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Comparative phytochemical analysis of the leaves of two Myrtaceae members- *Pimenta dioica* (L.) Merrill and *Syzygium aromaticum* (L.) Merrill and Perry

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Abstract

Spices are the rich sources of essential oils. All spice and clove belongs to the same family Myrtaceae. Leaves of these two plants possess a unique aroma when crushed. The aim of this study was to analyse and compare the major phytochemicals present in the leaves of *Pimenta dioica* (L.) Merrill and *Syzygium aromaticum* (L.) Merrill and Perry by Gas chromatography-Mass Spectrometry technique. Leaves were shade dried and pulverized with the help of an electric grinder. Through hydrodistillation the oils were separated and that was used for GC-MS analysis. The major constituent is eugenol. *Pimenta dioica* leaves contain 60.8% eugenol and that of *Syzygium aromaticum* contains 51.03%. In addition to eugenol other important components are chavicol, Palmitic acid, malonic acid and stearic acid. Eugenol has wide range of value such as antimicrobial, antioxidant, antiseptic anaesthetic etc. It is also used in the preparation of Ayurvedic medicines. Commercially these two leaves are not used for eugenol extraction. Present study revealed that it could be used as a promising one in Pharmaceutical industry.

Keywords: Clove, all spice, leaves eugenol, GC-MS

Introduction

Spices are rich sources of essential oils. Essential oils are natural, complex, multicomponent system composed mainly of terpenes in addition to other non-terpene compounds. Essential oils are commercially important especially for pharmaceutical, agronomic, food, sanitary, cosmetic and perfume industries. All spice tree or *Pimenta dioica* is a small sized tree belonging to the family Myrtaceae. The spice takes its popular name "All spice" from its resemblances in perfume and taste to a mixture of Cinnamon, Clove and Nutmeg. It is also known as Jamaica pepper. It is a small tree, about 8-10m tall with smooth, peeling bark. The leaves are oppositely arranged petiolate with narrowly elliptic or oblong blades having abundant oil glands and strong odour when they are crushed. They are dioecious, flowers are borne in axillary panicles, sepals are distinct in bud, petals and stamens are white coloured. The fresh fruits are green with purple black colour on ripening. Two seeds are found in each fruit^[1].

Cloves are the aromatic dried flower buds of a tree in the family Myrtaceae, *Syzygium aromaticum*. It is a small tree, about 12-15 m height Stem is usually forked. Leaves are lanceolate acute at both ends, or sub-acute at the tip and narrowed at the base in to slender leaf stalk. Above they are dark shining green below, paler, thinly coriaceous. Leaves are in pairs. The leaves are aromatic being full of minute oil glands. Presence of these oil glands makes the leaves very fragrant. Flowers are produced in numerous groups of terminal cymose clusters. Flowers are hermaphrodite and self-pollinating. Flower buds are first of pale in colour, gradually become green, after which they develop in into a bright red, when they are ready for collecting.

All spice and clove are widely used as a flavour enhancer for food such as fish, soups, sauces, cakes, rices and meat. In Pimenta, leaf oil contain eugenol, methyl eugenol, β - caryophyllene and myrcene as the major constituents^[2]. Eugenol is the main phyto constituents in the leaves of *S. aromaticum*. In addition to this acetyleugenol, β - caryophyllene, humulene are also present in it^[3]. Phyto chemical compounds present in this plants are phenyl propanoids, glycosides^[4] and tannins^[5].

Eugenol derived from the All spice and Clove have been in therapeutic use in dentistry as a local anaesthetic, antiseptic for teeth and gum.

