ABSTRACT

Nature has been a source of medicinal plants for thousands of years and an impressive number of modern drugs have been isolated from natural sources. It is estimated that 70 to 80% of the people worldwide rely predominantly on traditional health care system and largely on herbal medicines. Various medicinal plants have been used for years in daily life to treat various diseases all over the world. In last few decades, Plectranthus amboinicus is extensively studied for its medicinal properties by advanced scientific techniques and a variety of bioactive compounds have been isolated from the different parts of the plant and were analysed pharmacologically. In our present investigation phytochemical analysis of Plectranthus amboinicus young leaves has been evaluated for the presence of bioactive compounds using various polarity solvents including petroleum ether, chloroform, 80% ethanol and water. The study revealed the presence of alkaloids, flavonoids, aminoacids, terpenoids, phenolic compounds, glycosides, carbohydrates and tannins. The results also suggested that 80% ethanolic extract of Plectranthus amboinicus has a promising therapeutic potential.

Key words: Medicinal plants, Plectranthus amboinicus, Bioactive Compounds, Phytochemical Analysis.
INTRODUCTION

Historians from all around the world have produced evidence to show that apparently all primitive people used herbs—often in a sophisticated way. Knowledge of herbs has been handed down from generation to generation for thousands of years. Herbal drugs constitute a major part in all traditional systems of medicines and are a triumph of popular therapeutic diversity. In India, thousands of plant species are known to have medicinal properties and the use of different parts of several medicinal plants to cure specific ailments has been in vogue since ancient time. Traditional medicine using plant extracts continues to provide health coverage for over 80% of the world’s population, especially in the developing world. Plants above all other agents have been used for medicine from time immemorial because they have fitted the immediate personal need and are easily accessible, inexpensive. Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs.

_Plectranthus amboinicus_ (Lour.) Spreng is a tender, fleshy perennial herb, belonging to the family Lamiaceae and native to Southern and Eastern Africa. It is synonymous to _Coleus aromaticus_ Benth and is commonly known as Cuban oregano, Spanish thyme, Indian Borage, Mexican mint, etc. The herb has green, thick, succulent, heart shaped, leathery and juicy leaves with scalloped edges. The raw leaves emanate an oregano-like flavor and odour when cut or crushed. It is known to possess antimicrobial, antiepileptic and antioxidant properties. The leaves have been traditionally used for the treatment of chronic coughs, cold, bronchitis, asthma, nasal congestion as well as diarrhoea. The herb has also been reported to provide remedy for infections, rheumatism and flatulence. It can help in the treatment of cancer as it has anti-tumour and cytotoxic activities. Apart from these medicinal properties, _Plectranthus amboinicus_ also has culinary uses.

The medicinal value of the plants lies in some active chemical substances called phytochemicals that produce a definite physiological action on the human body. Phytochemicals are divided into two groups, which are primary and secondary constituents according to their functions in plant metabolism. Primary constituents comprise common sugars, aminoacids, proteins and chlorophyll while secondary constituents consists of alkaloids, terpenoids, flavonoids, tannins, phenolic compounds. It is necessary to focus
and develop these compounds to be more effective drugs. In view of its medicinal value, the present study is aimed to screen the pharmaceutically important bioactive substances from *Plectranthus amboinicus* young leaves that greatly contribute the ethnomedicinal properties.

**MATERIALS AND METHODS**

**Collection of plant material**

The young leaves of *Plectranthus amboinicus* were collected from Coimbatore and authenticated by Botanical Survey of India, Coimbatore, Tamilnadu, India. A voucher specimen has been deposited in the laboratory for future reference (BSI/SRC/5/23/2012-13/Tech.372). The specimen was later shade dried, powdered and stored in an air-tight container for further use. The powdered material was used for pharmacological investigation, while for phytochemical screening the powder was extracted with different solvents in their increasing order of polarity such as petroleum ether, chloroform, 80% ethanol and water on orbital shaker. All the extracts were concentrated by distilling the solvent in a rotary flash evaporator and stored at 4ºC. The crude extracts were collected in amber coloured sample bottles and stored. All chemicals and reagents used including the solvents were of analytical grade.

**Pharmacological Studies**

**Physicochemical parameters**

**Ash Values**

The determination of various physicochemical parameters such total ash, water-soluble ash, alkalinity of water soluble and acid insoluble ash values of the powdered material was determined as per the Indian Pharmacopoeia [17].

**Extractive Values**

Extract of the powdered leaves were prepared with different solvents for the study of extractive value [18].

**Fluorescence Analysis**

A small quantity of dried and finely powdered material was placed on a clean grease free microscopic slide and added 1-2 drops of the freshly prepared reagent solution, mixed gently by tilting the slide and waited for 1-2 minutes. Then the slide was viewed in day light and (365 nm) ultraviolet radiations. The colors observed by application of different reagents in different radiations were recorded [19].
**Phytochemical Screening**

Phytochemical screening of all extracts was carried out by following standard procedures [20-22].

**Test for alkaloids**

**Dragendroff’s test**

To 5 ml of the extract few drops of Dragendroff’s reagent was added for the formation of orange coloured precipitate.

**Mayer’s test**

To 5 ml of the extract few drops of Mayer’s reagent was added for the formation of cream coloured precipitate.

**Wagner’s test**

To 5 ml of the extract few drops of Wagner’s reagent was added for the formation of reddish brown coloured precipitate.

