

Extraction Methods of Medicinal Plants for Antimicrobial Textiles

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Medicinal plants are the product of natural drug discoveries and have gained traction due to their pharmacological activities. Pathogens are everywhere, and they thrive in ideal conditions depending on the nutrients, moisture, temperature, and pH that increase the growth of harmful pathogens on surfaces and textiles.

antimicrobial agents

textile finishings

extractions

solvents

1. Introduction

There are various extraction methods, e.g., solvent extraction, distillation method, pressing, and sublimation. Solvent extraction is the most widely used method where the natural products undergo a process where the solvent penetrates through the plant cell wall and the solute dissolves in the solvents the solute followed by collecting the extract. It has been reported that the size of the plant material, properties of the solvent solid to solvent ratio extraction temperature, and extraction time will affect the extraction efficiency ^{[1][2]}. The selectivity of the solvents, solubility, cost and safety play a crucial role in solvent extraction. Solvents with the same polarity as the polarity of the solute will result in a greater yield. High temperature affects dispersion and solubility. High temperatures may result in solvents being lost and extracts with impurities and the degradation of thermolabile compounds. The extraction efficiency increases with extraction time. Increasing time will not affect the extraction. The greater the solvent-to-solid ratio, the greater the extraction yield ^[3].

Various extraction methods are used to extract the desired bioactive compounds from the plant materials, e.g., solvent extraction, distillation method, pressing, and sublimation. Solvent extraction is the most widely used extraction method when extracting from plant material.

1.1. Cold Extraction

In this extraction process, the plant parts are dried in a controlled environment at low temperatures and milled into a powder and weighed. The powder is added to a beaker with solvents and kept at room temperature for thirty minutes. The contents are shaken every twenty-four hours for seven days. The extract is filtered using Whatman filter paper under vacuum and drying at room temperature in a watch glass dish. The weight of the powder is recorded before and after drying ^[4].

1.2. Plant Tissue Homogenization

Fresh plant parts are grounded in a blender. The solvent is added and shaken vigorously for 5–10 min or left for 24 h followed by filtration of the extract. The filtrate can be dried under reduced pressure and redissolved in the solvent to determine the concentration, or it can be centrifuged for clarification for further studies [5].

1.3. Serial Exhaustive Extraction

In this extraction method, the solvent of increasing polarity from a non-polar solvent (hexane) to a polar solvent (methanol) is used to ensure a broad polarity range of compounds being extracted and to prepare crude extracts [5].

1.4. Soxhlet Extraction

In this extraction method, solid material is placed in a thimble in the extractor. The solvent is heated until reflux. The vapour rises, and the solvent is condensed and fills up the thimble. The extraction is repeated [6][7].

1.5. Maceration

A whole or coarsely powdered plant is soaked in the solvent in a container for a period under continuous mixing until agitation until the biomass matter is dissolved [5].

1.6. Decoction

In this extraction method, the plant parts are brought to a boil in water followed by cooling, straining, and passing sufficient cold water through the drug to produce the required volume [6].

1.7. Infusion

In this extraction method, the plant parts are macerated with either cold or boiling water [6].

1.8. Digestion

In this extraction method, the plant parts are macerated under gentle heating [6].

1.9. Percolation

In this extraction method, the raw material is placed in an appropriate amount of solvent for approximately 4 h in a closed container. Additional solvent is added to the top of the raw material and macerated in a closed container for 24 h. The percolator is opened, and the extract is poured out drip-wise. Additional solvent is added until the percolate measures about three-quarters of the required volume of the finished product. The marc is pressed, and the pressed liquid is added to the percolate. Additional solvent is added to produce the required volume, and the mixed liquid is clarified by filtration or by decanting [6].

1.10. Sonication

This method uses ultrasound technology to assist in the extraction of the bioactive compounds under frequencies ranging from 20 kHz to 2000 kHz. The ultrasound increases the permeability of cell walls and produces cavitation and ruptures the plant cell wall [6].

