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"A Review on Extraction,Isolation and Separation Technique Studies of Lantana Camara"

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Abstract

Cymbopogon lantana (commonly known as Lantana camara Linn.) is a highly invasive weed distributed across tropical and subtropical regions worldwide. Consumption of lantana foliage by grazing animals leads to cholestasis and hepatotoxicity. Both ruminant and non-ruminant species, including guinea pigs, rabbits, and female rats, exhibit susceptibility to its toxic effects. The principal hepatotoxins responsible for these effects are pentacyclic triterpenoids known as lantadenes, whose molecular structures have been well characterized. The green, unripe fruits of this plant are also reported to be toxic to humans. Moreover, Lantana species possess allelopathic properties that adversely affect the growth of neighboring vegetation. The major allelochemicals identified include phenolic compounds such as umbelliferone, methylcoumarin, and salicylic acid, with lantadene A and B showing strong phytotoxic potential. Management of lantana-induced toxicosis in animals typically involves the administration of activated charcoal along with supportive therapy. Interestingly, studies have highlighted the bilirubin-clearing effect of the Chinese herbal formulation Yin Zhi Huang—a decoction prepared from Artemisia capillaris and other herbs—or its active compound, 6,7-dimethylesculetin, suggesting potential for therapeutic application in lantana-induced hepatic injury. Ongoing research continues to explore novel drug candidates and bioactive compounds derived from various parts of the lantana plant, emphasizing its dual role as both a toxic and pharmacologically valuable species.

Keywords:

Camara lucida, extraction methods, antioxidants, essential oils, antimicrobial properties.

Introduction

The essential oil extracted from *Cymbopogon citratus* (lemongrass) is widely used in flavoring, fragrance formulation, aromatherapy, medicinal teas, and as a culinary herb (1). It also has therapeutic applications, particularly in the management of various skin disorders (2). Traditionally, *C. citratus* has been recognized as an important source of ethnomedicines (3) and is extensively used in many parts of the world for the treatment of digestive disturbances, fever, menstrual irregularities, rheumatism, and joint pain (4).Belonging to the family

Gramineae (Poaceae), *C. citratus* is one of approximately 500 genera and 8,000 herbaceous species classified under this group. Lemongrass is a tufted perennial plant that grows up to 1 meter in height, bearing numerous stiff leafy stems that emerge from short rhizomatous roots (5). In traditional medicine, the use of whole herbs and crude extracts remains a common practice, with many practitioners asserting the effectiveness of such preparations against a wide variety of ailments, often without scientific validation (6). The strong lemon-like aroma of lemongrass oil is attributed to its high **citral** content, which gives the plant its characteristic fragrance and forms the basis for its application in soaps, detergents, perfumery, and related industries. As a rich source of citral, it is also employed in the **perfumery and food** sectors and serves as a precursor in the synthesis of **ionones**, intermediates used in the production of **vitamin A** (7). Because of its diverse industrial applications in **cosmetics, food, pharmaceuticals, agriculture, and flavor manufacturing**, *Cymbopogon* species are extensively cultivated in tropical and subtropical regions. The high aldehyde content of *C. citratus* contributes to its distinctive lemony odor. It contains two geometric isomers—**neral (citral B)** and **geranial (citral A)**—which naturally coexist and are responsible for the major aromatic properties of the oil (8). In addition to citral, the essential oils of *Cymbopogon* species contain various **bioactive compounds** that impart significant medicinal and pharmacological properties (9). According to the **World Health Organization (WHO)**, herbal medicine continues to play a major role in global healthcare, with more than two-thirds of the population in developing countries depending on it for primary health needs (10). Thus, lemongrass, an aromatic and perennial grass from the Gramineae family, remains a plant of both traditional and scientific importance due to its wide therapeutic potential.

Extraction Techniques

Extraction is a crucial preliminary step in the isolation and characterization of bioactive compounds from *Cymbopogon citratus*. Various extraction methods have been developed, each with distinct advantages and limitations depending on the nature of the target compounds and the desired extraction efficiency.

1. Maceration

Maceration is among the oldest and simplest techniques for extracting phytochemicals. The process involves soaking the powdered plant material in a suitable solvent at room temperature for a specific period, allowing the solvent to penetrate the plant matrix and dissolve the active constituents. This method is favored for its simplicity, cost-effectiveness, and minimal equipment requirements. However, it is often associated with prolonged extraction times, higher solvent consumption, and comparatively lower yields. In studies on *Moringa oleifera*, maceration has proven effective for the extraction of phenolic compounds, although optimization of solvent type, temperature, and duration is necessary to achieve maximum recovery. Similarly, when applied to *Cymbopogon citratus*, the method can efficiently extract essential oils and polar compounds, but may be less suitable for large-scale or time-sensitive applications.

