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ETHANOLIC EXTRACTION OF AEGLE MARMELOS LEAVES ACT AS A NATURAL ACTIVE POTENTIAL

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Abstract:
In many cultures around the world, herbal medicines have long been utilised to treat a variety of illnesses. In Indian traditional medicine, the Ayurveda and Siddha medical systems are extremely well-known. In recent years, researchers have worked to uncover and validate plant-derived substances that can be used to treat a variety of diseases. Similarly, it has been established that many plant parts, including leaves, fruits, seeds, etc., contain health- and nutrition-promoting substances that have historically been utilised to treat a variety of diseases. Aegle marmelos are utilised in ethnomedicine to take advantage of their therapeutic qualities, which include astringent, antidiarrheal, antidysenteric, demulcent, antipyretic, and anti-inflammatory actions. In India, it is a significant medicinal tree. During the present study, the extraction of the phytochemical was performed by Soxhlet extractor. Phytochemical screening of ethanolic extract leaves revealed the presence of alkaloids, terpenoids, tannins, etc. Within this view, the present study has explored the efficiency of the Aegle marmelos as a valuable natural source.

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INTRODUCTION:

Aegle marmelos is a rare species of tree that is indigenous to the Indian subcontinent and Southeast Asia. It is also referred to as bael (or bili or bhel), Bengal quince, golden apple, Japanese bitter orange, stone appl, or wood apple. As a naturalised species, it can be found in India, Bangladesh, Sri Lanka, and Nepal. Hindus and Buddhists both regard the tree as sacred.Aegle marmelos is a small to medium-sized tree or deciduous shrub that can grow up to 13 metres (43 feet) tall, with slender branches that droop and an open, uneven crown.The bark is a light brown or greyish colour, smooth or finely fissured and flaking, and furnished with long straight spines that can range in length from 1.2 to 2.5 centimetres (12-1 inch), singly or in pairs. Cut portions of the bark frequently ooze slimy sap. [1]. The flowers are 1.5 to 2 cm, pale green or yellowish, sweetly scented, bisexual, in short drooping unbranched clusters at the end of twigs and leaf axils. They usually appear with young leaves. The calyx is flat with 4 small teeth. The four or five petals of 6–8 millimetres (¼–⅜ in) overlap in the bud. Many stamens have short filaments and pale brown, short style anthers. The ovary is bright green with an inconspicuous disc.

The fruit typically has a diameter of between 5 and 10 cm (2 and 4 in). It is globose or slightly pear-shaped with a thick, hard rind and does not split upon ripening. The woody shell is smooth and green, gray until it is fully ripe when it turns yellow. Inside are 8 to 15 or 20 sections filled with aromatic orange pulp, each section with 6 (8) to 10 (15) flattened-oblong seeds each about 1 cm long, bearing woolly hairs and each enclosed in a sac of adhesive, transparent mucilage that solidifies on drying. The exact number of seeds varies in different publications. [2, 3]

The fruit matures in December after maturing on the tree for around 11 months. The largest varieties can grow to be the size of a giant grapefruit or pomelo. The shell must be broken with a hammer or machete because it is so tough. The golden, fibrous pulp has a strong scent. It has been said to have a marmalade-like flavour and a rose-like aroma. According to Boning (2006), the flavour is "While some types are acidic and slightly astringent, they are generally sweet, aromatic, and pleasing. It resembles a marmalade that contains both tamarind and citrus in varying amounts." Slimy mucilage encases a number of hairy seeds. [4]