1.11. Enzymatic Extraction

In this extraction method, enzymes are used to increase the yields during the extraction. Enzymes are used to soften the tissues of biomass and facilitate the degradation of the cells [8].

1.12. Microwave-Assisted Extraction

This extraction method uses microwave radiation and solvents to extract bioactive compounds. Microwave energy is generated through microwave radiation that heats the solvents whilst increasing the kinetics of the extraction. Moisture occurs in the plant cells when heat is applied and evaporates. The microwave effect generates pressure on the cell wall and results in cell rupture. Exudation occurs and leads to an increase in extraction yield [8].

1.13. Ultrasonic-Assisted Extraction

This is an extraction method using ultrasonic sound waves that pass through the solvent, producing energy by enhancing the diffusion of the solvent into the sample array. The Ultrasonic-Assisted Extraction is cost-effective in terms of the quantity of solvent used, temperature, and time [8].

1.14. The Supercritical Fluid Extraction

In this extraction method, supercritical fluids at high temperatures and pressures above the critical values are applied to the extraction material. The pressure is adjusted, and the supercritical fluids return to their gas phase and evaporate without leaving solvent residues [8].

1.15. Pressurised Liquid Extraction

This extraction method is conducted under high pressures and temperatures that aid in the high solubility of the compounds in the solvent and result in high diffusion of the solvent into the sample array [8]. **Table 1** shows the various extraction methods used when extracting biomass.

Table 1. Extraction methods used in biomass extractions [3].

Method	Solvent	Temperature	Pressure	Time	Volume Consumed	The Polarity of Natural Products
Maceration	Water, Aqueous and non-aqueous solvents	Room temperature	Atmospheric	Long	Large	Dependent on extracting solvent
Percolation	Water, Aqueous and non-aqueous solvents	Room temperature, occasional heat	Atmospheric	Long	Large	Dependent on extracting solvent
Decoction	Water	Under heat	Atmospheric	Moderate	None	Polar compounds
Reflux extraction	Aqueous and non-aqueous solvents	Under heat	Atmospheric	Moderate	Moderate	Dependent on the extracting solvents
Soxhlet extraction	Organic solvents	Under heat	Atmospheric	Long	Moderate	Dependent on extracting solvent
Pressurised liquid extraction	Water, aqueous and non-aqueous solvents	Under heat	High	Short	Small	Dependent on extracting solvent
Supercritical fluid extraction	CO ₂	Near room temperatures	High	Short	None or small	Non-polar to moderate compounds

Method	Solvent	Temperature	Pressure	Time	Volume Consumed	The Polarity of Natural Products
Ultrasound-assisted extraction	Water, aqueous and non-aqueous solvents	Room temperature or under heat	Atmospheric	Short	Moderate	Dependent on extracting solvent
Microwave-assisted extraction	Water, aqueous and non-aqueous solvents	Room temperature	Atmospheric	Short	Moderate	Dependent on extracting solvent
Pulsed electric field extraction	Water, aqueous and non-aqueous solvents	Room temperature or under heat	Atmospheric	Short	Moderate	Dependent on extracting solvent
Enzyme assisted extraction	Water, aqueous and non-aqueous solvents	Room temperature or heated after enzyme treatment	Atmospheric	Moderate	Moderate	Dependent on extracting solvent

Chromatography is a technique used to separate molecules based on their size, shape, and charge. The analyte in the solvent passes through a molecular sieve which leads to its separation. Paper and thin layer chromatography readily provide qualitative information and through which it becomes possible to obtain quantitative data.

2.2. Paper Chromatography (PC)

In this technique, a sheet of paper is used to carry out separations which act as both support as well a medium for separation. The sample is placed near the bottom of the filter paper and the filter paper is placed in the chromatographic chamber with solvent. The solvent moves forward by capillary action carrying soluble molecules along with it. Low porosity paper will produce a slow rate of movement of the solvent and thick papers have increased sample capacity [9](#).

