

Solvent-Based Qualitative Analysis Of Secondary Metabolites In *Aegle Marmelos* Leaves

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Abstract: *Aegle marmelos* (L.) Correa, commonly known as bael, is a medicinal plant widely used in traditional medicine. The present study aimed to carry out qualitative phytochemical analysis of *Aegle marmelos* leaves using different solvents, including ethanol, methanol, chloroform, water, and petroleum ether. Standard phytochemical screening methods were employed to detect major classes of secondary metabolites. The results indicated variation in metabolite distribution depending on the solvent used, highlighting the influence of solvent polarity on phytochemical extraction. This preliminary investigation provides baseline information on the phytochemical profile of bael leaves and supports their ethnomedicinal relevance.

Keywords: *Aegle marmelos*, bael leaves, qualitative phytochemical analysis, solvent extracts.

INTRODUCTION

Medicinal plants have been an integral part of human civilization since ancient times, serving as primary resources for the prevention and treatment of numerous diseases. The therapeutic potential of plants is largely attributed to their capacity to synthesize a wide variety of organic compounds, many of which are not directly involved in growth or development but act as protective secondary metabolites¹. The investigation of these compounds through phytochemical analysis provides a scientific basis for validating traditional claims, ensuring reproducibility of therapeutic applications, and creating opportunities for the discovery of novel drugs².

Phytochemical analysis is broadly classified into qualitative and quantitative approaches. Among these, qualitative analysis serves as the first and most fundamental step, offering insights into the types of secondary metabolites present in a plant extract. This preliminary screening not only identifies the chemical classes but also guides subsequent isolation, characterization, and pharmacological evaluation³. Such an approach is particularly crucial for plants that hold a strong

position in ethnomedicine, as it allows researchers to bridge the gap between traditional knowledge and modern scientific validation⁴.

Aegle marmelos (L.) Correa, commonly known as bael, is a medicinally important plant belonging to the family Rutaceae. Native to India and widely distributed across the Indian subcontinent, the plant has been extensively utilized in Ayurveda, Siddha, and other indigenous systems of medicine⁵. Every part of this tree—including the root, bark, fruit, and leaves—has been employed in traditional remedies for various ailments. The leaves, in particular, hold a prominent place in indigenous practices, being used in formulations for the management of diabetes, gastrointestinal disorders, inflammatory conditions, and microbial infections⁶. This long-standing ethnopharmacological use strongly indicates that the leaves contain bioactive principles, warranting systematic phytochemical investigation.

The importance of phytochemical screening of *Aegle marmelos* leaves also lies in its role as a bridge between folklore and evidence-based medicine. While traditional knowledge suggests their wide applicability, scientific validation requires systematic documentation of the chemical constituents through established laboratory procedures. Qualitative phytochemical analysis thus becomes an essential tool, providing preliminary evidence of the presence of broad classes of metabolites that may underlie the reported pharmacological effects⁷.

Moreover, qualitative phytochemical analysis is cost-effective, reproducible, and methodologically straightforward, making it highly suitable for initial screening of medicinal plants. It involves a series of well-established colorimetric and precipitation reactions that give rapid confirmation of metabolite classes without the need for sophisticated instrumentation⁸. Such initial evidence is indispensable for guiding further advanced studies, including chromatographic separation, spectroscopic characterization, and bioactivity evaluation. In addition, qualitative analysis enables comparisons of extracts prepared under different conditions, such as solvent polarity or extraction technique, thereby contributing to standardization and optimization of herbal formulations⁹.

The investigation of medicinal plants like *Aegle marmelos* also aligns with the global interest in natural products research. With the rising incidence of chronic diseases, the limitations of synthetic drugs, and the growing concern over adverse side effects, plant-derived products have gained renewed attention in recent decades¹⁰. Scientific exploration of ethnomedicinal plants not only contributes to drug discovery but also supports biodiversity conservation and sustainable use of natural resources. Within this framework, phytochemical analysis of bael leaves provides essential baseline data for integrating traditional medicine with modern pharmaceutical research¹¹.

This study involves the qualitative phytochemical analysis of ethanol extract of *Aegle marmelos* leaves using standard preliminary screening methods. The purpose of this investigation is to establish a scientific basis for the presence of broad classes of secondary metabolites that may contribute to the ethnomedicinal applications of the plant. By adopting a qualitative approach, the study provides preliminary but essential data that will support future pharmacological and phytochemical research on *Aegle marmelos*.



Fig-1: Visual Depiction of *Aegle marmelos* Plant and Foliage

MATERIALS AND METHODS

Collection and Preparation of Plant Material

Fresh leaves of *Aegle marmelos* were collected from Derisanamcope village, Kanyakumari district. The leaves were washed under running water, shade-dried, and ground into fine powder. The powdered material was stored in an airtight container until use.

Extraction of Plant Material

10 g of powdered leaves were successively extracted with 250 ml each of petroleum ether, chloroform, methanol, ethanol, and distilled water using the Soxhlet method. Extractions were continued until the solvent in the siphon tube became colorless. The extracts were concentrated under reduced pressure, air-dried, and stored at 4°C in airtight containers.

Qualitative Phytochemical Screening

Preliminary phytochemical analysis of the extracts was carried out using standard protocols to detect the major classes of secondary metabolites.

Alkaloids

The presence of alkaloids was tested using the **Iodine test**, where 3 mL of extract solution mixed with a few drops of iodine solution produced a **blue colour**, confirming alkaloids.

