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A Review on Extraction, Isolation and Separation Technique Studies of Aegle Marmelos

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Abstract: *Plants and their products are a major source for food and medicine that are Highly beneficial for various animals and humans. This article focuses on complete profile of Aegle marmelos L. which belongs to the family Rutaceae, is commonly known as bael, widely Available in several places in India. Ethnobotany: Traditional use of A. marmelos for various Diseases includes abdominal disorders, ulcer, cholera, diarrhea, nerve disorders, gonorrhea, Heart disorders, dog bite, jaundice, snake bite and many more. A number of biologically active Compounds isolated from various parts of A. marmelos which belongs to various chemical Groups. Phytochemistry: The isolated components belong to Alkaloids, Terpenoids, Vitamins, Coumarins, Tannins, Carbohydrates, Flavonoids, Fatty Acids, Essential Oils and some other Miscellaneous compounds. Pharmacological Activities: The plant also possess various Pharmacological activities such as Antioxidant, Antibacterial, Antifungal, Antidiarrheal, Antidiabetic, Antiproliferative, Cytoprotective, Hepatoprotective, Antifertility, Analgesic, Antiarthritis, Contractile, Antihyperlipidemic, Cardioprotective, Radioprotective, Anticancer, Antiviral, Antiulcer, Immunomodulatory and Wound Healing properties. Conclusion: Hence this review can Be a good reference for researchers who are willing to undertake further investigation about A.Marmelos.*

Keywords: Aegle marmelos, Phytochemistry, Pharmacological properties, Therapeutic potential, Toxicological studies

I. INTRODUCTION

The journey of life for birds, animals and humans had started with utilization of plants or plant parts as food. Various plant parts like root, steam, leaf, flower and fruits are used by animal kingdom for survival of their lives. Humans are considered as most developed among all living species on earth. They are adopting plants not only as an origin of food but also to delight various ailments of mankind since ancient age. Several plants or plant parts are used to heal a number of physical and mental disturbances and helps us to withstand successfully. Ancient literature such as Rigveda,Yajurveda, Atharvaveda, Charak Samhita and Sushrut Samhita also describes the use of plants for the treatment of various health problems.1 By considering the importance of plants and their role as medicine, several researches are going on to find the active chemical constituents. Traditional uses of plants or plant parts are showing the direction to the use of plants for specific kind of disease or diseases. In last five decades, these plants have been extensively studied by advanced scientific techniques and reported for various medicinal properties viz, anticancer activity, antibacterial activity, antifungal activity, antidiabetic activity, antioxidant activity, hepatoprotective activity, haemolytic activity, larvicidal activity and anti-inflammatory activity etc.2Aegle marmelos L. (Family: Rutaceae) is a widely available plant in various places in India

Extraction Techniques

Extraction is a critical initial step in isolating bioactive compounds from Aegle marmelosSeveral techniques have been utilized, each with its advantages and limitations, depending on the target compounds and the desired yield.

Maceration: Maceration is one of the oldest and simplest extraction methods, involving soaking plant materials in solvents at ambient temperature. The process is straightforward and requires minimal equipment, making it accessible. However, maceration has drawbacks, such as long extraction times and the potential for lower yields. For Moringa oleifera, maceration is effective in extracting phenolic compounds, though it may require high solvent volumes and longer durations to achieve satisfactory yields.

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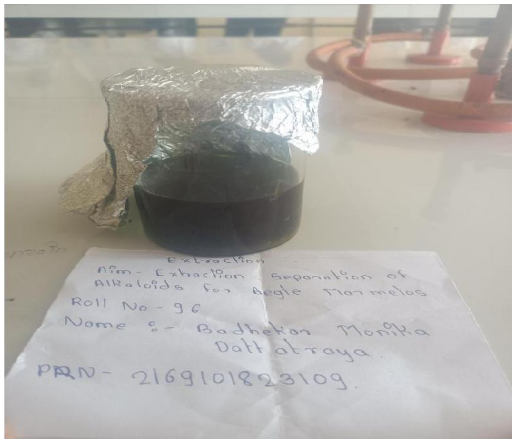
Procedure of Maceration

- The plant material is finely ground or crushed to increase the surface area for better solvent penetration.
- The material is then submerged in a solvent such as water, ethanol, methanol, or a solvent mixture.
- The solvent is kept in contact with the material for an extended period, typically ranging from a few hours to several days, depending on the type of material, solvent, and target compounds.
- Stirring or occasional shaking may be applied to enhance the extraction process.
- After maceration, the solvent containing the extracted compounds (the filtrate) is separated from the solid plant material by filtration or decantation.
- The solvent is often evaporated to obtain a concentrated extract, which can then be used for further studies or applications.



