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HPLC Analysis of phenolic compounds in methanolic extract of *lavandula bipinnata*

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Abstract

This study aimed to analyse the phenolic phytocompounds present in the methanolic extract of *Lavandula bipinnata* collected from the Panchmadi region of Madhya Pradesh. The phenolic profile was determined using methanol as the extraction solvent and optimised HPLC settings on a Shimadzu Prominence model with a PDA detector (SPD-M 20 A) for accurate detection and quantification of phytochemical standards. The methanolic extract of *Lavandula bipinnata* had a lot of phenolic compounds including chlorogenic acid, gallic acid, umbelliferone, luteolin 7-O-glucoside, vitexin, and isoquercitroside. Methanol extraction was found to be the most efficient, and the HPLC setup was optimised to ensure precise analysis of phytochemical compounds.

Keyword: HPLC; Lavandula; Phytocompunds; Extract; Solvent.

Introduction

Lavandula bipinnata (Roth) Kuntze belongs to the family Lamiaceae, often known as the Labiatae, a family of flowering plants with 264 genera and 6990 species. The Lamiaceae family is widespread across the world; in India alone, there are 350 species and 64 genera. It is indigenous to the southeast parts of India, the Mediterranean region, and tropical Africa. The genus includes tiny shrubs, herbaceous plants, and annuals with fragrant blooms and leaves. Italy, Spain, and France cultivate it. *Lavandula bipinnata* is less frequently used due to its therapeutic qualities. People grow lavender in large quantities primarily for its essential oils, which find application in perfumes, cosmetics, food processing, and, more recently,

"Ayurvedic" and "aromatherapy" goods [1]. Although some species are woody shrubs or subshrubs, the majority of the family's members are square-stemmed perennial or annual herbs. Most leaves have volatile oils and are aromatic, with the majority being simple and organized in the opposite manner. The flowers' five-lobed, bell-shaped calyxes (united sepals) and two-lipped, open-mouthed, tubular corollas (united petals) prefer to cluster together. People widely use lavender essential oil as a supplementary medicine on its own and as an ingredient in a variety of over-the-counter supplements and cosmetics [2]. People have used them as aromatherapists [3], antibacterials during World War I [4], sedatives [5], carminatives [6], antidepressants [7], anti-inflammatory agents [8], and antimicrobial and larvicidal agents [9] since ancient times. Its primary therapeutic uses stem from its advantages in the central nervous system [10]. Slender and upright, 40–100 cm tall, *Lavandula bipinnata* (Roth) Kuntze (Lamiaceae) grows in central and southern India [11].

2.0 Materials and methods

Lavandula bipinnata was collected in Panchmadi, Madhya Pradesh, in December 2022. Plant materials were gently rinsed with distilled water and dried in shade. The National Botanical Research Institute verified the *Lavandula bipinnata* collection number (348603) and accession number (113085). The plant material was dried under shade and ground by a mechanical grinder to make a powder. Then 20 g of powder was soaked with 95% methanol at room temperature (25 °C) for fractionation extraction. The whole extract was collected, filtered, and the solvent evaporated to dryness under reduced pressure and temperature (45 °C) by using a rotary evaporator. The yield of the *Lavandula bipinnata* leaf extract was calculated.

2.1 Preparation of Extracts: 20 g of powder was soaked with 95% methanol at room temperature (25 °C) for fractionation extraction. The whole extract was collected, filtered, and the solvent evaporated to dryness under reduced pressure and temperature (45 °C) by using a rotary evaporator. The yield of the aerial part of Lavandula bipinnata methanol extract was found to be 9.0429 gm [12].

