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HARNESSING THE POWER OF BAEL TREE: ANTITUBERCULAR PROPERTIES AND PHYTOCHEMICAL PROFILING OF *AEGLE MARMELOS* FRUIT-DERIVED COMPOUNDS

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ABSTRACT

Aegle marmelos, commonly known as Bael, is a sacred medicinal plant widely found in tropical and subtropical regions. This article focuses on the extraction, isolation, and evaluation of bioactive compounds from the fruit of *A. marmelos*, with a particular antitubercular activity. The fruits were subjected to acetone extraction followed by successive purification steps, leading to the isolation of four key bioactive compounds: Marmelosin, Marmin, Xanthotoxol, and Afzelin. These compounds were tested against *Mycobacterium tuberculosis* H37Ra and *Mycobacterium bovis* using the XRMA protocol. Notably, Compounds 1 and 2 demonstrated significant inhibitory activity against *M. bovis* at a concentration of 100 µg/mL. The findings suggest the potential role of *A. marmelos* fruit extracts in targeting both active and dormant forms of tuberculosis bacteria. Additionally, the fruit is rich in carbohydrates, vitamins, and phytochemicals like coumarins and flavonoids, which has wide-ranging pharmacological properties including antidiabetic, hepatoprotective, antimicrobial, anticancer, and radioprotective effects. One of the compounds, rutin, also shows the inhibitory potential against SARS-CoV-2 therapeutic targets, suggesting broader antiviral applications.

INTRODUCTION

Description: Aegle marmelos is an herbal plant, which is commonly found in our local areas. It grows in tropical and subtropical regions like India, Sri Lanka, Bangladesh, Nepal, Myanmar, Thailand. Which has various names such as bael, Bengal quince, golden apple, stone apple, wood apple, and bilva. It belongs to the **Rutaceae** family. Considered a sacred tree by Hindus and is offered to Lord Shiva while worshipping .

Extraction and Isolation of Bioactive Compounds from *Aegle marmelos* Fruit :

The fresh fruits of *Aegle marmelos* (1.9 kg) were initially crushed and subjected to extraction using acetone as the solvent. The extraction process was carried out at room temperature in three successive cycles, each lasting 14 hours, with a total volume of 6 liters of acetone. The combined acetone extracts were filtered and concentrated under reduced pressure using a rotary evaporator, yielding a greenish semi-solid residue weighing 237.4 g (representing a 12.59% yield based on the initial fruit weight). To remove lipophilic impurities such as fats and oils, the concentrated acetone extract was defatted using petroleum ether (300 mL × 3), resulting in a defatted extract weighing 230 g. This defatted material was further partitioned between water and n-butanol (300 mL each), producing a n-butanol soluble fraction. Upon concentration, this fraction yielded a yellowish solid residue weighing 194 g, which was selected for further fractionation. A portion of the n-butanol fraction (100 g) was subjected to column chromatography (CC) using a petroleum ether-acetone solvent system with a gradient ranging from 10% to 50% acetone. A

total of 60 fractions were collected, and based on similarities in their thin-layer chromatography (TLC) profiles, these were combined into 19 main fractions labeled AM1 through AM19. Fraction AM4 (3 g) was further separated by CC using an acetone gradient (25% to 50%) in petroleum ether to yield 36 sub-fractions (AM4-1 to AM4-36). Sub-fractions AM4-17 to AM4-36 were pooled together and crystallized in acetone to isolate **Compound 1** (1.0 g). Similarly, fractions AM8 and AM9 were combined, and **Compound 3** (20 mg) was isolated as white crystals. Fractions AM11 to AM14 were pooled and re-subjected to CC with a petroleum ether-acetone gradient (30% to 50%), yielding 26 sub-fractions (AM11-14-1 to AM11-14-26). From these, sub-fractions AM11-14-9 to AM11-14-17 were combined and crystallized to obtain **Compound 2** (100 mg). In a final purification step, fraction AM18 (2.5 g) was fractionated by CC using a methanol gradient (10% to 15%) in chloroform, collecting 12 sub-fractions (AM18-1 to AM18-12). Sub-fraction AM18-6 was further purified using preparative TLC, developed with a methanol:chloroform (15%) solvent system, resulting in the isolation of **Compound 4** (15 mg). This methodical extraction and purification process led to the successful isolation of four distinct bioactive compounds from *Aegle marmelos* fruit, which are under investigation for their potential antitubercular properties.