Procedure of Maceration

The maceration process involves several sequential steps designed to maximize the extraction of bioactive compounds from plant materials such as *Cymbopogon citratus*. The general procedure is as follows:

1. **Preparation of Plant Material:**
The plant material is finely ground or crushed to increase the surface area, thereby enhancing solvent penetration and mass transfer.
2. **Selection and Addition of Solvent:**
The powdered material is immersed in a suitable solvent such as water, ethanol, methanol, or an appropriate solvent mixture, depending on the polarity of the desired compounds.
3. **Soaking Period:**
The solvent and plant material are kept in contact for a prolonged period, typically ranging from several hours to a few days. The duration depends on factors such as the solvent type, temperature, and the nature of the target constituents.

4. **Agitation:**
Occasional stirring or shaking is applied to improve solvent diffusion and ensure uniform extraction throughout the mixture.
5. **Filtration or Decantation:**
After the maceration period, the liquid extract (filtrate) is separated from the solid plant residue using filtration or decantation techniques.
6. **Concentration of Extract:**
The filtrate is subjected to solvent evaporation, usually under reduced pressure, to obtain a concentrated extract that contains the bioactive components. This concentrated extract can then be stored or used for further analysis and formulation studies.

Fig:- Maceration

2. Soxhlet Extraction

Soxhlet extraction is a classical and highly efficient method used for the extraction of bioactive compounds from plant materials. The technique operates on a continuous solvent reflux principle, in which the solvent repeatedly passes through the plant matrix, ensuring thorough extraction of the desired constituents. This method is particularly suitable for heat-stable compounds and is widely applied in phytochemical and pharmacognostic studies.

Soxhlet extraction offers high extraction efficiency and reproducibility, making it ideal for isolating essential oils, alkaloids, flavonoids, and phenolic compounds from *Cymbopogon citratus*. However, the process has certain drawbacks, such as extended extraction times, high energy consumption, and significant use of organic solvents, which can raise environmental and economic concerns.

Procedure of Soxhlet Extraction

1. **Heating the Solvent:**
A round-bottom flask containing the chosen solvent (e.g., ethanol, methanol, or hexane) is heated to produce vapors.
2. **Condensation of Vapors:**
The vapors rise through the distillation arm and enter the condenser, where they cool and condense into liquid form.

3. **Percolation Through Plant Material:**
The condensed solvent drips into the extraction chamber containing the powdered plant material. As the solvent level rises, it dissolves the target compounds present in the sample.
4. **Automatic Siphoning:**
Once the chamber fills to a certain point, the siphon mechanism automatically drains the extract-laden solvent back into the boiling flask. This cycle repeats continuously, allowing fresh solvent to interact with the plant residue multiple times.
5. **Collection of Extract:**
After several cycles, the extraction is complete. The solvent in the boiling flask now contains the concentrated extract, which is then evaporated to obtain the purified bioactive constituents.

3. Ultrasound-Assisted Extraction (UAE)

Ultrasound-Assisted Extraction (UAE) is a modern and efficient technique that employs ultrasonic waves to enhance the extraction of bioactive compounds from plant materials. The ultrasonic waves generate cavitation bubbles within the solvent, which collapse near the plant cell walls, causing mechanical disruption and facilitating the rapid release of intracellular constituents. This method offers several advantages, including shorter extraction times, reduced solvent consumption, and lower operational temperatures, making it highly suitable for the extraction of thermolabile or heat-sensitive compounds. UAE has demonstrated excellent efficiency in extracting antioxidants, phenolic compounds, and essential oils from *Cymbopogon citratus*. Due to its mild processing conditions and enhanced mass transfer, UAE is increasingly preferred in both research and industrial applications. It provides a sustainable alternative to conventional methods by preserving the integrity of delicate compounds while minimizing energy costs and solvent waste.

Procedure of Ultrasound-Assisted Extraction (UAE)

The Ultrasound-Assisted Extraction process involves the following sequential steps:

1. **Preparation of Plant Material:**
The plant material is finely ground or cut into smaller pieces to increase the surface area, which enhances solvent penetration and improves extraction efficiency.
2. **Sonication Setup:**
The prepared sample is placed in an extraction vessel containing a suitable solvent such as ethanol, methanol, or water. An ultrasonic probe or ultrasonic bath is used to generate high-frequency sound waves within the solvent system.
3. **Extraction Process:**
The ultrasonic waves create cavitation bubbles that collapse near the plant cell walls, disrupting cellular structures and promoting the release of bioactive compounds. The sonication process is typically carried out for a specific duration—ranging from a few minutes to about an hour—depending on the nature of the plant material, solvent type, and target compounds.
4. **Filtration and Concentration:**
After completion of the sonication, the mixture is filtered to separate the solvent extract from the solid plant residue. The filtrate is then concentrated, often by evaporating the solvent under reduced pressure, to obtain a purified extract rich in bioactive constituents.

