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Research Article

Studies on the Evaluation of Flavonoids (Morin, Naringin, Quercetin & Rutin) in *Pongamia Pinnata* (L.) Pierre Leaves By HPLC

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ABSTRACT

Pongamia pinnata (L.) Pierre is recognized for its bioactive potential due to the presence of polyphenolic compounds, notably flavonoids, known for their antioxidant properties and associated health benefits. This study focused on the evaluation and quantification of specific flavonoids—Morin, Naringin, Quercetin, and Rutin—in the leaves of *P. pinnata*. High-Performance Liquid Chromatography (HPLC) was employed for qualitative analysis, utilizing a reverse-phase column with 0.1% phosphoric acid and acetonitrile in a 25:75 ratio. The standard retention times for Quercetin, Morin, Naringin, and Rutin were recorded as 2.513 min, 3.887 min, 7.130 min, and 4.780 min, respectively, with specific findings for *Pongamia pinnata* leaves indicating Morin at 3.547 min, Rutin at 4.853 min, Naringin at 6.507min, and Quercetin at 2.527 min. The peak areas for these compounds reflected their relative concentrations, underscoring the medicinal value of *P. pinnata* as a significant source of bioactive flavonoids for pharmacological research.

INTRODUCTION

Pongamia pinnata (L.) Pierre, belonging to the Fabaceae family, is a medium-sized glabrous tree extensively used in traditional medicine and valued for its wide range of applications, including green manure, animal fodder, wood, fuel, bio-pesticide, and as fish poison in India and neighbouring regions (1). Commonly known as Karanja in Hindi and Indian beech in English,

Pongamia pinnata thrives in tropical and subtropical climates, particularly in well-drained, sunny areas. This plant is a member of the Papilionoidea sub-family, notable for its nitrogen-fixing capabilities, a feature characteristic of many legumes, and is synonymous with *Millettia pinnata* (L.) Pierre, Panigrahi in botanical literature [2].

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Pongamia pinnata (L.) Pierre, a well-known tree in traditional medicine, has a rich history of therapeutic use rooted in Ayurveda, where its diverse medicinal applications have been recognized for decades. This versatile plant produces bioactive compounds, such as karanjin and pongamol, which not only impart a bitter taste deterring herbivores but also provide natural protection against insects, reducing management costs and enhancing sustainability. These compounds have practical uses beyond pest resistance, finding applications in crop sprays, cosmetics, and sunscreens due to their chemical properties [3]. Traditional uses of *Pongamia pinnata* extend to treating conditions such as malaria, bronchitis, whooping cough, and rheumatic joints, among others. The leaves, known for their digestive, laxative, and anthelmintic properties, are used in remedies for ailments like piles, wounds, and inflammation. Various extracts from the leaves, roots, and seeds are employed in managing diseases, including leprosy, rheumatism, and certain infections. *Pongamia pinnata* has also shown promising effects in wound healing, attributed to its antioxidative activity, moderate antimicrobial effects, and the induction of cytokine production, which may support its role in tropical wound care [4]. The plant's therapeutic potential has also gained modern recognition, as the Ministry of AYUSH has recommended formulations containing *Pongamia pinnata* for its antiviral, immunomodulatory, and anti-inflammatory properties, particularly prioritizing plants like this in COVID-19 research [5]. Beyond traditional uses, extracts of *Pongamia pinnata* demonstrate significant antihyperglycemic, hypolipidemic, and hepatoprotective activities, in some cases proving as effective as standard pharmaceuticals [6]. Phytochemical studies on *Pongamia pinnata* have uncovered a diverse range of bioactive compounds, prominently featuring flavonoids

with unique structural variations. The plant is abundant in prenylated flavonoids, including furano-flavones, furanoflavonols, chromenoflavones, and chalcones. Of these, flavones and their derivatives are the most common, distributed throughout all plant parts. These flavones exhibit various structural modifications, such as methylenedioxy groups and glycosidated forms, and include compounds like Furano chalcones and pyranochalcones. Subsequent studies expanded on findings, identifying furanoflavones, chromenoflavones, and other unique flavonoids across different plant parts, such as the flower, stem bark, and roots. This array of flavonoids contributes to *Pongamia pinnata*'s wide range of medicinal and agricultural applications due to their distinct bioactivities. [7] Morin is a natural flavonoid found in various plants, including those in the Moraceae family. It has a chemical structure of 2-(2,4-dihydroxyphenyl)-3,5,7-trihydroxychromen-4-one, characterized by a three-ring system with multiple hydroxyl groups. Naringin is a flavonoid glycoside primarily found in citrus fruits, especially grapefruits and bitter oranges. It is chemically classified as the 7-O-neohesperidoside of naringenin. With a flavonol backbone, quercetin has hydroxyl groups attached to positions 3, 5, 7, 3', and 4', and is known for its potential health benefits. Rutin, or quercetin-3-rutinoside, is a flavonol glycoside made of quercetin and the disaccharide rutinose, found in foods like buckwheat, citrus fruits, apples, and berries. [8-9] The aim was to evaluate and determine the presence of the flavonoids Morin, Naringenin, Quercetin, and Rutin using the HPLC method. The rationale for selecting leaves over fruits lies in their constant availability, making them a more sustainable and accessible resource for further research and practical applications in medicinal biotechnology.

