyclic fibres. These parameters were either absent or present in different forms in the test samples which help to differentiate different species of Cinnamomum. Though Cinnamomum bark is of great importance, very little work has been done on their structure and development. The earlier studies

on these aspects were by Birnstiel (1922), Santos (1930) and more recently by Bamber and Summerville (1979). All of them had shown prevalence of differences of bark structure in this genus.

Phytochemical studies

Qualitative tests for Phenols, Flavanoids, Terpenoids, and Saponins in three different culinary leaves in aqueous revealed the presence of those phytochemicals in them. The results observed are represented in Table 5 and Graph 1.

Qualitative analysis of the phytochemicals present in the above three culinary leaves had shown great variations in phenol, flavonoid, terpenoid and saponins. The amount of flavonoid was greater in *Pimenta diocia*. Amount of terpenoid was high in *Cinnamomum zeylanicum*. The amount of phenol was same in *Cinnamomum malabatrum*, *Cinnamomum zeylanicum* and *Pimenta dioica*. Flavanoid content was comparatively low in *Cinnamomum zeylanicum* compared to the other two plants. The presence of saponin was same in *Cinnamomum malabatrum* and *Pimenta diocia* and lower than *Cinnamomum zeylanicum*. The presence of terpenoid was same in *Cinnamomum malabatrum* and *Pimenta diocia*. Flavanoids exhibit inhibition of mutagenicity induced by chemical mutagens and have anticarcinogenic, antioxidant and anti-inflammatory activities (Miyazawa *et al.*, 2000).

Conclusion

The comparative studies were conducted on the morphology, leaf, stem and wood anatomy and phytochemical analysis of the three culinary plants – *Cinnamomum malabatrum* and *Cinnamomum zeylanicum* belonging to family Lauraceae and Pimenta dioica belonging to family Myrtaceae. Pathological observation and adulteration of Cinnamomum species was observed and analysed.

Morphologically, all the samples were showing variations in their leaf morphology. Significance differences were found in the anatomical characters of all the three samples. Stomatal studies reveal that it was present only on the lower side of the leaves. Here all the samples under study possess anomocytic type of stomata.

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Table 1 Morphological Parameters

Sl. No.	Characters	*Cinnamomum malabatrum*	*Cinnamomum zeylanicum*	*Pimenta dioica*
1	Habit	Tree	Tree	Evergreen small tree
2	Height of the plant	30-45 feet	30-50 feet	20-40 feet
3	Phyllotaxy	Simple, opposite or subopposite	Simple, slightly opposite	Simple, opposite
4	Morphology of leaves	Glabrous, oblong, lanceolate, basically trinerved , reticulate venation, apex acute to accuminate	Leaves are elongated ovate with a pointed tip, shiny, dark green on upper surface and light green below	Pinnately veined, slightly thick midrib is impressed on the upper surface and prominent beneath, lateral veins not very prominent
5	Colour	Yellowish green above, pale below	Deep green	Dark green
6	Taste	Pungent	Pungent	Pungent
7	Odour	Fragnent	Strongly aromatic	Aromatic
8	Bark	Smooth, brown, pustular with aromatic smell	1 mm thick, pale- yellowish brown, smooth, aromatic smell	Smooth, shiny, pale brown, aromatic smell

Table 2. Anatomical Characters of Leaf

Sl. No.	Characters	*Cinnamomum malabatrum*	*Cinnamomum zeylanicum*	*Pimenta dioica*
1	Cuticle	Thick cuticle	Thick cuticle	Thick Cuticle
2	Epidermis	Thick walled epidermis	Single layered epidermis	Cuticular epidermis
3	Trichomes	Glandular trichome	Microscopic unicellular, unbranched	Absent
4	Vascular bundle	Conjoint, collateral, oval shaped meristele	Oval or round shaped, oil cells are located in the phloem	Bicollateral, xylem appears slightly compressed and is surrounded by phloem
5	Stomata	Anomocytic	Anomocytic	Anomocytic