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All Spice Tree

Clove Tree

Materials and Methods

Healthy and mature leaves were collected from the clove tree and all spice tree in the month of October at Puthoor in Kollam district of Kerala. Shade dried leaves were coarsely grinded using an electrical grinder. The grounded leaves were subjected to hydro distillation using Clevenger apparatus for 4h for isolation of oils separately. The oil phase was separated and dried over anhydrous sodium sulphate and after filtration, kept in a dark glass bottle at 4 °C until used for GC-MS analysis

Dried leaves of *Pimenta dioica*Dried leaves of *Syzygium aromaticum*

Gas Chromatography-Mass Spectrometry (GC-MS)

The GC-MS analysis was carried out on a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Crop., USA) coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system was equipped with a PR5 MS column (30 m x 0.25 mm x 0.25 µm film thickness). Analyses were carried out using

helium as carrier gas at a flow rate of 1.0 mL/ min at a split ratio of 1:10 and the following temperature program: 40 °C for 1 min; raising 4.0 °C/min to 160 °C and held for 6 min; rising at 6 °C/min to 210 °C and held for 1 min. The injector and detector were held at 200 °C. Diluted samples (1:10 hexane, v/v) of 0.2µL of the mixtures were always injected. Mass spectra were obtained by electron ionization (EI) at 70 eV, Using a spectral range of m/z 40-50.

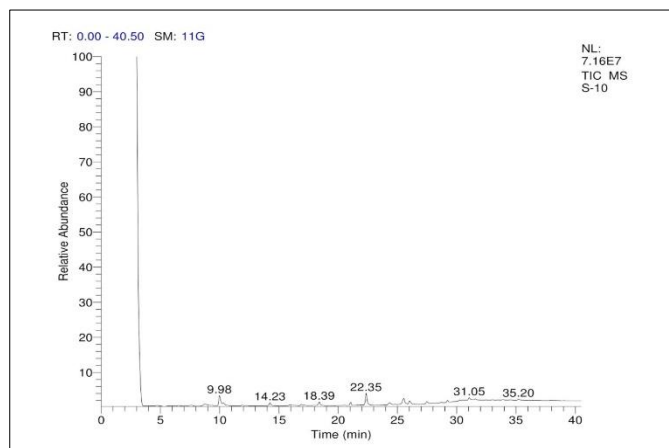
Most of the compounds were identified using mass spectra (authentic chemicals, Wiley spectral library Collection and National Institute of Standards and Technology (NIST) library). The separated components of the essential oil were identified by matching their mass spectra with the NIST published data.

Results and Discussion

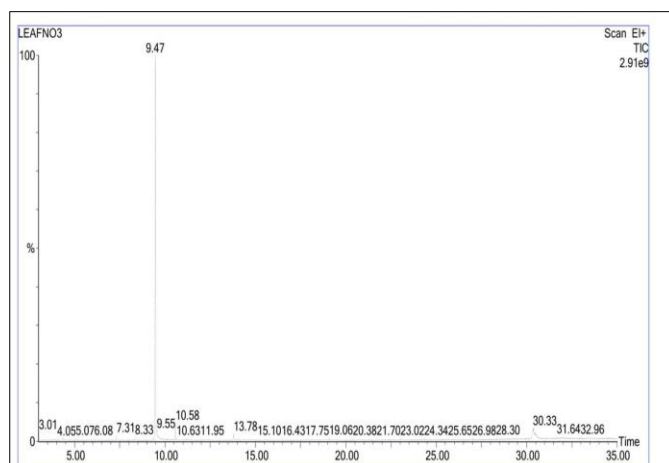
GC-MS analysis was carried out in the powdered leaves of *Pimenta dioica* and *Syzygium aromaticum*. The results are given in the table 1 and graphs. (1-3)

Table 1: Major Chemical components in the leaves of *Pimenta dioica* (L.) Merrill. and *Syzygium aromaticum* (L.) Merrill and Perry