Methodology



Preparation and extraction of plant extract:

Collect fresh *Pongamia pinnata* leaf samples and dry them using the shade-drying method. Once dried, grind the leaves with a mortar and pestle until they reach a fine powder consistency. Store the powder in a dark drawer until further use.

Soxhlation

Samples were prepared and weighed to 20 grams. The apparatus was cleaned with ethanol, and both the sample and solvent system were loaded. The apparatus was placed in the heating mantle at 30°C, where eight cycles of Soxhlation were completed. The extract was then stored at room temperature for further analysis [5].

HPLC (High-performance liquid chromatography)

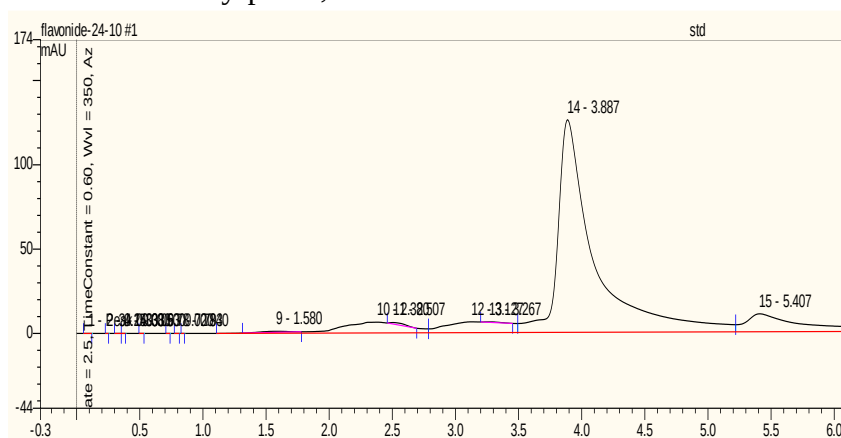
High-performance liquid chromatography (HPLC) was used to separate, identify, and quantify compounds within the sample. The mobile phase consisted of HPLC water, organic solvents, and buffers adjusted to a specific pH. The sample was prepared by dissolving it in a suitable solvent, then filtered through a 0.22 or 0.45 µm filter to remove any particulates. The HPLC system comprised a pump, injector, column, and detector. The mobile phase was pumped through the column at a constant flow rate, and the sample was injected using either an autosampler or a manual injector. The column separated the compounds based on their interactions with the stationary phase, and a

UV-Vis or other suitable detector identified the separated components [6].

Elution was monitored, and the retention times of the compounds were recorded. Data analysis was performed to determine the concentration and purity of the analytes by examining peak areas or heights. Following analysis, the column was flushed with a high proportion of organic solvent to remove residual compounds and ensure optimal column performance. Results were compared with the standard for further evaluation.

RESULTS

The HPLC analysis of the standard was conducted using an RP column, with a mobile phase composed of 0.1% H₃PO₄ and acetonitrile in a 25:75 ratio at a flow rate of 1 mL/min. Detection was performed at a wavelength of 350 nm, with the column temperature maintained at 37 °C and an injection volume of 20 µL. The retention times for Quercetin, Morin, Naringin, and Rutin were recorded at 2.513 min, 3.887 min, 7.130 min, and 4.780 min, respectively, as shown in Figures 1-4. The HPLC analysis of the sample followed the same protocol as that of the standard. The retention times observed for Morin, Naringin, Quercetin, and Rutin were 3.547 min, 6.507 min, 2.527 min, and 4.853 min, respectively, with peak areas of 6.7877, 53.6055, 109.5969, and 51.842 mAU*min, as illustrated in Figure 5.



