

QUANTIFICATION OF RUTIN FROM *AEGLE MARMELLOS* USING HIGH-PERFORMANCE THIN LAYER CHROMATOGRAPHY

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ABSTRACT

Aegle marmelos, commonly known as Bael or Bengal quince, is a medicinal plant belonging to the family Rutaceae. The leaves of *Aegle marmelos* are rich in bioactive compounds and are traditionally used in various herbal remedies. Medicinal properties of *Aegle marmelos* leaves include antidiarrheal, antipyretic, ulcer healing, anti-inflammatory, antidiabetic, hypolipidemic and antioxidant activities. Rutin is a flavonoid, specifically a glycoside of quercetin extracted from leaves of bael. In the present study, the ethanolic extract of leaves of *Aegle marmelos*, collected from Ethno Veterinary Herbal Garden of Veterinary College and Research Institute, Orathanadu was subjected to High Performance Thin Layer Chromatography (HPTLC) to quantify rutin, a flavonoid from this plant. The study revealed that *Aegle marmelos* sample was found to contain rutin @ 272.2 µg per 100 mg of sample.

Keywords: Rutin, *Aegle marmelos*, Flavonoid, High Performance Thin Layer Chromatography

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INTRODUCTION

Aegle marmelos, commonly known as Bael, is a deciduous tree belonging to the family Rutaceae, native to South Asia.

This plant has been utilized in traditional medicine for centuries, particularly in Ayurveda, where it is revered for its diverse health benefits. The leaves, fruits, and leaves of *Aegle marmelos* have been integral to various therapeutic practices. Like many medicinal plants, *Aegle marmelos* leaves are rich in phytochemicals, like alkaloids, tannins, saponins and glycosides which play a crucial role in plant defence mechanisms. *Aegle marmelos* is commonly used in Indian culture for its tasty fruit and leaves, which are believed to be presented

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to Lord Shiva in traditional practices. This plant is reported to have numerous pharmacological properties. The leaf extract possesses anti-inflammatory, anti-microbial and anti-cancer properties, similar to plant roots (Pandey and Pande, 2023). More than 80% of the world's population relies completely on medicinal plants for basic health care. Some of these herbs have been shown to provide symptomatic relief and help prevent secondary complications of diseases, while others have been shown to aid in the regeneration of aberrant cells and the overcoming of disease-causing microorganisms (Nyamai *et al.*, 2016). Bael leaves are laxative, and expectorant that are used to cure disorders such as ophthalmia, deafness, inflammations, cataract, diabetes, diarrhoea, dysentery, heart palpitations and asthmatic issues (Subedi and Bashyal, 2022). The leaves are also noted for their potential in regulating blood sugar levels, making them beneficial for diabetes management (Bhavani and Rajeshkumar, 2014). In many regions, the decoction of Bael leaves is consumed to enhance overall health and vitality.

Flavonoids are polyphenolic compounds which are one of the important classes of plant derived chemicals that contain benzopyrone moiety. About 4000 types of flavonoids have been reported to be present in plants. Rutin is a flavonol which is found abundantly in many plants. It has been demonstrated to have a number of pharmacological activities, including antioxidant, cytoprotective, vasoprotective, anticarcinogenic, neuroprotective and cardioprotective activities (Ganeshpurkar

and Saluja, 2017). Rutin's therapeutic potential is attributed to its ability to scavenge free radicals, thereby mitigating oxidative stress. Research indicates that rutin from *Aegle marmelos* exhibits significant antioxidant activity, contributing to cellular protection against damage induced by reactive oxygen species (Ahmad *et al.*, 2021). Additionally, studies have highlighted its anti-inflammatory effects, anti-diabetic effects which may aid in the management of various disorders.

Due to its pharmacological properties, rutin is increasingly being incorporated into dietary supplements and functional foods. Its presence in *Aegle marmelos* leaves positions it as a valuable ingredient in natural health products, contributing to their antioxidant and anti-inflammatory benefits.

Several studies have estimated phytochemicals using analytical technique such as High Performance Thin Layer Chromatography (HPTLC) which is considered to be sensitive, simple and accurate method (Ranganathan *et al.*, 2020). Thomas *et al.* (2020) estimated rutin in ethanolic and aqueous extracts of *Moringa oleifera* using a fixed composition of the mobile phase (toluene: ethyl acetate: menthol: formic acid solution (4.9:4.1:2:0.5 v/v/v/v) with densitometric analysis at 300 nm. Quantification of rutin level may support medicinal properties of *Aegle marmelos* and there were no studies involved in quantification of rutin from *Aegle marmelos* cultivated from Cauvery delta region of Tamil Nadu.