2.3. Thin Layer Chromatography (TLC)

This technique separates the samples based on the interaction between a thin layer of adsorbent attached to the plate with low molecular weight compounds. Different adsorbents are used to separate various compounds [9].

2.4. Gas Chromatography (GC)

This technique is used to separate volatile compounds. The rate of kinetics for the chemical species is determined through its distribution in the gas phase. Gas chromatography involves a sample being vaporized and injected onto the head of the chromatographic column. The sample is transported through the column by the flow of the inert, gaseous mobile phase. The column itself contains a liquid stationary phase which is adsorbed onto the surface of an inert solid [9].

2.5. High-Performance Liquid Chromatography (HPLC)

This technique separates compounds based on their interactions with solid particles of a tightly packed column and the solvent of the mobile phase. The Diode Array Detector measures the absorption spectra of the analytes to aid in their identification of the compounds [9].

3. Qualitative and Quantitative Phytochemical Screening

3.1. Introduction

The study of bioactive compounds encompass phytochemical and pharmacological approaches [10]. Many plant parts contain bioactive components, e.g., bark, leaves, stems, fruits, and seeds [11]. Phytochemicals are chemicals produced by the various parts of the plants namely, alkaloids, flavonoids, terpenoids, steroids, tannins, glycosides, etc. The bioactive compounds have various antimicrobial and antibacterial properties [12]. Qualitative phytochemical screening plays a crucial role in identifying various biochemical compounds produced by plants. The quantification of those metabolites may assist in the extraction, purification, and identification of the bioactive compounds for human use [12]. The preliminary qualitative phytochemical screening is carried out as per standard methods described by Trease & Evans 1989.

Detection of Alkaloids

The extracts are dissolved in dilute hydrochloric acid, filtered individually, and tested for the presence of alkaloids.

Mayers test: The extraction added to the Mayers reagent. A yellow cream precipitate formation indicates the presence of alkaloids.

Wagner's test: Wagner's reagent is added to the extraction of a brown-reddish brown formation observed, and it indicates the presence of alkaloids.

Detection of Flavonoids

Lead acetate test: A few drops of lead acetate solution is added to the extracts. A yellow-colour precipitate indicates the presence of flavonoids.

Sulfuric acid test: A few drops of sulfuric acid are added to the extracts, and the formation of orange colour indicates the presence of flavonoids.

Detection of Steroids

A few drops of acetic anhydride are added to the extracts and the formation of violet to blue to green in some samples indicates the presence of steroids.

Detection of Terpenoids

Salkowski's Test: Extract 5 mg of the selected plant part mixed with 2 mL chloroform and 3 mL concentrated sulfuric acid added carefully to form a layer. A reddish-brown colour indicates the presence of terpenoids.

Detection of Anthraquinones

Bontrager's Test: About 5 mg of the extract is boiled with 10% HCl for a few minutes in a water bath. It's filtered and allowed to cool. An equal volume of CHCl_3 is added to the filtrate. A few drops of 10% NH_3 are added to the mixture and heated. The formation of pink colour indicates the presence of anthraquinones.

Detection of Phenols

Ferric chloride test: A few drops of ferric chloride are added to the 10 mL extract. A bluish-black colour indicates the presence of phenol.

Lead acetate test: A few drops of lead acetate solution is mixed with 10 mg extract. A yellow colour indicates the presence of phenol.

Detection of Saponins

A 0.5 mg of the extract is mixed vigorously with 5 mL of distilled water. The formation of frothing indicates the presence of saponins.

Detection of Tannins

A few millilitres of the extract are mixed with a few millilitres of water and heated in a water bath. The mixture is filtered. Ferric chloride is added to the filtrate. The dark green colour indicates the presence of tannins.

