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A Review on Extraction, Separation and Isolation Technique of Alkaloids from Achyranthes Aspera

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Abstract: *Achyranthes aspera (Amaranthaceae) is an important medicinal herb found as a weed throughout India. Though almost all of its parts are used in traditional systems of medicines, seeds, roots and shoots are the most important parts which are used medicinally. The present article gives an account of updated information on its phytochemical and pharmacological properties. The review reveals that wide numbers of phytochemical constituents have been isolated from the plant which possesses activities like antiperiodic, diuretic, purgative, laxative, antiasthmatic, hepatoprotective, anti-allergic and various other important medicinal properties.*

The crushed plant is used in pneumonia and infusion of the root is used as mild astringent in bowel complaints. Decoction of powdered leaves with honey or sugar candy is useful in early stages of diarrhoea and dysentery. For the last few decades or so, extensive research work has been done to prove its biological activities and pharmacology of its extracts. Saponins, oleonolic acid, dihydroxy ketones, alkaloids, long chain compounds and many other chemical constituents have been isolated.

Keywords: Achyranthes aspera, Latjeera, Medicinal properties, chemical constituents, pharmacological activities.

I. INTRODUCTION

Achyranthes aspera L. (Amaranthaceae) is distributed as weed throughout India, tropical Asia and other parts of the world. Ayurvedic, Yunani practitioners and Kabirajes use different parts of the plant to treat leprosy, asthma, fistula, piles, arthritis, wound, insect and snake bite, renal and cardiac dropsy, kidney stone, diabetes, dermatological disorders, gynecological disorders, gonorrhea, malaria, pneumonia, fever, cough, pyorrhea, dysentery, rabies, hysteria, toothache etc. The plant is a popular folk remedy in traditional system of medicine throughout the tropical Asian and African countries.The plant is reported to be used as antimicrobial, larvicidal, antifertility, immunostimulant, hypoglycemic, hypolipidemic, anti-inflammatory, antioxidant, diuretic, cardiac stimulant, antihypertensive, anti-anasacra, analgesic, antipyretic, antinoiceptive, prothyrodic, antispasmodic and hepatoprotective. Phytochemical investigations revealed the presence of sterols, alkaloids, saponins, sapogenins, cardiac glycosides, ecdysterone etc from different parts of the plant. Some other species of the genus Achyranthes viz. A. fauriei, A. bidentata, A. japonica, A. ferruginea etc. have also been investigated for their active constituents and pharmacological potential. This review incorporates different aspects of A. aspera cited from the existing literature emphasizing on its phytochemistry and pharmacology.

TRADITIONAL USES:-Traditionally, the plant is used in asthma and cough. It is pungent, antiphlegmatic, antiperiodic, diuretic, purgative and laxative, useful in oedema, dropsy and piles, boils and eruptions of skin etc. Crushed plant is boiled in water and is used in pneumonia. Infusion of the root is a mild astringent in bowel complaints. The flowering spikes or seeds, ground and made into a paste with water, are used as external application for bites of poisonous snakes and reptiles, used in night blindness and cutaneous diseases. For snake bites the ground root is given with water until the patient vomits and regains consciousness.

Extraction Techniques:

Extraction in chemistry is a separation process consisting of the separation of a substance from a matrix.The distribution of a solute between two phases is an equilibrium condition described by partition theory

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1. 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.

Procedure of Maceration:

• Preparation of Plant Material: Grind the dried Achyranthes aspera plant material into a fine powder using a mill or grinder.
• Selection of Solvent: Choose a suitable solvent based on the desired bioactive compounds. Common solvents used for maceration include ethanol, methanol, and hexane.
• Maceration: Mix the powdered plant material with the solvent in a specific ratio (e.g., 1:10) in a glass container. Stir well to ensure uniform mixing.
• Incubation: Close the container with a lid and incubate it in a cool, dark place for a specified period (e.g., 3-7 days). Shake the container periodically to facilitate solvent penetration and compound extraction.
• Filtration: After the incubation period, filter the mixture using a filter paper or cloth to separate the solvent from the plant material. Discard the plant material and reserve the solvent.
• Evaporation: Evaporate the solvent using a rotary evaporator or vacuum distillation apparatus to obtain a concentrated extract.