In the present study, the leaves of *Aegle marmelos*, cultivated from the herbal garden of Ethno Veterinary Herbal Product Research and Development Centre, located at Veterinary College and Research Institute, Orathanadu, Thanjavur district, were used for extraction of rutin, a flavonoid which was quantified using High Performance Thin Layer Chromatography.

MATERIALS AND METHODS

Plant Source

The herbal garden, Ethno Veterinary Herbal Product Research and Development Centre, Tamil Nadu Veterinary and Animal Sciences University, Orathanadu, Thanjavur District, Tamil Nadu, provided fresh *Aegle marmelos* leaves. The leaves were cleaned, shade-dried, and crushed for additional analysis.

Preparation of ethanolic extract of *Aegle marmelos*

To extract bioactive components from *Aegle marmelos* leaves, a cold maceration approach was used. This approach involved macerating 280 g of finely powdered *Aegle marmelos* leaves with 70% ethanol in a 1:5 ratio for seven days. The mixture was held at -20°C and shaken intermittently to improve extraction efficiency. After maceration, the mixture was filtered through Whatman No. 1 filter paper to produce the clear extract.

The filtrate was then concentrated with a rotary evaporator to completely remove/condense the solvent, yielding a phytochemical-rich concentrated extract.

Cold maceration is a mild extraction

technique that minimizes the degradation of heat-sensitive compounds, making it well-suited for generating high-quality extracts from medicinal plants. This method effectively safeguards the integrity of bioactive components, such as flavonoids and phenolics, which are vital for the medicinal properties of *Aegle marmelos* leaves.

Characterization of rutin in *Aegle marmelos*: advanced HPTLC analysis

Sigma Aldrich (USA) provided standard rutin for comparative analyses. The chromatographic analysis was conducted using silica gel 60 F254 Thin Layer Chromatography (TLC) plates (Merck). Various volumes of rutin standard solution (1 mg/mL) were applied, specifically 1, 2, 3, 4, 5, 6, 7, and 8 µL, alongside 2, 4, 6, 8, 10, 12, and 14 µL of *Aegle marmelos* crude extract samples (100 mg/mL). The application was performed using a CAMAG Linomat 5 sample applicator, with banding as the application type, maintaining an 8.0 mm distance on the Y-axis. The length of the application was also kept at 8.00 mm. The plate was developed at room temperature in a CAMAG twin-trough vertical development chamber (20x10), which had been saturated with the mobile phase of ethyl acetate, formic acid, glacial acetic acid and water (20:1:1:2.6 v/v) for 20 minutes. The solvent front position was set at 70 mm. Following development, the plate was analyzed using a CAMAG visualizer2 with a tungsten light source at a wave length of 366 nm, employing a scanning speed of 20 mm/s and slit dimensions of

6.0 mm x 0.45 mm. The scanning process was managed with Vision CATS software (version 2.5.18262.1). The detection of rutin in the *Aegle marmelos* crude extract samples was identified on the HPTLC plates as bands exhibiting Retardation factor (Rf) values consistent with those of the rutin standard. The Retardation factor (Rf), which represents the ratio of the distance travelled by the compound to that of the solvent in a specified time frame, was measured for both the standard and sample spots.

Assessment of rutin levels in *Aegle marmelos* crude extract

The area calibration for the chemical rutin was performed at 366 nm with a CAMAG scanner (S/N: 250410). Eight reference standard samples and seven *Aegle marmelos* crude extract samples were tested to produce a standard curve for determining the rutin concentration of the *Aegle marmelos* crude extract samples.

