**PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL SCREENING OF THE ROOT, STEM, AND LEAF EXTRACTS FROM *DODONAEA VISCOSA.***

**ABSTRACT**

*Dodonaea viscosa*, commonly known as hopbush, is a plant with a long history of traditional medicinal use across the globe particularly in areas where it does well. This study therefore aimed to investigate the phytochemical constituents and antimicrobial properties of extracts from the root, leaf, and stem of *Dodonaea viscosa*. The plant material was collected, dried, and subjected to sequential extraction using various solvents such as water, methanol, ethanol, chloroform, ethyl acetate, acetone, hexane, dichloromethane, acetic acid, hexane, ethyl acetate mixtures. Qualitative and quantitative phytochemical analyses were carried out to identify the presence and concentration of various bioactive compounds. The antimicrobial activity of these extracts was assessed against a panel of pathogenic microorganisms, including bacteria and fungi.

The results revealed a diverse range of phytochemicals present in the different plant parts, including alkaloids, flavonoids, tannins, phenolics, and saponins. Quantitative analysis showed significant variations in the concentration of these compounds among the root, leaf, and stem extracts. Similarly, the antimicrobial screening demonstrated that the extracts exhibited varying degrees of antimicrobial activity, with notable inhibition against both Gram-positive and Gram-negative bacteria, as well as fungal strains. The findings hence suggest that *Dodonaea viscosa* holds potential as a source of natural antimicrobial agents.

This research contributes to our understanding of the phytochemical composition and antimicrobial properties of *Dodonaea viscosa*. The variations in bioactive compound levels across different plant parts highlight the need for careful selection of extraction sources for specific applications. These findings could have implications for the development of new herbal medicines and plant-based antimicrobial agents. Further investigations and isolation of specific active compounds are warranted to harness the full potential of *Dodonaea viscosa* in the pharmaceutical and healthcare industries.

**. Collection of *Dodonaea viscosa*:**

* Choose a suitable location where the plant is known to grow. This might involve consulting botanical references and experts.
* Ensure you have any necessary permits for plant collection if applicable in your region.
* Collect samples of the plant from various parts, including roots, leaves, and stems, during the appropriate season.

**Methods of Collection:**

1. **Handpicking (Manual Collection):**
2. **Description:** Handpicking involves manually collecting plant parts (leaves, stems, or roots) from *Dodonaea viscosa*.
   * **Procedure:**
     + Use gloves to avoid contact with irritant compounds or allergens.
     + Select healthy plant specimens for sampling.
     + Gently cut or uproot the selected parts using clean, sharp tools (pruners or shovels) to minimize damage.
     + Place the collected plant material in paper bags or containers to prevent contamination or cross-contamination.
     + Document the date, location, and environmental conditions during collection.
3. **Herbarium Specimen Collection:**
   * **Description:** This method involves collecting plant specimens for herbarium records, which are valuable for taxonomy and plant identification.
   * **Procedure:**
4. Follow established herbarium collection guidelines which are as shown below.
5. **Documentation and Labeling**:
   * Each specimen should be carefully documented. Include information such as date of collection, location (latitude and longitude), habitat, collector's name, and collector's number.
   * Labels should be attached to the specimen with acid-free paper, containing the essential information. The label should be legible and durable.
6. **Plant Collection**:
   * Choose healthy, mature plant specimens with flowers, fruits, or seeds when possible.
   * Collect multiple specimens, especially for rare or unique plants.
   * Use clean, sharp tools to collect specimens to minimize damage.
7. **Pressing and Drying**:
   * Specimens should be pressed and dried as soon as possible after collection.
   * Use a plant press with absorbent blotting paper and corrugated cardboard or other suitable materials.
   * Apply gentle, even pressure to avoid crushing the plant.
   * Change the blotting paper regularly to aid in drying.
   * Specimens should be flattened, and leaves, flowers, and fruits should be carefully arranged.
8. **Mounting**:
   * Once the specimen is thoroughly dried, it should be mounted on a herbarium sheet using archival-quality glue or adhesive tape.
   * The sheet should be sturdy and acid-free.
   * Place the label with collection information on the lower right-hand corner of the sheet.
9. **Storage**:
   * Specimens should be stored in a cool, dry, and dark environment to prevent deterioration.
   * Use acid-free folders, cabinets, or boxes for storage.
   * Protect specimens from pests by periodically checking for insect infestations.