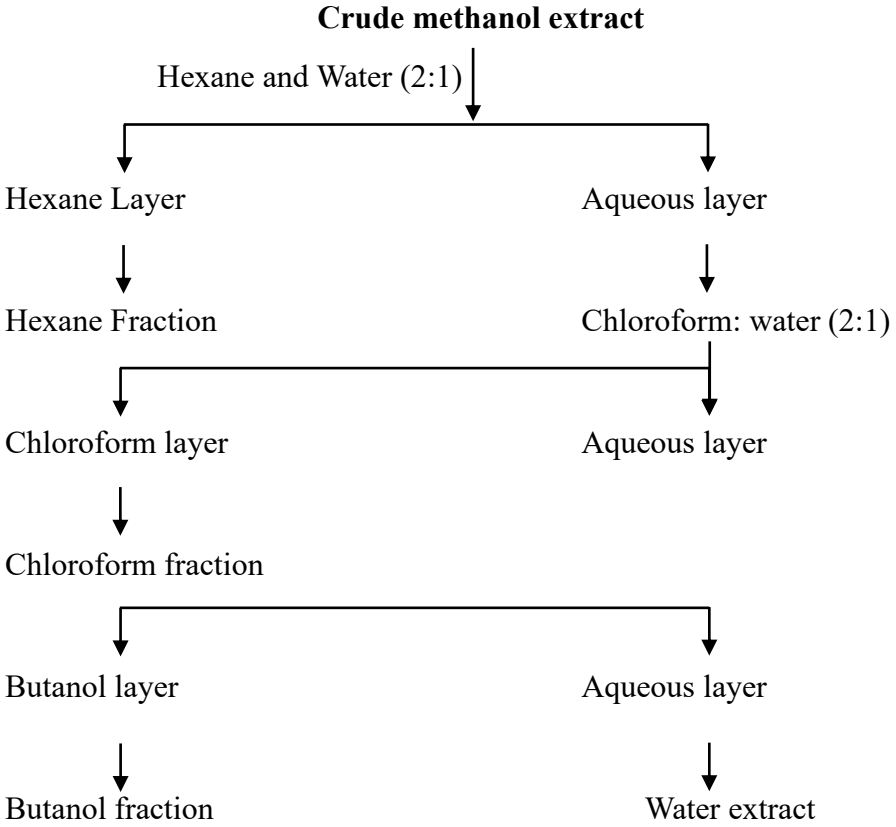


Table 1: Yield of Extracts with Different Solvents

S. No.	Solvents	Extraction yield in (gm)
1	Methanol	9.0429
2	Hexane	0.4063
3	Chloroform	0.1898
4	Butanol	1.728
5	Water	3.118

1. Chemicals and reagents

Acetonitrile (HPLC grade),) and acetic acid (HPLC grade) were used. All the chemicals and reagents are analytical grade and were purchased from Merck. Standard gallic acid, quercetin, caffeic and Rutin acid was purchased from Sigma Aldrich.

3.2 HPLC instrumentation

Analysis of phytochemicals were determined by High-Performance Liquid chromatography HPLC. The gallic acid, quercetin, rutin and caffeic acid is used as a standard for the qualitative and quantitative analysis. The HPLC analysis was performed on Shimadzu Prominence model with PDA detector (SPD-M 20 A). The chromatographic separation of metabolites was achieved on Shim-pack GWS C18 (250 x 4.6, 5µm) column with 0.6 mL/min flow rate and 20 µL injection volume was injected by auto sampler (SIL-20AC HT) working at 40°C in the column oven (CTO-10 AS VP). The mobile phase consisted of two solvents including 1 % Acetic acid in water (Solvent A) and Acetonitrile (Solvent B) of HPLC grade. Sample height is calculated by the standard external method [13].

3.3. Preparation of standard and sample solutions

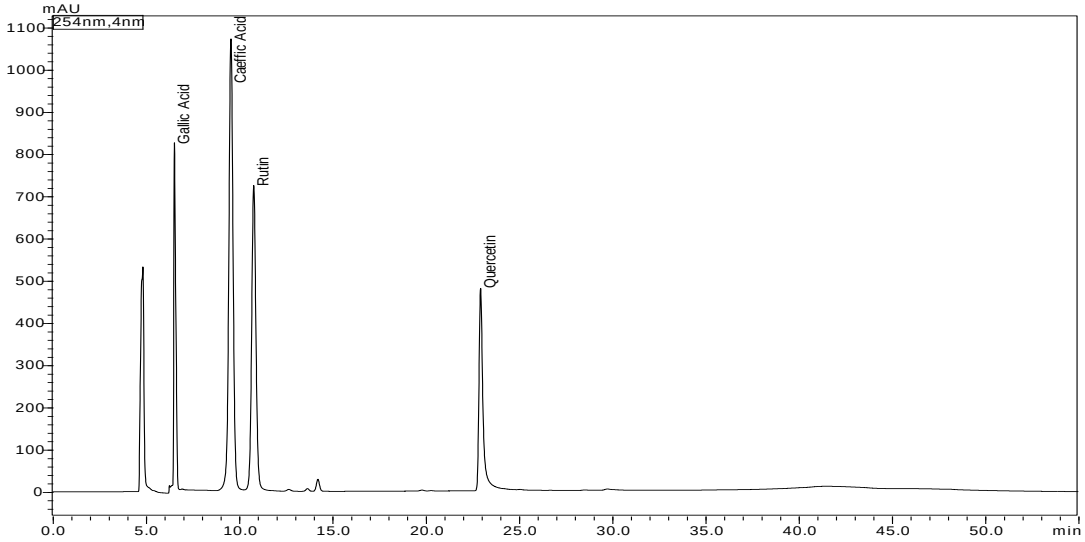
A standard solution of the gallic acid, quercetin, rutin and caffeic acid was prepared in methanol (1 mg/mL). The sample solution also was prepared by dissolving 10 mg of extract in 1 mL methanol. Both the standard and sample solutions were filtered through Whatman NYL 0.45 mm syringe filter. The responses were measured as peak areas vs concentration.