Testing compounds against Tb bacteria: The four compounds (Compound 1 to Compound 4) were tested to see how effective they are against tuberculosis bacteria in both their **active** (growing) and **dormant** (inactive) forms. To do this, scientists used a method called the **XRMA protocol**, which is a standard procedure for checking how well compounds can stop bacterial growth. Two types of tuberculosis bacteria were used in the experiment: **Mycobacterium tuberculosis H37Ra, Mycobacterium bovis-** These bacteria were first grown in a nutrient-rich liquid called **M. phlei medium** until they reached a good growth level (measured as O.D. 1.0 — this just means the bacteria had multiplied enough to start testing). The original bacterial stock was stored very cold at **–70°C**, and before starting the experiment, it was refreshed by growing it once in the same medium. For each compound, the experiments were done **three times (triplicates)** to ensure accuracy and reliability.

Taxonomical classification of Aegle marmelos : (Table-1)

Taxonomical Rank	Taxon
Kingdom	Plantae
Division	Magnoliophyta
Class	Magnoliopsida
Order	Sapindales
Family	Rutaceae
Sub-Family	Aurantioideae
Genus	Aegle
Species	A.Marmelos
Common Name	Bael Patra, Bael

Fig-1:Aegle marmelos

Compounds 1 and 2 at 100 µg/mL, showed growth inhibition of M. bovis –Anti-mycolytic.

Compounds isolated from Aegle marmelos fruits are shown in results and discussion (Table-2)

Phytochemical constituents of A. marmelos (FRUIT) : The fruit of **Aegle marmelos** has many useful natural compounds. It contains nutrients like carbohydrates, minerals, vitamins, and other helpful plant chemicals such as coumarins, phenolic acids, alkaloids, flavonoids, organic acids, oils, and fats.

It is a good source of: **Carbohydrates** – 31.80 g per 100 g, **Fiber** – 2.90 g per 100 g, **Minerals** – 1.70 g per 100 g, **Fats** – 0.39 g per 100 g. It also has important vitamins like: **Vitamin A** – 0.05 mg, **Vitamin B2** – 1.20 mg, **Vitamin C** – 8.0 mg, **Riboflavin** – 0.03 mg, **Thiamine (Vitamin B1)** – 0.13 mg, **Beta-carotene** – 55.0 mg

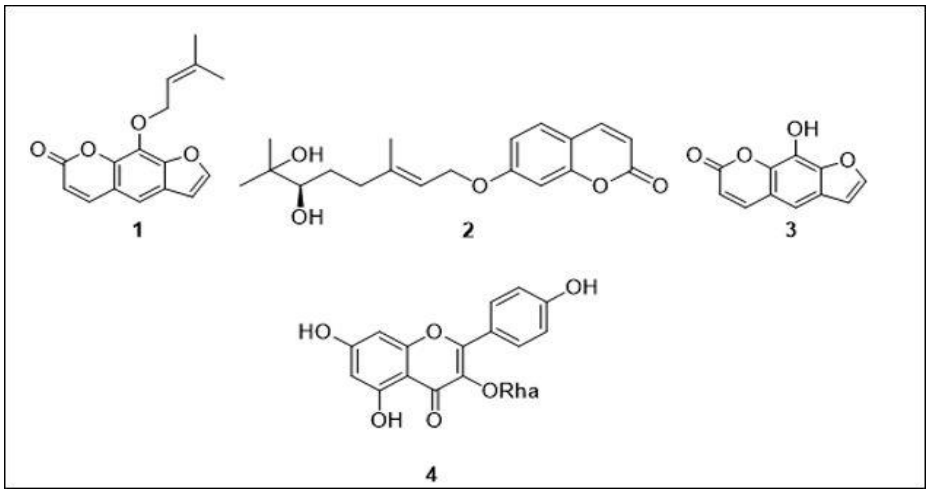

Fig-2: 1. Marmelosin , 2. Marmin. , 3.Xanthotoxol, 4. Afzelin

(All values are per 100 grams of the fruit.) (2)