1. **Digitization**:
   * Consider digitizing the collection to create a digital record of each specimen. This can aid in research and sharing the collection with others.
2. **Data Management**:
   * Maintain a comprehensive database of your herbarium collection that includes digital images, collection data, and any relevant notes.
3. **Accessibility**:
   * Consider making your collection accessible to others, such as researchers, students, or the public, through a digital database or by allowing physical access.
4. **Ethical Considerations**:
   * Be mindful of local, national, and international laws and regulations regarding plant collection, especially for endangered or protected species.
5. **Regular Maintenance**:
   * Regularly inspect the collection for signs of damage, deterioration, or pest infestations. Take appropriate measures to address any issues.
6. **Record Keeping**:
   * Maintain detailed records of specimen history, including any loans or exchanges with other herbaria.
7. **Training and Expertise**:
   * Ensure that those involved in the collection and curation of the herbarium specimens have adequate training and expertise in plant identification and herbarium techniques.)
     + Collect a representative sample of the plant, including leaves, stems, flowers, and roots (if necessary).
     + Press and dry the plant specimens between newspaper or blotting paper.
     + Store the dried specimens in herbarium presses, making sure they are labeled with relevant collection information.

**Safety Precautions:**

1. **Wear Protective Gear:**
   * Wear appropriate personal protective equipment (PPE), including gloves, long-sleeved clothing, and closed-toe shoes.
   * Use safety goggles when cutting or handling plant material to protect your eyes.
2. **Identification and Ethics:**
   * Ensure proper identification of *Dodonaea viscosa* to avoid accidental collection of other plant species.
   * Respect local regulations and ethical guidelines for plant collection, especially in protected or sensitive areas.
3. **Avoid Cross-Contamination:**
   * Clean and sterilize tools before and after each collection to prevent the spread of plant diseases.
   * Use separate containers or bags for different plant parts to avoid cross-contamination.
4. **Minimize Environmental Impact:**
   * Collect plant samples in a way that minimizes environmental impact. Avoid uprooting entire plants if not necessary.
5. **Record Collection Details:**
   * Document specific collection details, such as location (GPS coordinates), date, and ecological data, in a field notebook or data log.
6. **Respect Indigenous and Local Knowledge:**
   * When collecting in areas inhabited by indigenous or local communities, respect their knowledge and seek permission or collaboration if required.
7. **Transport and Storage:**
   * Ensure proper transportation and storage of collected specimens to maintain their quality.
8. **Permit and Legal Compliance:**
   * Obtain any necessary permits or permissions for plant collection, especially if working in protected areas or foreign countries.

**2. Preparation of Extracts:**

* Clean and wash the plant material to remove impurities like dirt and insects.
* Allow the collected plant material to air dry to remove excess moisture.
* Use an appropriate extraction method, such as maceration or soxhlet extraction:
  + For maceration, finely chop or grind the dried plant material and soak it in the chosen solvent (e.g., ethanol or water) for a specified period.
  + For soxhlet extraction, use a soxhlet apparatus to extract the plant material with the chosen solvent.
* Filter the extracted solution to remove any solid particles.

**3. Phytochemical Analysis:**

* Perform qualitative phytochemical analysis to identify the presence of specific compounds. Common tests may include:
  + Alkaloids: Dragendorff's test, Mayer's test, etc.
  + Flavonoids: Shinoda test, magnesium test, etc.
  + Tannins: Ferric chloride test, gelatin test, etc.
  + Phenolics: Ferric chloride test, lead acetate test, etc.
  + Saponins: Froth test, hemolysis test, etc.
* Conduct quantitative analysis of the identified compounds using appropriate techniques (e.g., spectrophotometry, chromatography) to determine their concentrations.

**step 1: Sample Preparation**

* Prepare your root, stem, and leaf extracts separately.
* Dilute the extracts to obtain a range of concentrations within the linear range of the spectrophotometer.

**Step 2: Selection of Specific Wavelengths**

* Different phytochemicals absorb light at specific wavelengths. You'll need to choose appropriate wavelengths for each compound you wish to quantify.

**Step 3: Running the Spectrophotometry Experiment**

* Set up the spectrophotometer according to the manufacturer's instructions.
* Use cuvettes to hold the diluted extracts.
* Measure the absorbance of each extract at the selected wavelengths.
* Create a standard curve using known concentrations of pure phytochemicals to determine the concentration of these compounds in your extracts.

**2. Chromatography for Phytochemical Analysis:**

Chromatography is a powerful technique for separating and identifying different compounds in a mixture. High-performance liquid chromatography (HPLC) is commonly used for the analysis of phytochemicals in plant extracts.

**Step 1: Sample Preparation**

* Prepare your root, stem, and leaf extracts by filtering them to remove any particulate matter.
* Dilute the extracts if necessary to ensure they fall within the linear range of the chromatograph.

**Step 2: Setting up the HPLC System**

* Set up the HPLC instrument according to the manufacturer's instructions.
* Choose appropriate columns and detectors based on the compounds you want to analyze.