ECOLOGY AND DISTRIBUTION

The believed origin of bael is India. The species reached the nearby countries in prehistorical times and recently to the other faraway lands through human movements. The bael trees thrive well in dry, mixed deciduous and dry dipterocarp forests and soils of India, Sri Lanka, Thailand, Pakistan, Bangladesh, Myanmar, Vietnam, the Philippines, Cambodia, Malaysia, Java, Egypt, Surinam, Trinidad, and Florida (S Figure). Bael occurs in India since 800 B.C. as a crop according to the historical reports. Bael is a subtropical species, although it can grow well in tropical environments. Bael can thrive well in high altitude as high as 1,200 m and withstand without any significant growth retardation at 50°C and −7°C. In the prolonged droughts, fruiting may cease, but the plant can survive with shallow soil moisture. Bael trees generally require well-drained soil (pH: 5–8), but many studies and grower-reports suggest that it can grow equally well in alkaline, stony, and shallow soils. Bael grows well and produces bountiful harvests of fruits in the “oolitic-limestone” soils of southern Florida. In India and Sri Lanka, bael is famous as a fruit species, which can grow in very tough soils where other trees and other crops cannot grow [5].

LEAF MORPHOLOGY

The leaves are alternate, single, or compound with one or occasionally two pairs of shortly stalked opposite leaflets the leaf petioles are glabrous and long, and with no wings absent. The leaves are trifoliate and aromatic, mostly when chopped. The leaves are deciduous, alternate, and borne as single or compound.[6] In compound leaves, the leaflets appear in 2–5 oval-ovate or ovate shaped, pointed, and frivolously toothed leaflets (i.e., shallowly serratocrenate). A leaflet is 4–10 cm long and 2–5 cm wide. The leaflets are thin, and their midribs are prominent from the beneath. [7]The terminal leaflet has a longer petiole. The new foliage emerging after a dormant or a reproductive phase is glossy in appearance and pink or burgundy in color [9].

Fig no. 1 Aegle Marmelos leaves

CHEMISTRY

In addition to flavonoids like rutin and marmesin, furocoumarins like xanthotoxin and the methyl ester of alloimperatorin, as well as a number of essential oils, the bael tree also contains the alkaloids á-fargarine (also known as allocryptopine), O-isopentenylhalfordinol, and O-methylhafordinol. A component called aegeline, also known as N-[2-hydroxy-2(4-methoxyphenyl) ethyl]-3-phenyl-2-propenamide, can be isolated from bael leaves.Aeglemarmelosine, a viscous orange oil with the chemical formula C16H15NO2 27D+7.89° (c 0.20, CHCl3), has been discovered.Thebael tree contains furocoumarins, including xanthotoxol [8]and the methyl ester of alloimperatorin, as well as flavonoids, rutin and marmesin; a number of essential oils; and, among its alkaloids, á-fargarine(=allocryptopine), *O*isopentenylhalfordinol, *O*-methylhafordinol.[13] Aegeline (N-[2-hydroxy-2(4-methoxyphenyl)ethyl]-3-phenyl-2-propenamide) is a constituent that can be extracted from bael leaves.[14][15]. Aeglemarmelosine, molecular formula $C_{16}H_{15}NO_2$ [α] ^{27D}+7.89° (*c* 0.20, $CHCl_3$), has been isolated as an orange viscous oil. [6]

MATERIAL AND METHODS:

Chemicals

Pyridine, conc. /dil. Sulphuric acid (H2SO4), CuSO4, HgCl2, MHA, lead acetate, ferric chloride, sodium nitroprusside, Mayer's reagent, Benedict's solution, Ninhydrin solution, NaOH, HNO_3etc.[11]

Glass wares

Beaker, Funnel, Glass, Tubes, Rods, Measuring cylinder, Pipettes, Round bottom flask, Watch glass, Condenser, etc.

Instruments

Grinder, Digital water bath, Electronic heater, Electronic balance, Soxhlet apparatus, Autoclave, Hot air, etc.

EXTRACTION METHOD

- **Plant Materials**

  The healthy and fresh leaves of the Aegle marmelos were collected from Herbal garden in Etawah city, U.P state. The collected leaves were washed in tap water for 2-3 times. The excess water was removed. The leaves were shade-dried at room temperature (30-35ºC). 500g of leaves of Aegle marmelos were coarsely powdered using a grinder. The powder was dried in an oven at 40°C for 24 h.