Maceration Process

Soxhlet Extraction: This method involves a continuous solvent reflux, where the solvent repeatedly passes through the plant material, enhancing extraction efficiency. Soxhlet extraction is widely used due to its high yield potential for heat-stable compounds. However, it requires long extraction times and large amounts of organic solvents, which may limit its environmental sustainability and cost-effectiveness. Soxhlet extraction has been effectively used to isolate various bioactive compounds from Aegle marmelos ,including essential oils and other phenolic constituents.

Process:

- The round-bottom flask containing the solvent is heated, causing the solvent to evaporate and travel upwards.
- The vapor reaches the condenser, where it cools and condenses, then drips into the extractor chamber containing the plant material.
- The solvent gradually fills the chamber, allowing the target compounds to dissolve into the solvent.
- Once the chamber reaches a certain level, it siphons back down to the flask, carrying the dissolved compounds with it.
- cycle repeats continuously, with fresh solvent contacting the plant material until the extraction process is complete.

Ultrasound-Assisted Extraction (UAE)

UAE utilizes ultrasonic waves to disrupt cell walls, enhancing the release of bioactive compounds. The method is particularly advantageous due to its speed, low solvent requirement, and ability to extract heat-sensitive compounds, as it operates at lower temperatures. UAE has shown high efficacy in extracting antioxidants, phenolics, and essential oils from Aegle marmelos, making it a suitable choice for preserving delicate compounds while reducing extraction time and energy costs.

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Procedure of Ultrasound-Assisted Extraction

- **Preparation**: The plant material is usually ground or cut into smaller pieces to increase surface area.
- **Sonication Setup:** The sample is placed in an extraction vessel with a suitable solvent, and an ultrasonic probe or bath generates ultrasonic waves.
- **Extraction Process:** Ultrasonic waves are applied for a specified duration, typically ranging from a few minutes to an hour, depending on the plant material, solvent, and target compounds.
- **Filtration and Concentration:** After extraction, the solvent containing the dissolved compounds is filtered to separate the solid residue. The solvent may then be evaporated to yield a concentrated extract.
- **Microwave-Assisted Extraction (MAE):** MAE employs microwave energy to heat solvents and plant materials, improving the efficiency of the extraction process. The method is known for its rapid processing times, reduced solvent usage, and high yields of bioactive compounds. MAE has been successfully applied in extracting Aegle marmelos bioactive compounds, such as glucosinolates and saponins, due to its ability to disrupt plant cell matrices and release intracellular compounds effectively. One limitation is the potential degradation of heat-sensitive components if the temperature is not carefully controlled.

Procedure of Microwave-Assisted Extraction

- Preparation: The plant material is often dried and ground to increase surface area.
- Microwave Setup: A microwave reactor or microwave-assisted extraction system is used, containing the plant material and solvent in an extraction vessel.
- Extraction Process: The vessel is subjected to microwave irradiation, rapidly heating the solvent and plant matrix for a specified time (usually a few minutes).
- Filtration and Concentration: After extraction, the solvent is filtered to separate the plant residue. The solvent may then be evaporated or further processed to concentrate the extract.

Supercritical Fluid Extraction (SFE): SFE uses supercritical fluids, typically carbon dioxide (CO_2), to extract non-polar compounds, offering selectivity, rapid extraction times, and minimal solvent residue. SFE is particularly effective for extracting lipophilic compounds such as essential oils and lipids Aegle marmelos, and CO_2's non-toxic and non-flammable nature makes it an environmentally friendly choice. However, SFE requires specialized equipment and may have limited effectiveness for polar compounds unless co-solvents are used.