4. Microwave-Assisted Extraction (MAE)

Microwave-Assisted Extraction (MAE) is an advanced extraction technique that utilizes microwave energy to heat both the solvent and the plant material simultaneously, thereby enhancing the efficiency of mass transfer and accelerating the extraction of bioactive compounds. The microwaves cause rapid heating of polar molecules within the plant matrix, leading to cell wall rupture and the subsequent release of intracellular constituents into the solvent. MAE is widely recognized for its **short extraction times**, **reduced solvent consumption**, and **high extraction yields**. The technique has been successfully applied to extract a variety of phytoconstituents from *Cymbopogon*

citratus, including glucosinolates, saponins, phenolics, and essential oils. Its efficiency arises from the uniform heating and enhanced solvent penetration achieved through microwave irradiation. However, a key limitation of MAE is the **potential degradation of thermolabile (heat-sensitive) compounds** if the temperature and exposure time are not carefully optimized. Therefore, precise control of process parameters—such as microwave power, extraction duration, and solvent type—is essential to maintain the integrity of the extracted bioactive compounds.

Procedure of Microwave-Assisted Extraction (MAE)

The general procedure for Microwave-Assisted Extraction involves the following steps:

1. **Preparation of Plant Material:**
The plant material is first cleaned, dried, and finely ground to increase its surface area, which enhances solvent contact and extraction efficiency.
2. **Microwave Setup:**
The prepared sample is placed in an extraction vessel containing a suitable solvent such as ethanol, methanol, or water. A microwave reactor or microwave-assisted extraction system is then used to generate controlled microwave energy.
3. **Extraction Process:**
The sample-solvent mixture is subjected to microwave irradiation for a predetermined duration—typically ranging from a few seconds to several minutes—depending on the solvent polarity, sample type, and target compounds. The rapid heating facilitates cell wall rupture and accelerates the release of intracellular constituents into the solvent.
4. **Filtration and Concentration:**
After extraction, the mixture is allowed to cool and then filtered to separate the plant residue from the solvent extract. The filtrate is subsequently concentrated, often through solvent evaporation under reduced pressure, to obtain a purified extract rich in bioactive compounds.

5. Supercritical Fluid Extraction (SFE)

Supercritical Fluid Extraction (SFE) is an advanced extraction technique that utilizes supercritical fluids—most commonly carbon dioxide (CO_2)—to extract bioactive compounds from plant materials. When CO_2 is subjected to temperatures and pressures above its critical point (31.1°C and 73.8 bar), it exhibits both gas-like diffusivity and liquid-like solvating power. This unique property allows it to penetrate plant matrices efficiently and dissolve non-polar compounds with high selectivity.

SFE offers several advantages, including **short extraction times**, **high selectivity**, and **minimal solvent residue** in the final extract. It is particularly effective for isolating **lipophilic compounds**, such as essential oils, waxes, and lipids, from *Cymbopogon citratus*. Moreover, CO_2 is **non-toxic, non-flammable, and environmentally benign**, making the process a sustainable alternative to conventional solvent extraction methods. However, the technique requires **specialized high-pressure equipment** and may show reduced efficiency for **polar compounds** unless a suitable co-solvent (modifier), such as ethanol or methanol, is added to enhance solubility and extraction performance.

Procedure of Supercritical Fluid Extraction (SFE)

1. **Preparation of Plant Material:**
The plant material is carefully cleaned, dried, and finely ground to increase the surface area, thereby improving solvent interaction during extraction.
2. **Supercritical Setup:**
The processed material is loaded into an extraction chamber. Carbon dioxide is then compressed and heated to reach its supercritical state, in which it exhibits both gas-like and liquid-like properties.

3. **Extraction Process:**
The supercritical CO_2 is passed through the plant matrix, where it solubilizes the target bioactive compounds. The CO_2 containing the dissolved compounds is then transferred to a separation chamber, where pressure reduction causes CO_2 to revert to its gaseous state.
4. **Collection and Recovery:**
As the CO_2 returns to the gaseous phase, it releases the extracted compounds, which are collected from the separator. The gaseous CO_2 can be condensed and **recycled** for subsequent extractions, minimizing environmental impact and operational costs.