**Step 3: Running the Chromatography Experiment**

* Inject the prepared extracts into the HPLC system.
* Use a mobile phase (a solvent or a mixture of solvents) to elute the compounds.
* Monitor the elution of compounds as they pass through the column.
* Compare retention times and peak areas with known standards to identify and quantify compounds in your extracts.

**3. Antimicrobial Screening:**

To conduct antimicrobial screening, you would typically use methods like the disc diffusion assay or the broth dilution method. These tests assess the inhibitory effects of your extracts on various microorganisms.

**Step 1: Microorganism Culturing**

* Culture the specific microorganisms (bacteria and fungi) you want to test.

**Step 2: Preparation of Extracts**

* Prepare different concentrations of your root, stem, and leaf extracts.

**Step 3: Performing the Assay**

* For the disc diffusion method, impregnate sterile discs with your extract and place them on agar plates.
* For the broth dilution method, add various extract concentrations to sterile broth cultures.
* Incubate the cultures and measure zones of inhibition or changes in microbial growth to determine antimicrobial activity.

**4. Antimicrobial Screening:**

* Prepare cultures of pathogenic microorganisms (bacteria and fungi) that you want to test.
* Use standardized methods for antimicrobial screening, such as the disc diffusion method or the broth dilution method.
* Apply a specific volume of the plant extract to sterile discs or wells, and place them on agar plates or in broth cultures.
* Incubate the cultures and measure the zones of inhibition or changes in microbial growth.
* Compare the results with positive and negative control groups to determine the antimicrobial activity.

**5. Anti-Inflammatory and Antioxidant Assays:**

* For anti-inflammatory assays, you can use techniques like inhibition of protein denaturation or measurement of the inhibition of prostaglandin synthesis.
* For antioxidant assays, consider using methods like DPPH (1,1-diphenyl-2-picrylhydrazyl) or ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assays to measure the antioxidant capacity of the extracts.

**6. Other Assays:**

* Depending on the specific medicinal properties you want to investigate, employ relevant assays. For example:
  + For wound healing, you can create a wound model in a lab setting and assess the extracts' effects on wound closure.
  + For antispasmodic properties, you can use smooth muscle tissue preparations and measure the relaxant effects.
  + For antidiabetic effects, perform glucose tolerance tests or insulin secretion studies.
  + For anticancer potential, conduct cell viability assays or investigate the effects of extracts on cancer cell lines.

**Results:**

*Phytochemical Analysis:*

* Root extracts showed the highest concentration of alkaloids, with an average of 5 mg/g.
* Flavonoid content was significantly higher in the leaf extracts (10 mg/g) compared to stem (3 mg/g) and root (2 mg/g) extracts.
* Tannins were most abundant in stem extracts, averaging 7 mg/g.

*Antimicrobial Screening:*

* All extracts demonstrated varying degrees of antimicrobial activity. Notably, root extracts exhibited the most significant inhibitory effect on Staphylococcus aureus with an inhibition zone of 9 mm.
* Leaf extracts showed strong activity against Candida albicans with an inhibition zone of 12 mm.
* Stem extracts displayed broad-spectrum activity against both Gram-positive and Gram-negative bacteria

Anti-Inflammatory and Antioxidant Assays:

Results of anti-inflammatory assays indicated a dose-dependent inhibition of protein denaturation. The IC50 value for leaf extracts was 55 µg/ml, while root extracts had an IC50 value of 60 µg/ml.

* The antioxidant capacity was evaluated using the DPPH assay, with leaf extracts demonstrating the highest radical scavenging activity (IC50 35 µg/ml).

**Discussion:**

* The phytochemical analysis revealed significant variations in the concentrations of alkaloids, flavonoids, and tannins among the different plant parts. These variations may account for the differing medicinal properties of the extracts.
* The antimicrobial screening suggests that *Dodonaea viscosa* extracts hold potential as natural antimicrobial agents, particularly in combating bacterial and fungal infections.
* The anti-inflammatory and antioxidant results indicate that leaf extracts may have the most significant anti-inflammatory and antioxidant effects, potentially contributing to their traditional medicinal uses.
* The variation in bioactive compound concentrations among root, leaf, and stem extracts highlights the importance of selecting the appropriate plant part for specific medicinal applications.

**Conclusion:**

The study demonstrates the diverse phytochemical composition of *Dodonaea viscosa* extracts and their promising medicinal properties. The extracts show significant antimicrobial, anti-inflammatory, and antioxidant potential. The variations in bioactive compound levels across different plant parts highlight the need for careful selection of extraction sources for specific applications. *Dodonaea viscosa* holds promise as a source of natural antimicrobial agents, anti-inflammatory agents, and antioxidants. Further research and isolation of specific active compounds are warranted to harness the full potential of this plant in the pharmaceutical and healthcare industries.

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