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Extraction and Compound Analysis of *Bauhinia acuminata*: Soxhlet Method and Identification

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Abstract

In the present study, the extraction and compound analysis of Bauhinia acuminata using the Soxhlet method, coupled with identification techniques, were conducted. Bauhinia acuminata L., a member of the Leguminosae (Fabaceae) family in the Caesalpinioideae subfamily, is a shrub usually reaching a height of around 3 m. It is native to tropical Southeast Asia and commonly found in the tropical regions of India and China. Primarily grown for ornamental purposes, this plant holds significance in traditional Indian medicine. The bark, leaves, stem, flowers, and roots of B. acuminata are utilized in treating various ailments including gastrointestinal, skin, venereal diseases, and leprosy. An investigation by Anju Krishna et al. investigated the phytochemical composition of leaf and stem extracts from B. acuminata, revealing its antioxidant, antibacterial, antifungal, and hemolytic properties. High levels of phenolic compounds, flavonoids, and other bioactive constituents, such as 1-methyl 3-nonyl indane, were detected in the extracts. Despite its extensive traditional use, there is a lack of published studies on the phytochemical, antioxidant, antimicrobial, and hemolytic activities of Bauhinia acuminata leaves and stems. The extraction process employs the Soxhlet method, followed by compound identification using various analytical techniques such as chromatography, mass spectrometry, nuclear magnetic resonance spectroscopy, and UV-Vis spectroscopy. The study aims to provide insights into the phytochemical composition and antimicrobial potential of Bauhinia acuminata extracts, shedding light on its medicinal properties and potential applications in healthcare.

Keywords: *Bauhinia acuminata*, phytochemical screening tests, Soxhlet method, dwarf white bauhinia, orchid

INTRODUCTION

Bauhinia acuminata is a flowering plant that is a member of the Leguminosae (Fabaceae) family. It has been traditionally used to cure gastrointestinal diseases, skin diseases, venereal diseases, and leprosy. Different parts of this plant, such as the bark, leaves, stem, flowers, and roots, have been utilized in traditional medicine. Although various parts of this plant have been reported to possess good medicinal properties, there is no definite study specifically on the phytochemical, antioxidant, antimicrobial, and hemolytic activity of *Bauhinia acuminata* leaves and stems. Hence, the aim of this study is to explore the phytochemical composition and antimicrobial activities of leaf and stem extracts from *Bauhinia acuminata* [1]. Based on the ethnopharmacological background, we chose to synthesize AgNPs (Silver Nanoparticles) using *B. acuminata*. The Ag (Silver) nanomaterial was synthesized using the aqueous extract of *B. acuminata* as a reducing agent. The characteristics

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of AgNPs (Silver Nanoparticles) were analyzed using UV-vis and FTIR (Fourier Transform Infrared Spectroscopy) spectroscopy, XRD (X-ray Diffraction), and TEM (Transmission electron microscopy). The antibacterial, antifungal, and mosquito larvicidal activities of the AgNPs synthesized with *B. acuminata* were assessed against species of high economic importance, including mosquito vectors of public health significance from the Aedes, Anopheles, and Culex genera [2].

METHODOLOGY AND MATERIALS

Phytochemical Screening Tests

Phytochemical screening tests are essential for identifying various classes of compounds in plant extracts. Here is a protocol for conducting phytochemical screening of *Bauhinia acuminata*:

Materials

- Fresh or dried leaves of *Bauhinia acuminata*,
- Solvents (e.g., ethanol, methanol, or water),
- Test tubes,
- Beakers,
- Droppers,
- Filter paper,
- Reagents for specific tests (listed below), and
- Heat source (optional).