RESULTS AND DISCUSSION

Table 1 presents the Rf values obtained from High-Performance Thin Layer Chromatography (HPTLC) analyses, indicating the presence of rutin in the *Aegle marmelos* crude extract samples. Figures 1, 2, and 3 illustrate the development of the TLC plate with fluorescence at 366 nm and confirm the identification of rutin in the *Aegle marmelos* crude extract, along with its corresponding Rf value. Numerous studies have employed HPTLC for the identification and evaluation of medicinal phytochemicals due to its sensitivity and precision. The current research effectively quantified key

phytochemicals in individual plant extracts, aligning with the findings of Salvi and Khan (2023), which also confirmed the presence of rutin in *Aegle marmelos*. Previous studies using CAMAG HPTLC relied on win CATS software, while this study utilized the more advanced vision CATS software. Additionally, there are no existing reports on the evaluation of rutin from *Aegle marmelos* grown in the Thanjavur district of Tamil Nadu.

A calibration curve was produced in the CAMAG scanner using four reference samples and five *Aegle marmelos* samples for quantification. Coefficient variation (CV) was 1.78%, and the correlation coefficient was $R = 0.993032$. The calibration curve revealed that the *Aegle marmelos* sample contains 272.2 μg of rutin per 100 mg of extract. The calibration curve was linear within a range of 147.7 $\mu\text{g/ml}$ - 344.7 $\mu\text{g/ml}$ for *Aegle marmelos* samples. The mobile phase made of ethyl acetate, formic acid, glacial acetic acid, and water (20:1:1:2.6 v/v/v/v) solvent system with intermediate to high polarity were used for the study. Arora and Itankar (2017) also identified rutin from *Chenopodium album* with similar solvent system. Ethyl acetate is a semi polar solvent which effectively dissolves flavonoid aglycones and acetic acid has the capacity to enhance the extraction of flavonoid components. Salvi and Khan (2023) identified rutin in *Aegle marmelos* leaves with the solvent system of Ethyl acetic acid derivation: frosty acidic corrosive: formic corrosive: water in 10:1.1:1.1:2.5 (v/v/v/v) with Rf of 0.38. Typically, the Rf value for a particular compound falls between 0 and

1 and the present study also demonstrated the Rf value of 0.27 while identifying rutin compound from *Aegle marmelos* leaves. Figure 4 depicts the calibration curve for relative quantification of rutin in the *Aegle marmelos* sample.

CONCLUSION

The findings of the present study indicate that the leaves of *Aegle marmelos* grown in the herbal garden of the Ethno

Veterinary Herbal Product Research and Development Centre, Veterinary College and Research Institute, Orathanadu, contain 272.2 µg of rutin per 100 mg of extract.

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Table 1: HPTLC profile of rutin in the ethanolic extract of *Aegle marmelos* samples (rutin-1mg/ml; *Aegle marmelos* samples-100mg/ml)

Track	Rf	Height	Area	Assigned substance
Rutin standard	0.274	0.5055	0.01904	Rutin
Rutin standard	0.276	0.5734	0.023.04	Rutin
Rutin standard	0.274	0.6231	0.02646	Rutin
Rutin standard	0.274	0.6511	0.02875	Rutin
<i>Aegle marmelos</i> sample	0.268	0.2962	0.01478	Rutin
<i>Aegle marmelos</i> sample	0.261	0.3428	0.01840	Rutin
<i>Aegle marmelos</i> sample	0.253	0.3374	0.01957	Rutin
<i>Aegle marmelos</i> sample	0.245	0.3515	0.02191	Rutin
<i>Aegle marmelos</i> sample	0.239	0.3461	0.0226	Rutin
<i>Aegle marmelos</i> sample	0.234	0.3243	0.02199	Rutin
<i>Aegle marmelos</i> sample	0.231	0.2846	0.01966	Rutin