Table 3. Macroscopial Characters of Cinnamon

Parameters	Sample 1	Sample 2
Colour	Greyish brown	Light brown
Surface character	Curls from one side only, thick	Curl inward from both side towards centre, smooth
Texture	Rough	Smooth
Odour	Aromatic	Fragrant
Taste	Sweet	Sweet and delicate

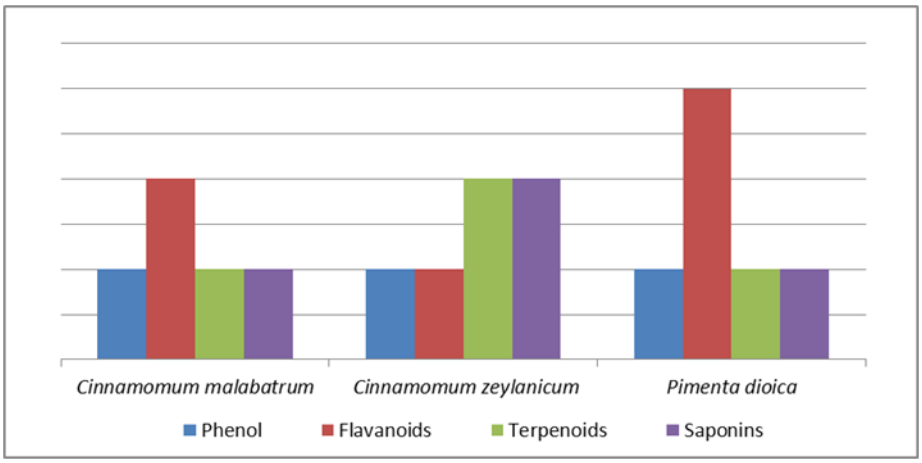
Table 4. Microscopial Characters of Cinnamon

Parameters	Sample 1	Sample 2
Oil cells	Bigger and grater in number	Lesser in number
Starch grains	Lesser in number	Greater in number
Pericyclic fibres	Present	Absent
Fibres	Wide	Thin

Table 5. Expression of Phytochemicals in Aqueous

Sl. No.	**Tests**	***Cinnamomum malabatrum***	***Cinnamomum zeylanicum***	***Pimenta dioica***
1	Phenols	+	+	+
2	Flavanoids	++	+	+++
3	Terpenoids	+	++	+
4	Saponins	+	++	+

Number of '+' indicates the presence and strength of phytochemicals



Graph 1. Presence of Phytochemicals in Aqueous

References

Agbagwa O. I. and Ndukwu B. C. (2004). The value of morphoanatomical features in the systematic of Cucurbita L. (Cucurbitaceae) species in Nigeria. Afr. J. Biotechnol.3 (10): 541-546.

Bamber R K and Summerville R (1979). Taxonomic significance of scle reified tissue in the bark of Lauraceae. TAWE Bull No.4: 69-75.

Birnstiel W (1922). Cited from Metcalfe and Chalk 1950.
Eminagaoglu O., Ozcan M. AND Kultur S. (2012). Contributions to the leaf and stem anatomy of Tradescantia fluminensis Vell. (Commelinaceae): An aleien species new to the flora of Turkey. ACU. J. Fort. Fac. 13(2): 270-277.

Kharazian N. (2007). The taxonomy and variation of leaf anatomical characters in the genus Aegilops L. (Poaceae) in Iran. Turk. J. Bot. 31:1-9.

Miyazawa M., Okuno Y., Nakamura S. and Kosaka H. (2000). Antimutagenic activity of flavonoids from Pogostemon cablin. J Agric Food Chem 48: 642-647.

Ozcan M., Unver M. C. and Eminagaoglu O. (2014). Comparitive anatomical and ecological investigations on some Centaurea S. L. (Asteraceae) taxa from (Turkey) and their taxonomic significance. Pak. J. Bot. 46(4): 1287-1301.

Santos, J K (1930). Leaf and bark structures of some cinnamon trees with special references to Philippine trees. Philippine J. Sci. 43: 305-365.