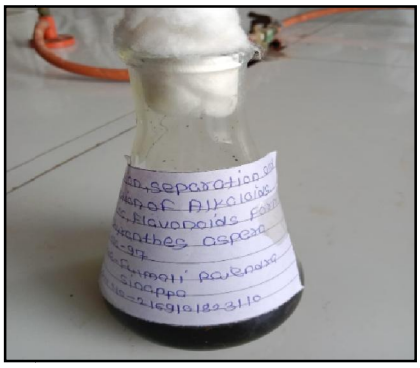

Figure: Maceration Process

2. Soxhlet Extraction: Soxhlet extraction is a laboratory technique used to extract compounds from solid materials using a solvent. This technique is often used when the desired compound has limited solubility in the solvent, and the impurity is insoluble in that solvent.

Procedure of Soxhlet Extraction:

- Preparation of Plant Material: Grind the dried Achyranthes aspera plant material into a fine powder using a mill or grinder.
- Preparation of Soxhlet Apparatus: Assemble the Soxhlet apparatus by attaching the round-bottom flask to the condenser and heating mantle.
- Add Solvent and Plant Material: Add the solvent to the round-bottom flask and place the powdered plant material in the Soxhlet thimble.
- Heating and Extraction: Heat the solvent using the heating mantle, causing it to evaporate and rise into the condenser.
- Condensation and Siphoning: The condensed solvent drips back into the Soxhlet thimble, where it extracts the bioactive compounds from the plant material. The extracted compounds are then siphoned back into the round-bottom flask.

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- Repetition of Cycles: Steps 4-5 are repeated for several cycles (e.g., 5-10 cycles) to ensure complete extraction of bioactive compounds.

3.Microwave Assisted Extraction: Microwave-assisted extraction (MAE) is a technique based on the use of the microwave energy to help the transfer of the solutes from the matrix into the solvent. As consequence, extraction yield is increased, whereas time and solvent consumption are decreased.

Procedure of Microwave Assisted Extraction:

- Preparation of Plant Material: Grind the dried Achyranthes aspera plant material into a fine powder using a mill or grinder.
- Selection of Solvent: Choose a suitable solvent based on the desired bioactive compounds.
- Preparation of Extraction Vessel: Place the powdered plant material in the extraction vessel and add the solvent.
- Microwave Irradiation: Place the extraction vessel in the microwave oven and irradiate with microwave energy at a controlled power level (e.g., 300-600 W) and time (e.g., 30-90 seconds).
- Cooling and Filtration: Remove the extraction vessel from the microwave oven and allow it to cool. Filter the extract using filter paper or cloth to separate the solvent from the plant material.

4. Pressurized Fluid Extraction: Pressurized fluid extraction (PFE) is one of several sample preparation methods that can be used to extract targeted analytes from a sample matrix into a solvent, to permit subsequent analysis. With PFE, samples are extracted with solvent in a pressurized cell reminiscent of a liquid chromatographic column.

Procedure of Pressurized Fluid Extraction:

- Preparation of Plant Material: Grind the dried Achyranthes aspera plant material into a fine powder using a mill or grinder.
- Preparation of Extraction Cell: Load the powdered plant material into the extraction cell, and add a small amount of solvent to moisten the material.
- Assembly of PFE Apparatus: Assemble the PFE apparatus by attaching the extraction cell to the heating and cooling system, pressure gauge, and collection vessel.
- Extraction: Close the extraction cell and apply pressure (e.g., 100-200 bar) using the pressure gauge. Heat the extraction solvent to a high temperature (e.g., 100-150°C) using the heating system.
- Static Extraction: Hold the pressure and temperature for a specified period (e.g., 5-10 minutes) to allow for static extraction.
- Dynamic Extraction: Release the pressure and allow the solvent to flow through the extraction cell, collecting the extract in the collection vessel.
- Repeat Extraction Cycles: Repeat steps 4-6 for multiple extraction cycles (e.g., 2- cycles) to achieve efficient extraction.