Other Pharmacological Benefits of *Aegle marmelos :*

Anti diabetic Activity: Aqueous extracts of Aegle marmelos leaves and fruits show potential in managing diabetes by reducing blood glucose and enhancing antioxidant levels.

Hepatoprotective Activity: Aegle marmelos leaf and fruit extracts demonstrate against liver injuries induced by alcohol and CCI4

Anti microbial Activity: Extracts from Aegle marmelos fruit and leaves exhibit bactericidal activity against pathogens like Shigella, E. coli, and Salmonella

Analgesic, Anti-inflammatory, & Antipyretic Activity: Various extracts of Aegle marmelos leaves show significant anti-inflammatory, analgesic, and antipyretic effects, helping reduce swelling, pain, and fever in animal models.

Anti fungal Activity: Aegle marmelos extracts, particularly from its leaves, show antifungal effects by inhibiting spore germination

Anti cancer Activity: Aegle marmelos extracts exhibit cytotoxicity and apoptosis induction in cancer cell lines.

Radioprotective Activity: Extracts of Aegle marmelos enhance radiation tolerance and reduce radiation-induced damage in animal models

Anti spermatogenic Activity: Studies indicate that Aegle marmelos extracts reduce sperm motility and quantity

Anti ulcer Activity: Aegle marmelos demonstrates significant protective effects against various forms of gastric ulcers.

Anti thyroid Activity: Scopoletin from Aegle marmelos leaves reduces thyroid hormone levels.

Toxicity Studies: No significant toxicity was found in rats after prolonged exposure to Aegle marmelos extracts, indicating a high safety margin.

Other Medicinal Values: Aegle marmelos exhibits additional benefits, including antidiarrheal, insecticidal, and antioxidant activities. (4)

Other use:

The compound rutin in the extract has an inhibitory effect on multiple therapeutic targets of SARS-CoV-2. Further *invitro* and *in vivo* studies are required to propose these compounds as efficient drugs. (5)

RESULTS AND DISCUSSION:

Compounds isolated from Aegle marmelos fruits: (Table-2)

Compound	Appearance	Molecular Formula	Identified
Compound-1	White Crystals	$C_{16}h_{14}o_4$	Marmelosin (Or Imperatorin)
Compound-2	White Crystals	$C_{19}h_{24}o_5$	Marmin
Compound-3	White Needles	$C_{11}h_6o_4$	Xanthotoxol
Compound-4	Yellow Gum	$C_{21}h_{20}o_{10}$	Afzelin

CONCLUSION:

The study highlights the promising antitubercular potential of *Aegle marmelos* (Bael) fruit through the isolation and evaluation of four bioactive compounds—Marmelosin, Marmin, Xanthotoxol, and Afzelin. Among these, Marmelosin and Marmin exhibited significant inhibitory activity against *Mycobacterium bovis* at a concentration of 100 µg/mL, indicating their potential to target tuberculosis-causing bacteria. In addition to its antitubercular properties, *A. marmelos* is rich in nutrients and exhibits a wide range of therapeutic effects such as antidiabetic, hepatoprotective, antimicrobial, anti-inflammatory, anticancer, and radioprotective actions. The identification of rutin as a compound with inhibitory potential against SARS-CoV-2 targets .Overall, the study bridges traditional herbal knowledge with contemporary scientific investigation, suggesting that *Aegle marmelos* may serve as a valuable source for developing effective, natural treatments for tuberculosis and other diseases.

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