3.4. Preparation of mobile phase

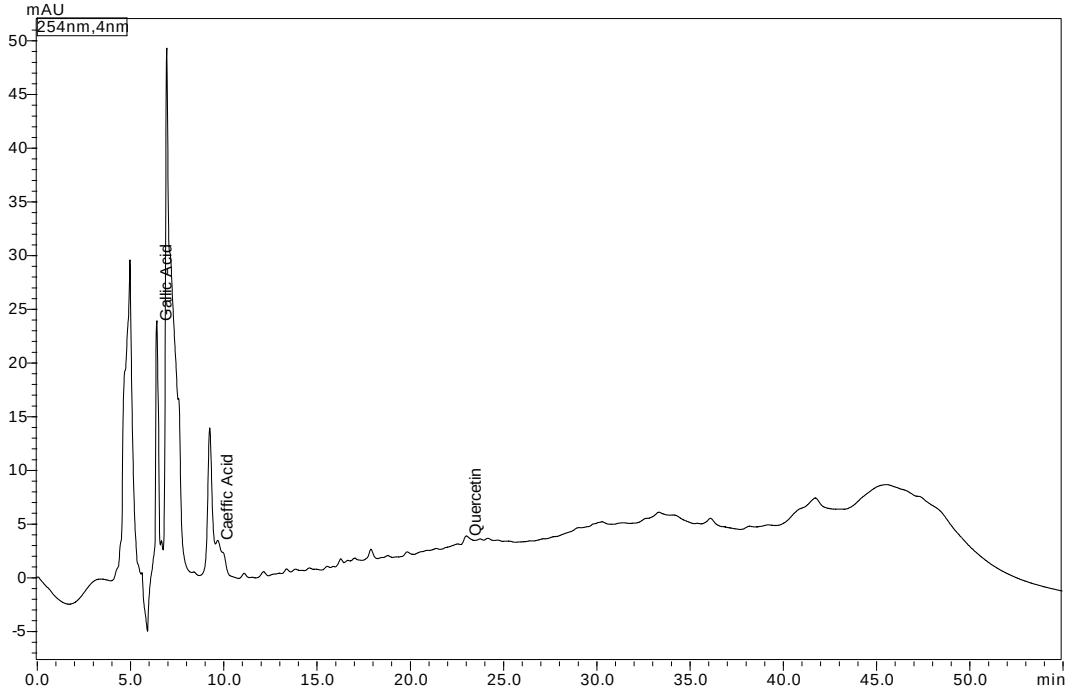
Mobile phase was prepared by using 1 % Acetic acid in water (Solvent A) and Acetonitrile (Solvent B) of HPLC grade. The pH was adjusted 3.5 with 1% acetic acid and then each solvent was filtered through 0.45 mm Millipore membrane filter followed by ultra-sonication to de-gas the solvent.

3. Results

HPLC Analysis in Standard



HPLC Analysis in methanolic plant extract *Lavandula bipinnata*



LB-M				
Compound name	Retention time	Area	% content	µg/g
Gallic acid	6.45	438273	3.14	6289.08
Caffeic acid	9.288	352468	1.02	2037.97
Rutine	11.127	13877	0.06	115.77
Quercitine	23.04	12593	0.09	171.61

4. Discussion

Various solvents were used for the extraction of bioactive compounds from Lavandula bipinnata. Methanol yielded the highest extraction amount (9.0429 gm), while water extraction yielded a value of 3.118 gm, whereas butanol and hexane yielded values of 1.728 gm and 0.4063 gm, respectively, and chloroform yielded only 0.1898 gm. This suggests that the best solvent for these studied phytochemical compounds is methanol. The study conducted a qualitative and quantitative analysis of the standard compound's quercetin, rutin, gallic acid, and caffeic acid. The HPLC analysis was performed using a PDA detector, whose stability and precision were assured for metabolite separation and quantification. An optimised chromatographic environment consisting of a Shim-pack GWS C18 column was used for efficient analysis with a 0.6 mL/min flow rate and a mobile phase composition containing 1% acetic acid in water (solvent A) and acetonitrile (solvent B). To ensure their purity and consistency, the compounds were dissolved in methanol and filtered through a 0.45-mm syringe filter to prepare the standard and sample solutions. The HPLC system's accuracy and efficiency were maintained by meticulously preparing and releasing the mobile phase.

5. Conclusion

This HPLC may be useful for quantitative estimation of the chemical constituents present in the plant extract, as well as quality assessment of the phenolic compounds. The technique developed for phytochemical extraction and HPLC analysis is accurate and efficient. Methanol proved to be the most effective extraction solvent, and we perfected the HPLC setup to provide precise phytochemical standard detection and quantification. This work provides a solid basis for additional studies into the extraction and analysis of plant metabolites.

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