Amino Acids

The **Ninhydrin test** was employed by adding 2 mL filtrate with 2 drops of ninhydrin solution, resulting in a **purple colour**, which indicated amino acids¹².

Flavonoids

Flavonoids were detected by the **Ammonia test**, where the filtrate treated with 5 mL dilute ammonia solution and concentrated H₂SO₄ produced a **yellow colour**.

Glycosides

The **Concentrated H₂SO₄ test** was performed by mixing 5 mL plant extract with 2 mL glacial acetic acid, a drop of 5% FeCl₃, and conc. H₂SO₄, forming a **brown ring**, confirming glycosides.

Carbohydrates

Carbohydrates were identified by **Molisch's test**. Here, 2 mL filtrate mixed with 2 drops of alcoholic α -naphthol and 1 mL conc. H₂SO₄ formed a **violet ring**.

Phenols

The **Ferric chloride test** was performed with aqueous extract and 5% ferric chloride solution, producing a **dark green/ bluish-black colour**, indicating phenols.

Saponins

The **Foam test** was done using 0.5 g extract with 2 mL water, shaken vigorously. The appearance of **persistent foam for 10 minutes** confirmed saponins.

Phytosterols

Phytosterols were identified by the **Salkowski's test**, where the filtrate treated with conc. H₂SO₄ produced a **red colour in the lower layer**.

Tannins

The **Bromine water test** was used, where 10 mL bromine water decolorized upon adding 0.5 g extract, confirming tannins.

Terpenoids

Terpenoids were detected by another **Salkowski's test**, where the filtrate with conc. H₂SO₄ gave a **golden-yellow layer at the bottom**.

Coumarins

The **NaOH test** with plant extract, 10% NaOH, and chloroform resulted in a **yellow colour**, confirming coumarins.

Phlobatannins

The **HCl test** was carried out by boiling 2 mL aqueous extract with 2 mL of 1% HCl, producing a **red precipitate**, which indicated phlobatannins.

Proteins

Proteins were confirmed using the **Xanthoproteic test**, where treatment with conc. nitric acid resulted in a **yellow solution**.

Anthocyanins

The **HCl test** was done by treating 2 mL plant extract with 2 mL 2N HCl followed by ammonia, producing a **pink-red solution**, confirming anthocyanins.

Cardiac Glycosides

The **Keller-Killani test** was performed by adding 1 mL filtrate to 1.5 mL glacial acetic acid, 1 drop of 5% FeCl₃, and conc. H₂SO₄ along the side of the tube, which gave a **blue solution**, confirming cardiac glycosides.

RESULT AND DISCUSSION

The preliminary phytochemical screening of *Aegle marmelos* leaf extracts using solvents of varying polarity (ethanol, chloroform, methanol, water, and petroleum ether) revealed the presence of several secondary metabolites (Table-1). The qualitative results indicate that solvent polarity significantly influences the solubility and detection of phytochemicals, with ethanol and methanol extracts showing the widest spectrum of bioactive compounds.

Table-1: Phytochemical screening of *A. marmelos* crude extracts

S.no	Extract	Ethanol	Chloroform	Methanol	Water	Petroleum Ether
1.	Alkaloids	+	+	+	+	-
2.	Aminoacids	-	+	-	-	-
3.	Flavanoids	+	+	+	+	-
4.	Glycosides	+	-	+	-	-
5.	Carbohydrates	-	-	+	+	-
6.	Phenols	+	+	+	-	-
7.	Saponins	+	-	-	+	+
8.	Phytosterols	-	-	-	+	-
9.	Tannins	+	-	-	+	-
10.	Terpenoids	+	-	-	-	-
11.	Coumarins	+	+	-	-	+
12.	Phlobatannins	-	-	-	-	-
13.	Proteins	+	-	-	-	-
14.	Anthocyanin	+	+	-	-	-
15.	Cardiac glycosides	+	-	-	-	-

[(+) Present, (-) Absent]

Alkaloids were present in all extracts except petroleum ether, while amino acids were detected only in chloroform. Flavonoids were found in ethanol, methanol, chloroform, and water extracts, whereas glycosides appeared in ethanol and methanol. Carbohydrates were present in methanol and water, and phenols were observed in ethanol, methanol, and chloroform extracts. Saponins were detected in ethanol, water, and petroleum ether, and phytosterols were observed only in water. Tannins appeared in ethanol and water, while terpenoids were confined to ethanol extract. Coumarins were found in ethanol, chloroform, and petroleum ether extracts, whereas phlobatannins were absent in all extracts. Proteins and anthocyanins were identified in ethanol, with anthocyanins also detected in chloroform. Cardiac glycosides were found exclusively in ethanol extract. Among the solvents, ethanol yielded the richest phytochemical profile, followed by methanol and water, while chloroform and

petroleum ether were less effective. These results demonstrate that solvent polarity plays a critical role in extracting bioactive compounds and provide a foundation for further phytochemical and pharmacological studies of *Aegle marmelos* leaves.

CONCLUSION

The qualitative phytochemical analysis of *Aegle marmelos* leaf extracts revealed the presence of various secondary metabolites, including alkaloids, flavonoids, glycosides, phenols, saponins, tannins, terpenoids, coumarins, proteins, anthocyanins, and cardiac glycosides. Ethanol was the most effective solvent, extracting the widest range of compounds, followed by methanol and water. The study confirms that solvent polarity strongly influences phytochemical extraction and provides a scientific basis for the traditional medicinal use of *Aegle marmelos* leaves. These findings serve as a foundation for further pharmacological and bioactive compound studies.

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