5. Supercritical Fluid Extraction: Supercritical fluid extraction (SFE) is a method of extracting a sample utilizing the unique properties of supercritical fluids. A supercritical fluid diffuses quickly as a gas and is capable of dissolving materials as a liquid. Supercritical carbon dioxide is mainly used.

Procedure of Supercritical Fluid Extraction:

- Preparation of Plant Material: Grind the dried Achyranthes aspera plant material into a fine powder using a mill or grinder.
- Preparation of Extraction Vessel: Load the powdered plant material into the extraction vessel.
- Assembly of SFE Apparatus: Assemble the SFE apparatus by attaching the extraction vessel to the CO2 cylinder, heating and cooling system, pressure gauge, and collection vessel.

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- Pressurization: Pressurize the system with CO2 to a pressure above the critical point (e.g., 100-300 bar).
- Heating: Heat the extraction vessel to a temperature above the critical temperature (e.g., 40-60°C).
- Extraction: Allow the supercritical CO2 to flow through the extraction vessel, extracting the bioactive compounds from the plant material.
- Separation: Separate the extracted compounds from the CO2 using a separator or a collection vessel.
- Depressurization: Depressurize the system to release the CO2.
- Collection of Extract: Collect the extracted bioactive compounds in a collection vessel.

6. Enzyme Assisted Extraction: Enzyme-assisted extraction method uses the specificity of enzymes to break down the dense structure of cell walls, enabling efficient extraction of active ingredients [51]. It offer advantages of energy saving, high quality, high yield, mild reaction conditions, and does not damage polysaccharide structures.

Procedure of Enzyme Assisted Extraction:

- Preparation of Plant Material: Grind the dried Achyranthes aspera plant material into a fine powder using a mill or grinder.
- Selection of Enzymes: Choose suitable enzymes based on the type of bioactive compounds to be extracted.
- Preparation of Enzyme Solution: Dissolve the enzymes in a solvent (e.g., water) to create an enzyme solution.
- Incubation: Mix the powdered plant material with the enzyme solution and incubate at a controlled temperature (e.g., 40-50°C) and pH (e.g., 5-7) for a specified period (e.g., 1-24 hours).
- Centrifugation: Centrifuge the mixture to separate the liquid extract from the solid plant material.
- Filtration: Filter the liquid extract using filter paper or cloth to remove any remaining plant material.

7. Ultrasound Assisted Extraction: Ultrasound-assisted extraction (UEA) typically works on the mechanism of cavitation, which generates the compression and expansion of the matrix, causing the permeabilization of the cell wall and enhanced extraction yield of desired compounds.

Procedure of Ultrasound Assisted Extraction:

- Preparation of Plant Material: Grind the dried Achyranthes aspera plant material into a fine powder using a mill or grinder.
- Selection of Enzymes: Choose suitable enzymes based on the type of bioactive compounds to be extracted.
- Preparation of Enzyme Solution: Dissolve the enzymes in a solvent (e.g., water) to create an enzyme solution.
- Incubation: Mix the powdered plant material with the enzyme solution and incubate at a controlled temperature (e.g., 40-50°C) and pH (e.g., 5-7) for a specified period (e.g., 1-24 hours).
- Centrifugation: Centrifuge the mixture to separate the liquid extract from the solid plant material.
- Filtration: Filter the liquid extract using filter paper or cloth to remove any remaining plant material.

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.

1. 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 alkaloids ,flavonoids and Saponins from Achyranthes aspera extracts.

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2. Electrophoretic: 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 Achyranthes aspera 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 Achyranthes aspera constituents.

II. CONCLUSION

In this study, total alkaloid content from methanol extracts of Achyranthes aspera (seeds) and Cissus quadrangularis (stem) was investigated. Alkaloid containing plants have many therapeutic properties like antimicrobial activity, antioxidant activity, free radicals scavenging activity. This study is given a scientific basis to plants already used for traditional purposes to treat various ailments and metabolic disorders.

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