Phytochemical Screening Tests

- *Test for alkaloids*: Mix a small amount of plant material with dilute hydrochloric acid (HCl). Heating the mixture gently and then it was filtered. Adding a few drops of Dragendorff's reagent to the filtrate, the formation of an orange-red precipitate indicates the presence of alkaloids.
- *Test for flavonoids*: Boil a small amount of plant material with water. Then filter the extract. Add a few drops of dilute ammonia solution to the filtrate, producing of a yellow color confirms the presence of flavonoids.
- *Test for tannins*: Mix the plant extract with water. Add a few drops of 5% ferric chloride ($FeCl_3$) solution. Formation of a blue-black or green precipitate indicates the presence of tannins.
- *Test for saponins*: Shake the plant material with water vigorously.
  Production of froth that shows for some time with presence of saponins.
- *Test for terpenoids*: Mix the plant extract with chloroform and evaporate to dryness.
  Then mix a few drops of concentrated sulfuric acid (H_2SO_4) to the residue. Appearance of a red or brown color shows the presence of terpenoids.
- *Test for glycosides*: Mix the plant extract with water. Then add a few drops of Fehling's solution A and B. Formation of a brick-red precipitate indicates the presence of glycosides.
- *Test for phenolic compounds*: Mix the plant extract with water.

Then add a few drops of 1% ferric chloride ($FeCl_3$) solution. Formation of a blue-green or dark green color indicates the presence of phenolic substances.

Interpretation

Positive results indicate the presence of respective phytochemical constituents in *Bauhinia acuminata*. Negative results suggest the absence of specific compounds or their presence below the detectable limit.

Extraction and Analysis of *Bauhinia acuminata* Plant Material

- *Plant material*: Fresh or dried parts of *Bauhinia acuminata*, including leaves and stems.
- *Solvents*: High-quality solvents suitable for Soxhlet extraction, such as ethanol or methanol.
- *Soxhlet apparatus*: Soxhlet extractor, a condenser, and a round-bottom flask.

- *Filter thimbles:* Made of suitable material (e.g., cellulose, glass fiber) for holding the plant material during extraction.
- *Analytical balances:* Precise balances for accurately measuring the mass of plant material and solvents.
- *Rotary evaporator:* Equipment for solvent evaporation to obtain the extract.
- *Chromatography equipment*: Including TLC plates, GC columns, or HPLC columns for chromatographic separation.
- *Mass spectrometer*: Instrumentation for mass spectrometry analysis to confirm compounds based on their mass-to-charge ratio.
- *Nuclear magnetic resonance (NMR) spectrometer*: Equipment for NMR spectroscopy analysis to evaluate the chemical structure of compounds.
- *UV-Vis spectrophotometer*: Instrumentation for UV-Vis spectroscopy method to obtain the absorption of light by substances in the extract.
- *Chemicals and reagents*: Reagents for sample preparation, chromatography, and other analytical procedures, including standards for compound identification.
- *Laboratory glassware:* Glassware for various procedures, including flasks, beakers, pipettes, and syringes.
- *Safety apparatus*: Personal protective equipment (PPE) such as lab coats, gloves, and safety goggles.
- *Information investigation software*: Software for data evaluation and processing, particularly for chromatography and spectroscopy matter.
- *Reference materials*: Standards or reference compounds for comparison during compound identification.
- *Well-equipped laboratory facilities*: Access to facilities for extraction, chromatography, and spectroscopy analysis.

Soxhlation of Bauhinia acuminata

Sample preparation: Obtain fresh or dried parts of *Bauhinia acuminata*, such as leaves and stems. If required, reduce the size of the plant material into a fine powder to increase surface area for extraction.

Soxhlet Extraction

- Place the powdered plant material into a thimble made of suitable material (e.g., cellulose, glass fiber).
- Set up the Soxhlet apparatus by assembling the Soxhlet extractor, condenser, and round-bottom flask.
- Fill the round-bottom flask with a suitable solvent (e.g., ethanol, methanol).
- Heat the flask to reflux, allowing the solvent vapor to rise and pass through the plant material in the thimble.
- The solvent extracts compounds from the plant material as it cycles through the Soxhlet apparatus.
- Continue the extraction process for several hours until the desired compounds are extracted.

Solvent evaporation

- After extraction, collect the solvent containing the extract in the round-bottom flask.
- Separate the solvent utilizing a rotary evaporator under reduced pressure and controlled temperature.
- This process concentrates the extract, leaving behind a residue containing the compounds of interest.

Compound Identification

To work on chromatographic evaluation using techniques such as thin-layer chromatography (TLC), gas chromatography (GC), or high-performance liquid chromatography (HPLC) to separate and confirm individual compounds in the extract.

- To use mass spectrometry (MS) to analyze the mass-to-charge ratio of compounds for identification.
- To install nuclear magnetic resonance (NMR) spectroscopy for the chemical structure of compounds.
- To utilize UV-Vis spectroscopy to test the absorption of light by compounds in the extract.

