Detection of Herbal Biocontrol Agents by Qualitative Methods

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ABSTRACT
Plants and plant-based medicaments are the basis of many of the modern pharmaceuticals we use today for our various ailments. Traditional herbal medicines are moving from fringe to mainstream use with a large number of people seeking remedies and health approaches free from side effects caused by synthetic chemicals. In the present study the three plants that are *Eucalyptus globulus* (family-Myrtaceae), *Lawsonia inermis* (family-Lythraceae) and *Polyalthia longifolia* (family-Annonaceae) were taken for phytochemical study. The present investigation based on find out bioactive phytochemicals by qualitative analysis in all three plants. *Eucalyptus globulus, Lawsonia inermis* and *Polyalthia longifolia* leaves extract were prepared by hot extraction method with soxhlet assembly. It was performed for the detection of alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins and reducing sugar etc. The highest yield of extract was found in acetone fraction of *Lawsonia inermis* (21%) and the lowest in chloroform fraction of *Eucalyptus globulus* (0.2%). In *Eucalyptus globulus* leaf extract, saponins are absent in petroleum ether fraction and aqueous fraction. Tannins are present in all fractions of *Lawsonia inermis*. In *Polyalthia longifolia* alkaloids are present in only methanolic fraction and in absolute alcohol fraction. On the basis of results the herbal formulation can be prepared for control of several biological crop deterioration factors.

Keywords: Crop deterioration, hot extraction, herbal medicine, phytochemical, soxhlet assembly

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INTRODUCTION
Plants have been used to treat or prevent illness since before recorded history. The sacred Vedas dating back between 3500 B.C and 800 B.C give many references of medicinal plants. One of the remotest works in traditional herbal medicine is "Virikshayurveda", compiled even before the beginning of Christian era. Plants and plant-based medicaments are the basis of many of the modern pharmaceuticals we use today for our various ailments [1]. The discovery of medicinal plants has usually depended on the experience of the populace based on long and dangerous self experiment. Progress over the centuries towards a better understanding of a plant derived medicine has depended on two factors that have gone hand in hand. One has been the development of increasingly strict criteria of proof that a medicine really does what it is claimed to do and the other has been the identification by chemical analysis of the active compound in the plant [2].

The plant kingdom has served as an inexhaustible source of useful drugs, foods, additives, flavouring agents, lubricants, colouring agents and gums from time immemorial. The therapeutic power of herbs had been recognized since creation of the universe and botanic medicine is one of the oldest practiced professions by mankind. Medicinal plants have been found useful as antimalarial, antisickling, anti-helminthic, anti-microbial, anti-convulsant, anti-hypertensive and anti-schistosomal (molluscicidal) agents [3]. The therapeutic potential of plants is due to the presence of secondary metabolites such as alkaloids, flavonoids, sterols and tannins etc. [4].
These secondary metabolites are responsible for the alteration of biochemistry and cytology of microbes [5, 6] and inhibitory activity of saponins on fungal growth has been reported [7]. Plants are nature’s “Chemical factories” providing the richest source of organic chemicals on earth. The world is blessed with a great variety of natural vegetation some of which are used as a traditional medicine to cure various sicknesses and diseases [8, 9]. They do not play a vital role in preventing various diseases but also serve as useful flavouring agents while others act as food additives and preservatives [10-11]. In addition, some plants have been used in the manufacture of adhesives, textiles, dyes, paints, plastic and rubber industries [12]. Biologically active compounds from natural sources have always been in great interest for scientists working on infectious diseases [13-16]. As a matter of fact, it has been estimated that about 25% of all prescribed medicines today are substances derived from plants [17, 18].

According to world health organization (WHO), more than 80% of the world’s population relies on traditional medicines for their primary health care needs. The medicinal value of plants lies in some chemical substances that produce a definite physiologic action on the human body. The most important of these bioactive compounds of plants are alkaloids, flavonoids, tannins and phenolic compounds. The phytochemical research based on ethno-pharmacological information is generally considered an effective approach in the discovery of new anti-infective agents from higher plants [19].

The aim of this study was to find out the bioactive chemical constituents and to evaluate the potential of plants that are used readily by communities for curative purposes. A qualitative phytochemical analysis was performed for the detection of alkaloids, carbohydrate, volatile oils, terpenoids, steroids, flavonoids, saponins and tannins [20-21].