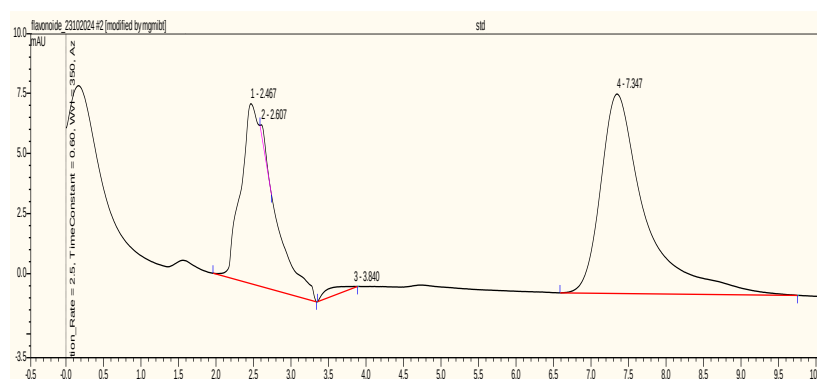


Fig.2 Standard Chromatogram of Naringin

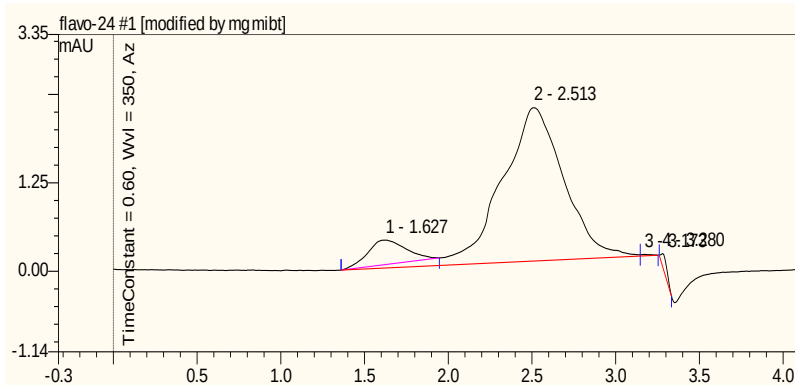


Fig.3 Standard Chromatogram of Quercetin

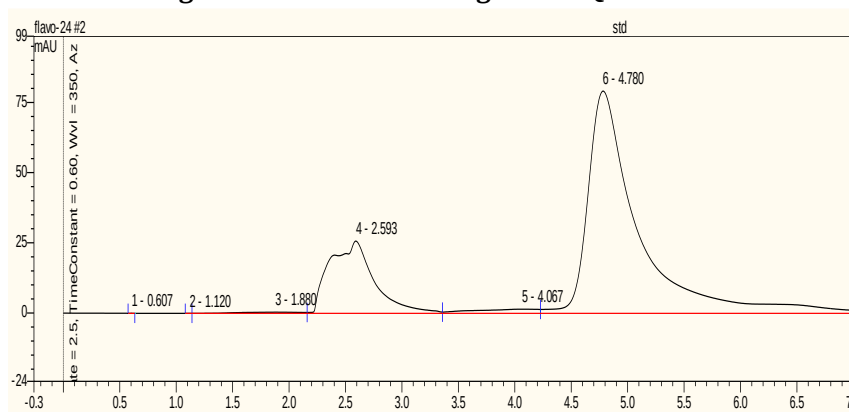


Fig.4 Chromatogram of Standard rutin

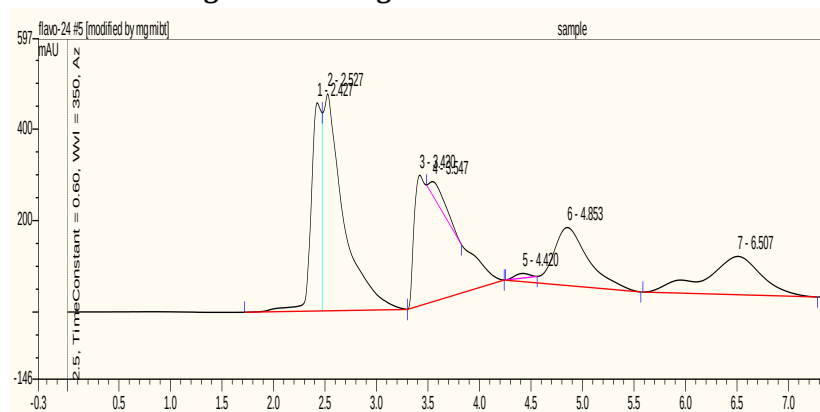


Fig.5 Chromatogram of *Pongamia pinnata* (L.) Pierre

DISCUSSION

The evaluation and quantification of flavonoids from *Pongamia* involved Soxhlet extraction followed by High-Performance Liquid Chromatography (HPLC) analysis. Soxhlet extraction efficiently isolated flavonoids, while HPLC provided precise identification and quantification of morin, rutin, quercetin, and naringin. The standard retention times for the four flavonoids were 2.513 min for Quercetin, 3.887 min for Morin, 7.130 min for Naringin, and 4.780 min for Rutin. In *Pongamia* leaf extracts Quercetin, Morin, Naringin, and Rutin are identified at retention times of 2.527 min, 3.547 min, 6.507 min, and 4.853 min respectively. These results confirm the presence of key flavonoids with potential health benefits in *pongamia* leaves. Previous studies have also reported the presence of flavonoids in *pongamia* leaves and their potential antioxidant and antimicrobial properties. The current study provides a detailed analysis of the flavonoid composition in *Pongamia* leaves, Results indicated that *Pongamia* leaves contain significant amounts of morin, rutin, quercetin, and naringin. HPLC demonstrated high selectivity and sensitivity, effectively separating the flavonoid compounds. The detection wavelength used is 350 nm to capture optimal absorbance

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