- **Extraction Procedure**

  The dried and powdered plant materials (500 g) were extracted successively with 200 ml of each solvent separately by using soxhlet extractor for 5h. The solvents used for the study was ethanol and water. The extracts were filtered and then concentrated to dryness using a steam bath at 37ºC. Aqueous extract was prepared at the Shri RLT Institute of Science and Technology, Etawah. Once the process was finished, the hydro-alcohol was evaporated using a rotary evaporator leaving a small yield of extracted plant material in the glass bottom flask [12, 13]. The hydro-alcoholic extract yielded dark greenish residues. The extracts were then kept in sterile bottles and put in refrigerator at 2-4o c until further use. Then the extract was diluted with measure the amount of distilled water prior to use to get the required concentration. [14] Yield of the extract obtained was calculated as

- **Yield% = (weight of extract covered / weight of dried powder) 100**

Each extract were transferred to glass vials and kept at 4º C before use. The extracts were dissolved in 25% aqueous dimethyl sulfoxide (DMSO) to produce a stock solution of 100 mg/ml.

- **Aqueous Extraction**

The method was adopted for extraction with little modification; 500g of the powdered plant were soaked separately in 200 ml of distilled water at ambient temperature for 24 hour at 130 rpm (revolution per minute). The extract was then filtered using Whatman filter paper No 1 .Each extracts were transferred to glass vials and kept at 4 ºC before use.

Fig no. 2 Extraction of leaves through Soxhlet Extractor in SRLT College

PHYTOCHEMICAL SCREENING

Identification tests were performed as follows:

1. **Alkaloid test**: A little chunk of the extract was mixedwith a limited amount of dil. HCl and is filtered with various alkaloid reagents like Mayer's reagent to test for the presence of alkaloids which showed the appearance of yellowish or orange insoluble pigments.
2. **Carbohydrate test:** A little portion of the extract was dissolved in 5 ml of distilled water followed by filtration. The filtrate then obtained was treated with Benedict's solution which gave red or orange precipitate to test for the presence of Carbohydrates.
3. **Saponins test:** About 1 ml solution of leaves extractwas diluted with distilled water to near 20 ml and was shaken in a graduated cylinder for almost 15 minutes. The process is known as the foam test.
4. **Phenol test:** A few drops of 10 % solution of lead acetate were added to the test solution. This resulted in the formation of a white precipitate which confirmed the presence of phenolic compounds.
5. **Tannins test:** Same as in the phenol test, whiteprecipitate confirmed the presence of tannins.
6. **Proteins and amino acids**: A few portions of the extract were treated with drops of conc. HNO3. This process of testing the presence of proteins and amino acids is known as the xanthoproteic test. Formation of yellow-colored precipitate determined the presence of protein.
7. **Terpenoids test**: A little portion of extracts were treated with chloroform and was filtered. The filtrates were then treated with few a drops of conc. H2SO4, shaken well and allowed to stand. The lower layer appeared to be somehow red which indicated the presence of steroids. After adding conc. H2SO4 to the side carefully (without shaking), the color changed to reddish brown which indicated the presence of terpenoids[15]

Table no. 1 List of Phytochemical screening test

Sr.no.	Test	Present /Absent
1	Carbohydrate	+
2	Proteins	-
3	Tannin	+
4	Alkaloids	+
5	Phenol test	+
6	Terpenoids	+
7	Saponins	+

+ Present; – Absent

RESULTS AND CONCLUSION

Our observations revealed tha ethanolic extracts of leafs of Aegle marmelos contain alkaloids, carbohydrates, proteins, glycosides and phenolics, saponin, terpenoids qualitatively, whereas amino acids are not found present in extracts after several trials. It is quite evident from this review that Aegle marmelos contains a number of phytoconstituents which reveals its uses for various therapeutic purposes. Looking upon wide prospects and potential of bael for various purposes, this will help in financial upliftment of the poor and landless farmers besides providing base for the Research and Development. This study contributes to the present knowledge of the presence of different active phytochemical test of Aegle marmelos leaves possessing potential chemical ingredients. It is confirmed that the plant extracts possess various active constituents. So it is concluded that the A. marmelos leaves can be considered as an ideal for holistic medical application.

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