Detection of Carbohydrates

A 0.5 mg of the extract is dissolved individually in five ml of distilled water and filtered. The filtrate is used to test the presence of carbohydrates [13].

3.2. Quantitative Phytochemical Analysis

Estimation of Total Alkaloids

One gram of extract sample is added to a 250 mL beaker, and 200 mL of 10% acetic acid in ethanol is added, covered, and left for settling for 4 h. The extract is filtered and concentrated in a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide is added dropwise to the extract until the precipitation is complete. The solution is allowed to settle, and the precipitate is collected and washed with dilute ammonium hydroxide, followed by filtration. The residue is dried and weighed [14].

Estimation of Total Flavonoids

A gram of sample is extracted repeatedly with 100 mL of 80% aqueous methanol. The mixture is filtered through Whatman no.1 filter paper into a pre-weighed 250 mL beaker. The filtrate is transferred to a water bath and allowed for evaporation to dryness and followed by weighing off the sample [12].

Estimation of Total Phenols

The sample is placed in a beaker and boiled for 15 min with 50 mL of ether for the extraction of phenolic compounds. five mL of the extract is pipetted out into a 50 mL flask followed by the addition of 10 mL of distilled water, 2 mL of ammonium hydroxide solution, and 5 mL of concentrated amyl alcohol. The samples are left to react for 30 min for colour development and read at 505 nm [12].

4. Textiles Analysis

4.1. Biocidal Analysis

The biocidal analysis evaluates the effectiveness of antimicrobial textiles. Several test methods have been established through quantitative antimicrobial tests. The number of microbes present on the finished fabrics can be counted and expressed as a percentage or as a log reduction. The test methods for quantitative determination are ATCC TM100, JIS L1902, AATCC90 percentage reduction, and ISO 20743 shake flask reduction methods [15].

The Parallel Streak Method (AATCC TM147) is a qualitative method used to determine the antibacterial activity of diffusible antimicrobials agents on treated textile materials. The Parallel Streak Method has proven to be effective. This method shows antibacterial activity against both Gram-positive and Gram-negative bacteria. The sterilised agar is dispensed (cooled to 47 °C (117 °F) by pouring 15 mL into each standard (15 × 100 mm) flat-bottomed petri dish. Allow agar to gel firmly before inoculating. The inoculum is prepared by transferring 1.0 mL of a 24-h broth culture into 9.0 mL of sterile distilled water containing it in a test tube or small flask. A 4 mm inoculating loop is

used, loaded with one loopful of the diluted inoculum and transferred to the surface of the sterile agar plate by making five streaks approximately 60 mm in length, spaced 10 mm apart by covering the central area of a standard petri-dish without refilling the loop. The specimen is pressed onto the agar surface with a sterile spatula. After 18 to 24 h of incubation at 37 °C, the plates are examined for bacterial growth directly underneath the textiles and around the edges of the textiles. If the antimicrobial substance diffuses into the agar, an inhibition area is formed, and its size indicates the effectiveness of the antimicrobial effect or the rate at which the active agent is released [16][17]. AATCC 100 (Suspension Test) is a quantitative antimicrobial test method used to determine the antibacterial activity of the textiles and fabrics against bacteria. The bacterial counts are recorded, and a percent reduction is measured using initial count and remaining count data [17].

4.2. Durability Analysis

Durability by washing method (ASTM E3162-18 or AATCC61-2A) is used to determine the durability of laundering. This test method is an accelerated laundering test method to measure the durability of antibacterial agents applied to textiles under simulated home laundering conditions. Ten grams of the coated fabric for laundering is prepared, followed by adding a 500 mL defined detergent solution. Set the washing machine at a temperature of 50 °C under abrasive action using stainless steel balls to simulate five home launderings for a 45-min laundering cycle at 40 revolutions per minute. After each cycle, remove the fabric and rinse with water thoroughly by hand. Repeat, depending on the total number of washes required.

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