Procedure of Supercritical Fluid Extraction

- Preparation: The plant material is dried and ground to increase the extraction surface area.
- Supercritical Setup: The material is placed in an extraction chamber, and CO_2 is pumped under high pressure, becoming supercritical in state.
- Extraction Process: Supercritical CO_2 passes through the plant matrix, dissolving target compounds. The CO_2 and extracted compounds then flow into a separation chamber, where $CO_2$is depressurized back to its gaseous state
- Collection and Recovery: As CO_2 becomes a gas again, it releases the extracted compounds, which can then be collected. The CO_2 can be recycled for additional extractions, making the process environmentally efficient.

Hydrodistillation: Commonly used for extracting essential oils, hydrodistillation involves passing steam through the plant material to vaporize volatile compounds, which are then condensed and collected. Although efficient for isolating essential oils, hydrodistillation is limited in extracting non-volatile bioactive compounds. For Aegle marmelos , hydrodistillation has been used to extract essential oils from seeds and leaves, though it may not be ideal for other bioactives.

- Preparation: Plant material is often dried and sometimes chopped or ground to improve extraction efficiency.

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- Loading: The plant material is loaded into a distillation chamber with either water or directly exposed to steam, depending on the specific method.
- Heating and Extraction: The chamber is heated, releasing essential oils and volatile compounds as steam.
- Condensation and Separation: The vaporized essential oils and water pass through a condenser, turning into a liquid that collects in a separator. Since essential oils are typically immiscible with water, they form a separate layer that can be easily isolated.

Isolation and Separation Techniques

Once the compounds are extracted, further isolation and separation are required to purify specific bioactive compounds. Techniques used for Moringa oleifera include chromatography, electrophoresis, and other advanced methods, each with varying degrees of specificity and resolution.

Chromatography: Chromatography techniques, including thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), and gas chromatography (GC), are widely used for separating and purifying bioactive compounds. HPLC is particularly effective in isolating Moringa compounds due to its high resolution and versatility in separating different types of compounds based on polarity and molecular weight. TLC is often used for initial screening and rapid qualitative analysis, while GC is suitable for volatile compounds such as essential oils. Chromatographic methods have been widely applied in isolating geraniol, isogeranial and citronellol from Aegle marmelos extracts.

Electrophoresis: Electrophoretic techniques, such as capillary electrophoresis (CE), offer high- resolution separation based on the charge-to-size ratio of molecules. Capillary electrophoresis is suitable for analyzing Aegle marmelos smaller bioactive compounds and offers rapid analysis with minimal sample and solvent requirements. However, it may be less effective for larger, non-ionic compounds, limiting its application for certain Aegle marmelos constituents.

Liquid-Liquid Extraction (LLE)

LLE separates compounds based on their solubility in different solvents. It is a simple yet effective technique for fractionatin Aegle marmelos extracts into polar and non-polar components. LLE has been used as a preliminary separation step before further purification, particularly for isolating hydrophilic and lipophilic compounds in Cymbopogon extracts.4. Preparative HPLC: This technique is an advanced form of HPLC used to isolate large quantities of purified compounds for further study or application. Preparative HPLC has proven effective in isolating Cymbopogon Citratus bioactive constituents with high purity, though it requires sophisticated equipment and expertise.

II. CONCLUSION

Several parts of A. marmelos have been reported as various traditional healers for treating various ailments of mankind. These contains Antioxidant, Antibacterial, Antifungal, Antidiarrheal, Antidiabetic, Antiproliferative, Cytoprotective, Hepatoprotective, Antifertility, Analgesic, Antiarthritis, Contractile, Antihyperlipidemic, Cardioprotective, Radioprotective, Anticancer, Antiviral, Anti-ulcer, Immunomodulatory and Wound Healing properties. A number of biologically active compounds isolated from various parts of A. marmelos which belongs to various chemical groups. The isolated components belong to Alkaloids, Terpenoids, Vitamins, Coumarins, Tannins, Carbohydrates, Flavonoids, Fatty Acids, Essential Oils and some other miscellaneous compounds. This review mainly focused on several phytochemical and pharmacological studies which have explained phytoconstituents and therapeutic potential of

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