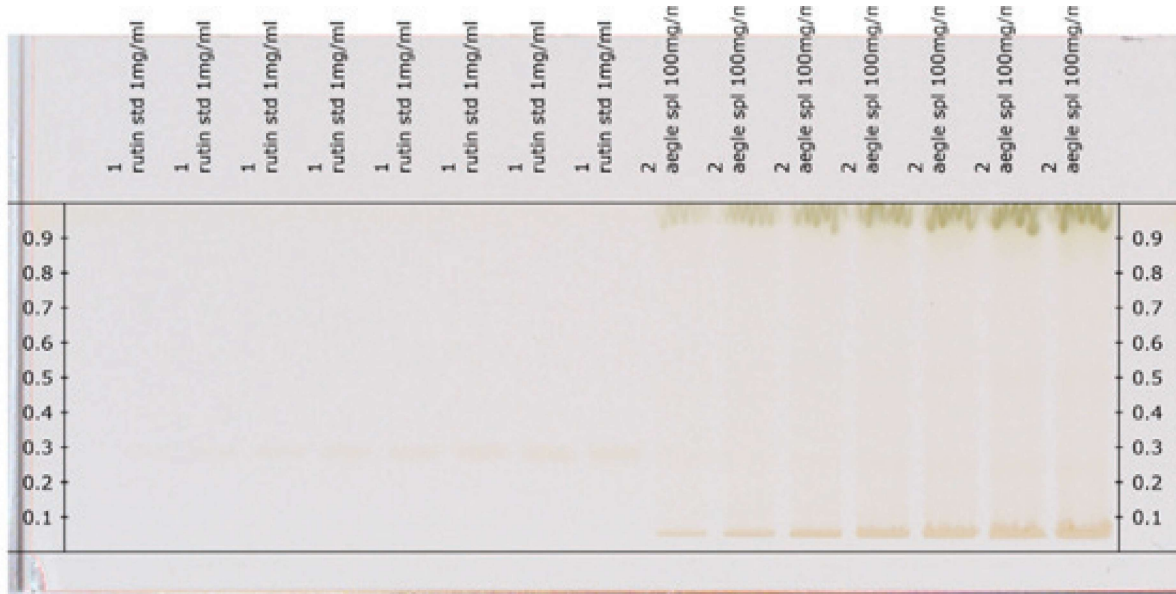


Fig 1: HPTLC Plate developed showing bands of standards and samples of rutin

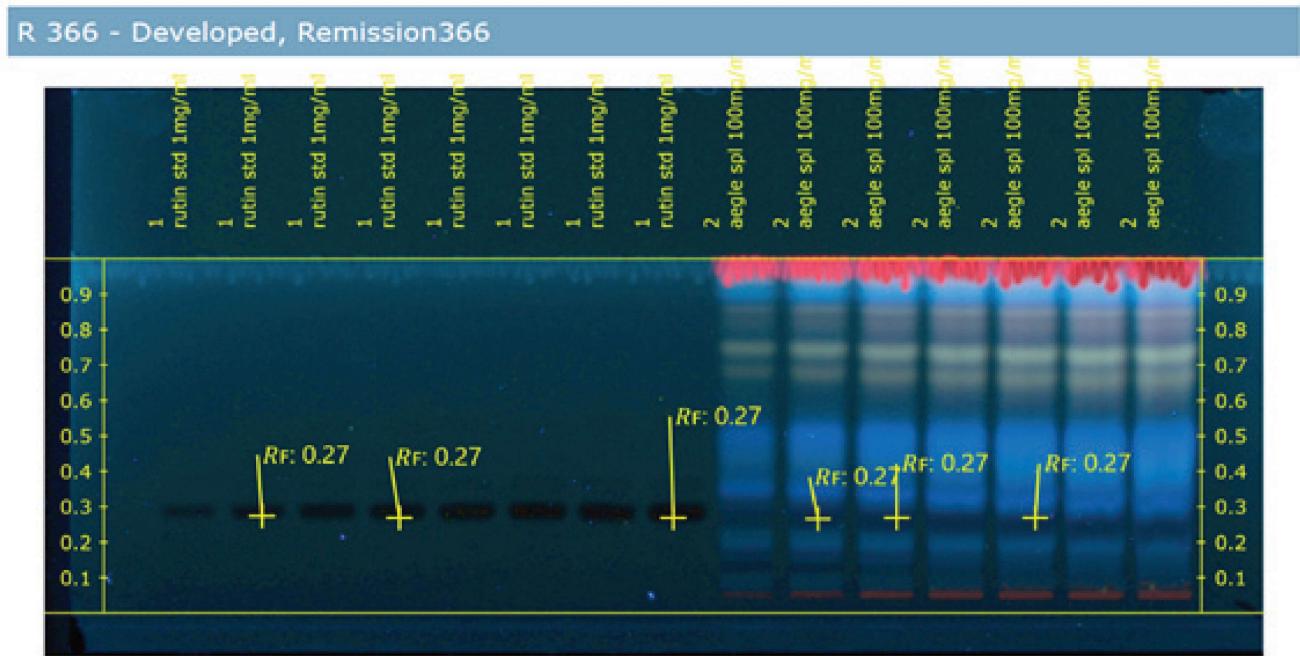


Fig 2: HPTLC Plate developed under visible remission (366 nm) showing bands of standards and samples of rutin