Hydrodistillation

Hydrodistillation is one of the most traditional and widely used methods for extracting essential oils from aromatic plants. The technique involves the use of water or steam to volatilize the plant's aromatic compounds, which are subsequently condensed and collected as liquid distillates. During this process, volatile compounds are vaporized along with water vapor and later separated based on differences in immiscibility and density. Hydrodistillation is particularly efficient for isolating **essential oils and volatile constituents**, though it is generally **ineffective for non-volatile or thermolabile bioactive compounds**. In the case of *Cymbopogon citratus*, this technique has been successfully employed to extract essential oils from both the **leaves and seeds**, yielding aromatic compounds rich in citral and other terpenoids. However, for extracting phenolics or other non-volatile compounds, alternative solvent-based or modern extraction techniques are preferred.

Procedure of Hydrodistillation

1. **Preparation of Plant Material:**
The plant material is typically cleaned, dried, and sometimes chopped or ground to increase the surface area and improve extraction efficiency.
2. **Loading into the Distillation Unit:**
The processed material is loaded into the distillation chamber, which contains water (in water distillation) or is exposed to steam (in steam distillation), depending on the selected hydrodistillation method.
3. **Heating and Extraction:**
The chamber is heated to generate steam, which passes through the plant matrix. The heat causes the essential oils and volatile compounds to evaporate along with the steam.
4. **Condensation and Separation:**
The steam containing the vaporized essential oils is passed through a condenser, where it cools and converts into a liquid mixture of water and oil. This distillate is collected in a separator, where the essential oils naturally separate as a distinct layer due to their immiscibility with water and lower density. The isolated essential oils are then collected and stored for further analysis or formulation.

Isolation and Separation Techniques

After extraction, further purification is required to isolate individual bioactive compounds. Techniques such as **chromatography**, **electrophoresis**, and **liquid–liquid extraction** are commonly employed for *Cymbopogon* and *Moringa oleifera* due to their accuracy and efficiency.

1. **Chromatography:**
Methods like **TLC**, **HPLC**, and **GC** are widely applied for separation and purification. **HPLC** provides high resolution and is effective for diverse compounds differing in polarity and molecular weight. **TLC** serves as a quick screening and qualitative tool, while **GC** is ideal for volatile constituents such as essential

oils. These methods are successfully used to isolate **citral, geraniol, isogeranial,** and **citronellol** from *Cymbopogon* extracts.

2. **Electrophoresis:**

Capillary electrophoresis (CE) enables high-resolution separation based on molecular charge-to-size ratio. It is efficient for small bioactive molecules of *Cymbopogon*, offering rapid analysis with minimal solvent use, though it is less suitable for large or non-ionic compounds.

3. **Liquid–Liquid Extraction (LLE):**

LLE separates compounds according to their solubility in immiscible solvents and is useful for fractionating extracts into **polar and non-polar** portions. It often serves as a preliminary step before chromatographic purification of *Cymbopogon* constituents.

4. **Preparative HPLC:**

An advanced version of HPLC, **Preparative HPLC** enables large-scale isolation of high-purity compounds from *Cymbopogon citratus*. Although precise and effective, it demands specialized instrumentation and technical expertise.

Calculation :-

$$Rf = \frac{\text{Distance travelled by the sample}}{\text{Distance travelled by the solvent}}$$

Where, Distance travelled by the sample =2.54

Distance travelled by the solvent =6.5

$$Rf = \frac{2.54}{6.5}$$

Rf = 0.39

Standard RF value: 0.3-0.6

Conclusion

The essential oil of *Cymbopogon citratus* has gained significant attention for its potential use in food preservation systems. It contains key phytoconstituents such as **citral, citronellol, isoneral**, and various **phenolic compounds**, which exhibit strong **antimicrobial and antifungal** properties. Studies indicate that lemongrass essential oil can act as a **natural preservative**, enhancing meat product shelf life and protecting against microbial contamination. However, further research is required to determine optimal concentrations and application methods for industrial use. Beyond meat preservation, lemongrass oil also shows promise in controlling **storage fungi**, which degrade food quality and pose health risks. Overall, *C. citratus* essential oil represents a **safe, eco-friendly alternative** to synthetic additives, offering potential benefits for **food preservation**, particularly in **rural regions** lacking modern storage infrastructure.

Result-

Ethanolic extraction of 5 g plant powder by maceration yielded a clear filtrate rich in bioactive compounds. TLC analysis using silica gel G and a methanol : chloroform (8 : 2) solvent system showed an Rf value of 0.39, confirming the presence of flavonoids. Phytochemical tests (alkaline reagent, NH_4OH, Mg-turning, and Zn tests) all produced

positive color reactions, further indicating flavonoid content. These results show that ethanol efficiently extracts polar phytochemicals, and Lantana camara contains flavonoids with potential antioxidant and antimicrobial activity.

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