**MATERIALS AND METHODS**

**Collection and extract preparation of test plants**

Leaves of *Eucalyptus globulus, Polyalthia longifolia* and *lawsonia inermis* were collected from University College of Science campus, Udaipur. Collected plant parts were disinfected with 0.1% HgCl$_2$ and subsequently washed with distilled water. All plant parts were shade dried at room temperature and powdered mechanically. The ground material was passed through sieve No. 240 so as to obtain powder of mesh size 60, which was used to prepare extract. Partially purified extracts in different solvents were prepared. Hot extraction methods [22] were used to prepare partially purified extract respectively. Extraction in different solvent series used for successive separation from non-polar to polar was done using glass soxhlet assembly. The extract was vacuum dried in vacuum evaporator.

**Percent Extractive Value**

Partially purified fractions obtained at every step including aqueous fraction were vacuum dried in a rotary evaporator. The dried extract and fractions were weighed and their percentage in terms of the dry weight of the plant material was estimated by the following formula:

\[
\text{Percent extractive} = \frac{\text{Weight of dried extract}}{\text{Weight of dried plant material}} \times 100
\]

**Phytochemical study of the test plants**

Various fractions obtained by hot extraction of leaves and seed of all test plants were than subjected to qualitative test for the identification of phytochemical constituents. Phytochemical analysis of all the extracts was studied by the qualitative tests suggested by [23]. All reagents used for phytochemical testing are listed in annexure no. 2. Tests done for detection of secondary metabolites were as follows:
Alkaloids
Alkaloids are compounds having one or more nitrogen containing heterocyclic ring. Presence of alkaloids in methanolic extract was tested by performing Wagner's test. Small amount of extract was taken in a test tube and few drops of dilute HCl was added to it and filtered. Wagner's reagent was added to filtrate. Development of red color indicated presence of alkaloids.

Carbohydrate
Carbohydrates are widely distributed in plants and can be detected by Fehling test. Small amount of extract was dissolved in 5 ml distilled water and filtered. The filtrate was collected. The filtrate was taken and few drops of equal quantity of Fehling A and Fehling B solution were added and heated. Development of brick-red color indicates presence of carbohydrate.

Tannins
Chemically tannins contain the mixture of complex organic substances in which polyphenols are present. Ferric chloride test used to detect presence of tannins. Extract was mixed with ferric chloride and lead acetate. Development of white precipitate indicated presence of tannins.

Saponins
Saponins are complex glycosidal compounds in which the aglycone is tri-terpenoid or steroidal in nature. Foam test was used to detect presence of saponins. Small amount of extract was taken in a test tube and 20 ml distilled water was added to it. Then it was shaken for 15 minutes. Formation of layer of foam at surface indicated presence of saponins.

Flavonoids
Flavonoids usually occur in plants as glycosides in which one or more of phenolic hydroxyl groups are combined with sugar residues. Extract was taken in a test tube and concentrated H₂SO₄ was added to it. Development of yellow-orange color indicated presence of flavonoids.

Phytosterols
Sterols are tri-terpenes and their structure is based on cyclopentane perhydroxy phenanthrene ring system. Libermann's test was used for detection of phytosterols. Small amount of extract was taken in a glass test tube and alcoholic koh was added to it. As a result saponification takes place. The solution was then diluted with the mixture of distilled water and ether (1:1). Subsequently ether was evaporated and residue was collected. Further libermann's test was done with this residue. The residue was mixed with 2 ml chcl₃ and 1 ml of acetic anhydride and 1 ml of concentrated h₂so₄ under room temperature. Formation of brown colored ring at junction of two layers indicates presence of sterols.

Volatile Oils
The odorous volatile principles of plants are known as volatile or essential oils. Sudan III test was used to detect presence of volatile oils. Small amount of extract was taken in a test tube and alcoholic Sudan III added to it. Development of red color indicated presence of volatile oils.

RESULTS AND DISCUSSION
The percent extractive values of partially purified extracts of test plants are shown in (Table 1). The highest yield of extract was found in acetone fraction of Lawsonia inermis leaf (21%) and lowest in chloroform fraction of Eucalyptus globulus leaf (0.2%). The phytochemical constituents of all partially purified fractions of test plants are given in (Table 2-4). In Polyalthia longifolia alkaloids are present in only methanolic fraction and in absolute alcohol fraction and in rest of all it is absent. In the similar conclusion, carbohydrates are present in methanolic fraction only. Flavanoids are absent only in benzene fraction. In Eucalyptus globulus, saponins are absent in petroleum ether fraction and in aqueous fraction. Carbohydrate are absent in all fraction except petroleum ether. Volatile oil is present in all groups but absent in aqueous fraction. Alkaloids are absent in aqueous fraction only.