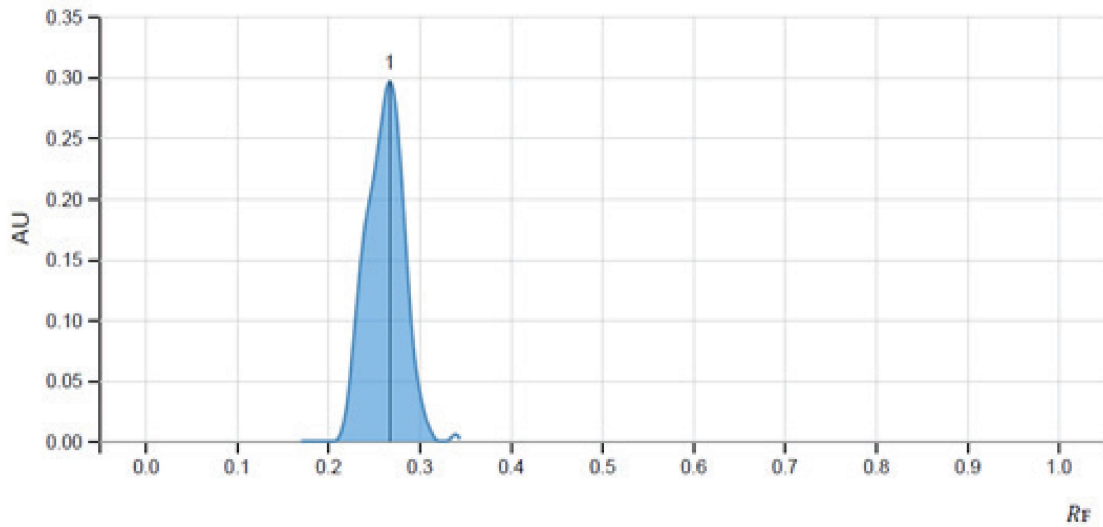


Fig 3: HPTLC plate track peak for identification of rutin in *Aegle marmelos* (Rf - 0.27)

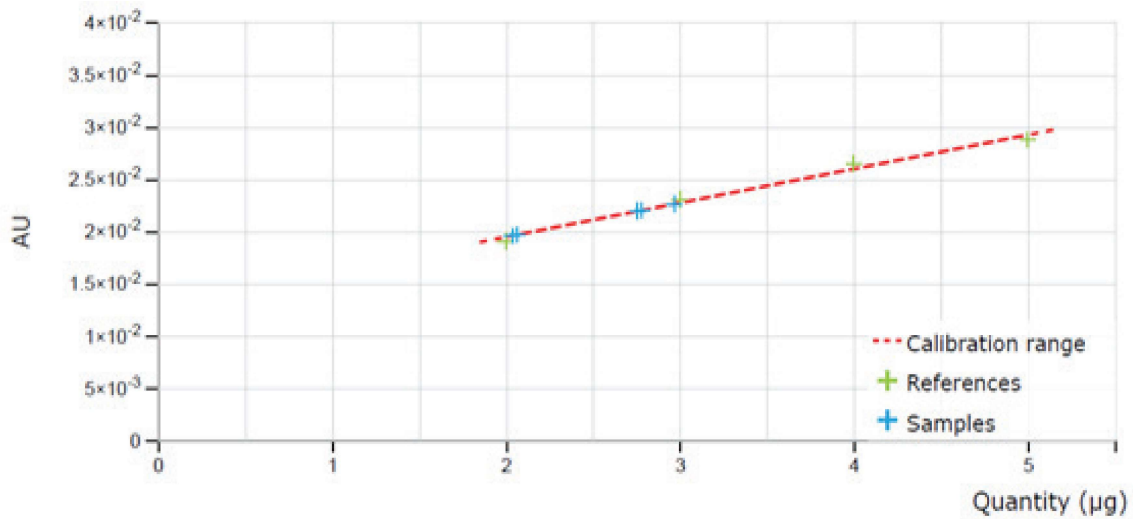


Fig 4: HPTLC calibration curve for relative quantification of rutin in *Aegle marmelos* Samples

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