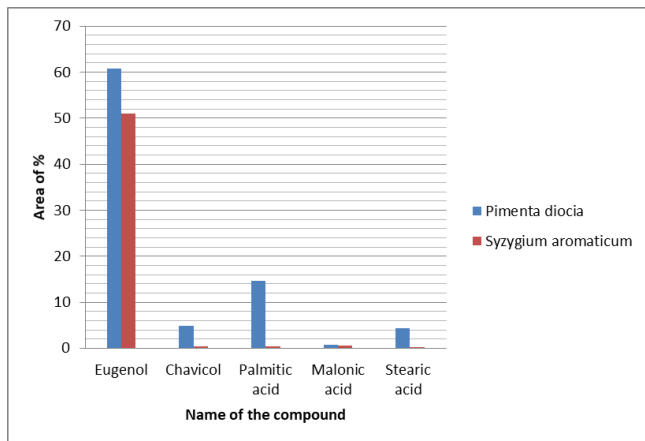
S. No	Name of the Compound	Plants (Area %)	
		<i>Pimenta dioica</i>	<i>Syzygium aromaticum</i>
1	Eugenol	60.8	51.03
2	Chavicol	4.8	0.33
3	Palmitic acid	14.61	0.37
4	Malonic acid	0.67	0.57
5	Stearic acid	4.39	0.195



Graph 1: GC-MS Chromatogram profile of leaves of *Pimenta dioica* (L.) Merrill



Graph 2: GC-MS Chromatogram profile of leaves of *Syzygium aromaticum* (L.) Merrill and Perry



Graph 3: Comparison of Major Phytochemicals in the leaves of *Pimenta dioica* (L.) Merrill and *Syzygium aromaticum* (L.) Merrill and Perry

Eugenol is the main compound present in the leaves of *Pimenta dioica* and *Syzygium aromaticum*. In *P. dioica* leaves eugenol is 60.8% and in that of *S. aromaticum* it is 51.03%. In addition to this, other important compounds present in both of them include chavicol, palmitic acid, malonic acid and stearic acid. In addition to the above mentioned compounds *P. dioica* leaves contain myrcene, limonene, inalool, alpha-copaene, caryophyllene, geraniol etc in lower concentration. The quantity of oil obtained from the leaves varies from area to area, and with the time of the year they gathered. Oil yields from dried leaves range from 0.7 to 2.9 percent while fresh leaves provide from 0.35 to 1.25 percent [6]. Leaves in the male tree contain oil higher in quality and quantity than that of female [7]. Antifungal activity of *P. dioica* leaves extracts have been described by Siju *et al.* [8]. In India it is used as an aromatic stimulant and as an adjuvant to tonics and purgative [9]. Antioxidant and hepato protective activity of *P. dioica* leaves extracts have been described by Sharma. R., [10]. *P. dioica* leaf oil and fruits oil is also used in perfumes, after shaves. *P. dioica* plant oil shows nematicidal activity against pinewood nematodes [11].

In addition to the above mentioned compounds other compounds are present in lower concentration in the leaves of *S. aromaticum* include Memantine, alfa copaene, allo aromadendrane, arachidonic acid, 2, 4, 6- trihydroxy aceto phenone etc. Two ellagitannins namely Syzygin A and Syzygin B were isolated from the leaves [12]. Clove leaves yield to 3.0-4.8% essential oil. Percentage of the essential oil content in the leaves increased from 38.3 to 95.2% with maturity, while the contents eugenyl acetate (51.2 to 1.5%) and caryophyllene (6.3 to 0.2%) decreased [12]. Eugenol inhibited 5-lipoxygenase activity and leukotriene C-4 in human PMNL cells [13].

The therapeutic properties of the all spice oil and clove oil are anaesthetic, analgesic, antimicrobial, antioxidant, antiseptic, acaricidal, carminative, muscle relaxant and rubefacient. It can be helpful for the digestive system for cramp, flatulence, indigestion and nausea. Further the essential oils can help in case of depression, tension, neuralgia and stress.

Conclusion

The chemical composition of the dried leaves of *P. dioica* & *S. aromaticum* were investigated. Leaves of *Pimenta dioica* shows highest concentration of eugenol than *Syzygium*. Commercially these two leaves are not also used for eugenol extraction. This study revealed that it could be used as a promising one to pharmaceutical industry.

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Conflict of interest

Authors have no conflict of interest

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