In the continuation of photochemical screening, tannins are present in all fractions but alkaloids are absent in all group of Lawsonia inermis.
Table 1: Percent extractive of partially purified extracts of all test plants

<table>
<thead>
<tr>
<th>S. No</th>
<th>Name of Plant</th>
<th>Partially purified fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PE</td>
</tr>
<tr>
<td>1.</td>
<td><em>Eucalyptus globulus</em></td>
<td>7.0%</td>
</tr>
<tr>
<td>2.</td>
<td><em>Lawsonia inermis</em></td>
<td>7.3%</td>
</tr>
<tr>
<td>3.</td>
<td><em>Polyalthia longifolia</em></td>
<td>8.1%</td>
</tr>
</tbody>
</table>

Table 2: Phytochemical Screening of Various Fractions of Lawsonia inermis Leaf Extract

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Fractions</th>
<th>Alkaloids</th>
<th>Steroids</th>
<th>Volatile oil</th>
<th>Tannins</th>
<th>Carbohydrate</th>
<th>Saponins</th>
<th>Flavonoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>PE</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>2.</td>
<td>Benzene</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>+ve</td>
<td>-ve</td>
<td>-ve</td>
<td>+ve</td>
</tr>
<tr>
<td>3.</td>
<td>Chloroform</td>
<td>-ve</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
<td>-ve</td>
<td>-ve</td>
<td>+ve</td>
</tr>
<tr>
<td>4.</td>
<td>Acetone</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
</tr>
<tr>
<td>5.</td>
<td>Methanol</td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>6.</td>
<td>Aqueous</td>
<td>-ve</td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
</tbody>
</table>

+ve - Present, -ve - Absent

Table 3: Phytochemical Screening of Various Fractions of Polyalthia longifolia Leaf Extract

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Fractions</th>
<th>Alkaloids</th>
<th>Phytosterols</th>
<th>Volatile oil</th>
<th>Tannins</th>
<th>Carbohydrate</th>
<th>Saponins</th>
<th>Flavonoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>PE</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>+ve</td>
</tr>
<tr>
<td>2.</td>
<td>Benzene</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>+ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
</tr>
<tr>
<td>3.</td>
<td>Chloroform</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>+ve</td>
</tr>
<tr>
<td>4.</td>
<td>Acetone</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>-ve</td>
<td>+ve</td>
</tr>
<tr>
<td>5.</td>
<td>Methanol</td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>6.</td>
<td>Aqueous</td>
<td>-ve</td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
</tbody>
</table>

Table 4: Phytochemical Screening of Various Fractions of Eucalyptus globulus Leaf Extract

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Fractions</th>
<th>Alkaloids</th>
<th>Phytosterols</th>
<th>Volatile oil</th>
<th>Tannins</th>
<th>Carbohydrate</th>
<th>Saponins</th>
<th>Flavonoids</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>PE</td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
</tr>
<tr>
<td>2.</td>
<td>Benzene</td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>3.</td>
<td>Chloroform</td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
<td>-ve</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
</tr>
<tr>
<td>4.</td>
<td>Acetone</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>5.</td>
<td>Methanol</td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>6.</td>
<td>Aqueous</td>
<td>-ve</td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
</tr>
</tbody>
</table>

+ve - Present, -ve- Absent

CONCLUSION

The test plants mentioned in the study that is *Eucalyptus globulus*, *Lawsonia inermis* and *Polyalthia longifolia* contained many bioactive chemical constituents including Alkaloids, Phytosterols, Volatile oil, Tannins, Carbohydrate, Saponins, Flavonoids and Tannins. In This finding give credence to the traditional medicinal application of the plants as remedies for measles, internal and external wounds and infections and revealed their potential in the treatment of typhoid fever. The organic solvent showed positive result for the presence of volatile oils and fatty acids which were absent in the water extract. However water and chloroform were positive for saponins, a class of compounds known to be effective for the treatment of syphilis and other venereal diseases [24].
REFERENCES