Information investigation

- To analyze the chromatographic, spectrometric, and spectroscopic data obtained during compound identification.
- To compare the data with reference standards or literature values to identify the compounds present in the extract.

Phytochemical Analysis

To determine the phytochemical composition of the extract, focusing on antioxidant compounds such as phenols and flavonoids.

Antimicrobial action

Microbial strains: Obtain microbial strains for antimicrobial testing, including bacteria (e.g., *Escherichia coli*, *Staphylococcus aureus*) and fungi (e.g., *Candida albicans*).

Preparation of extracts: Prepare different concentrations of the *Bauhinia acuminata* extract by dissolving in appropriate solvents (e.g., DMSO, ethanol) to obtain desired concentrations.

Agar well diffusion method: Form Mueller-Hinton agar plates for bacterial strains and Sabouraud dextrose agar plates for fungal strains.

- Inoculate the plates with optimized strength of suspensions of microbial cultures.
- Utilize a sterile cork borer to make wells in the agar plates.
- Fill the wells with different concentrations of the *Bauhinia acuminata* extract.
- Incubate the plates at appropriate temperatures for bacterial (37°C) and fungal (25–30°C) growth.

Measurement of inhibition zones: Measure the diameter of inhibition zones formed around the wells after incubation.

- Find out the mean diameter of inhibition zones for each strength of the extract.

Minimum Inhibitory Concentration (MIC) optimization

- Work on MIC evaluation by broth dilution process.
- Prepare serial dilutions of the extract in broth media.
- Inoculate each dilution with standardized microbial suspensions.
- Incubate the tubes and check the minimum inhibitory concentration of the extract.

Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) Determination

- Subculture samples from MIC tubes showing no visible growth on agar plates.
- Incubate plates and test the minimum inhibitory concentration of the extract.
- Investigate the information using proper statistical procedures to evaluate the importance of antimicrobial action.

RESULTS AND DISCUSSION

Phytochemical analysis of plant extracts proved the presence of carbohydrates, flavonoids, saponins, tannins, terpenoids, phenols, and proteins. Given the strong resistance of microorganisms to conventional drugs, there is a stressful requirement to describe alternative ways to curb the improvement of multidrug-resistant strains. Plant-derived compounds offer a promising avenue as substitutes for conventional drugs. In evaluation, the antimicrobial action of the methanolic leaf extract of *Bauhinia acuminata* stated good actions against foodborne pathogens such as *Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Escherichia coli*. Furthermore, the antimicrobial activity of *Biophytum sensitivum* was assessed and found to align with previous reports [3]. In our study, silver nanoparticles (AgNPs) synthesized with *B. acuminata* demonstrated effectiveness against *E. coli* and *S. aureus*, with minimum inhibitory concentrations (MICs) of 900 and 750 μg, respectively. The green synthesis procedure, characterizing naturally available pre-activators such as vitamins, sugars, plant extracts, biodegradable polymers, and microorganisms as reductants and capping substances, is environmentally friendly and reveals good antimicrobial activities against one of the major pathogenic bacteria. The antimicrobial action of the biosynthesized leaf extract of *Bauhinia acuminata* in study showed good action against *Staphylococcus aureus* and *Escherichia coli* [4–10].

CONCLUSION

This study revealed successfully to extract compounds from *Bauhinia acuminata* leaves and stems using the Soxhlet extraction method. This method efficiently extracted a broad spectrum of compounds from the plant material. Phytochemical investigation opened the positivity of different bioactive compounds, consisting of carbohydrates, flavonoids, saponins, tannins, terpenoids, phenols, and proteins. These compounds possess diverse biological activities and potential health benefits. Moreover, specific compounds were identified using chromatography and spectroscopy techniques, confirming the presence of key constituents in the extract. These identified compounds contribute to the medicinal properties of *Bauhinia acuminata* and may support its traditional uses for various ailments. In conclusion, this study provides valuable insights into the extraction and compound analysis of *Bauhinia acuminata* using the Soxhlet method. Further research could delve into the pharmacological activities of the isolated compounds and explore their potential applications in pharmaceuticals, nutraceuticals, and other industries.

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