**Hager’s test**

To 3 ml of the extract few drops of Hager’s reagent was added for the formation of prominent yellow precipitate.

**Test for flavonoids**

To 3 ml of the extract few magnesium ribbons are dipped and conc. HCl was added over them and observed for the formation of magenta (brick red) colour indicating the presence of flavonoids.

**Test for Aminoacids**

To 3 ml of the extract few drops of 0.2% ninhydrin reagent was added and heated. Formation of violet colour indicated the presence of aminoacids.

**Test for proteins**

**Biuret test**

To 3 ml of the extract few drops of 10% sodium chloride and 1% copper sulphate was added for the formation of violet or purple colour. On addition of alkali, it becomes dark violet.
Millon’s test
To 3 ml of the extract few drops of Millon’s reagent was added for the formation of red colour.

Test for carbohydrates
Molisch’s test
To a small amount of the extract few drops of Molisch’s reagent was added followed by the addition of conc. H₂SO₄ along the sides of the test tube. The mixture was then allowed to stand for 2 min and then diluted with 5 ml of distilled water. Formation of red or dull violet colour at the inter phase of two layers indicates the presence of carbohydrates.

Fehling’s test
The extract was treated with 5 ml of Fehling’s solution (A and B) and kept in boiling water bath. The formation of yellow or red colour precipitate indicates the presence of reducing sugar.

Test for tannins
A fraction of the extract was dissolved in water and then it was subjected to water bath at 37°C for 1 h and treated with ferric chloride solution and observed for the formation of dark green colour.

Test for sterols
Liebermann-Burchard test
To a small amount of the extract few drops of chloroform, acetic anhydride and H₂SO₄ was added along the sides of the test tube to observe the formation of dark red or pink colour.

Test for glycosides
Baljet’s Test
To 5 ml of the extract few drops of sodium picrate was added to observe yellow to orange colour.

Keller-Killiani test
To 5 ml of the extract few drops of ferric chloride solution was added and mixed, then sulphuric acid containing ferric chloride solution was added, it forms two layer showed reddish brown while upper layer turns bluish green indicates the presence of glycosides.
Test for phenols

Ferric chloride test
A fraction of the extract was treated with 5% ferric chloride solution and observed for the formation of deep blue or black colour.

Test for saponins

Foam test
To a small amount of the extract few drops of distilled water was added and shaken vigorously until persistent foam was observed.

Test for terpenoids

Chloroform test
To 5 ml of the extract few drops of chloroform and conc. \( \text{H}_2\text{SO}_4 \) was added carefully along the sides of the test tube to form a layer and observed for the presence of reddish brown colour.

RESULTS AND DISCUSSION

The traditional knowledge of Ayurvedic medicine is being explored and widely accepted. Studies on plants used in the traditional medicine systems are investigating their pharmacological and chemical constituents in order to generate ideas for new drugs, as well as to understand the scientific basis of their folkloric uses.

Ash value
The powdered material was evaluated for its physico-chemical parameters like Ash values, Water soluble ash, Acid Insoluble ash and the results are shown in Table I.

Table I: Physico-chemical studies of *Plectranthus amboinicus* young Leaves.

<table>
<thead>
<tr>
<th>Types of Ash value</th>
<th>Observation (%) w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total ash</td>
<td>13.54</td>
</tr>
<tr>
<td>Water soluble ash</td>
<td>6.18</td>
</tr>
<tr>
<td>Acid insoluble ash</td>
<td>2.49</td>
</tr>
</tbody>
</table>
Extractive values

Extractive values of the successive extracts of *Plectranthus amboinicus* are shown in Table II.

**Table II: Percentage of successive extracts of *Plectranthus amboinicus* young Leaves.**

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Extract values (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petroleum ether</td>
<td>2.36</td>
</tr>
<tr>
<td>80% Ethanol</td>
<td>14.92</td>
</tr>
<tr>
<td>Water</td>
<td>18.07</td>
</tr>
</tbody>
</table>

Fluorescence Analysis

The powdered material was subjected to fluorescence analysis as per the standard procedure and the results are shown in Table III.

**Table III: Fluorescence analysis of *Plectranthus amboinicus* young Leaves.**

<table>
<thead>
<tr>
<th>Plant sample</th>
<th>Day light</th>
<th>UV light (365nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Powder</td>
<td>Pale brown</td>
<td>Dark brown</td>
</tr>
<tr>
<td>Powder + Distilled water</td>
<td>Brown</td>
<td>Brownish black</td>
</tr>
<tr>
<td>Powder + NaOH</td>
<td>Brown</td>
<td>Greenish black</td>
</tr>
<tr>
<td>Powder + H₂SO₄</td>
<td>Dark brown</td>
<td>Greenish black</td>
</tr>
<tr>
<td>Powder + HCl</td>
<td>Greenish yellow</td>
<td>Greenish black</td>
</tr>
<tr>
<td>Powder + HNO₃</td>
<td>Light brown</td>
<td>Green</td>
</tr>
<tr>
<td>Powder + Ammonia</td>
<td>Green</td>
<td>Greenish black</td>
</tr>
<tr>
<td>Powder + CHCl₃</td>
<td>Green</td>
<td>Yellowish green</td>
</tr>
</tbody>
</table>

Phytochemical Screening

Powdered young leaves of *Plectranthus amboinicus* were subjected to various qualitative tests for the identification of phytochemical constituents includes tests for alkaloids (Dragendoff’s test, Mayer’s test, Hager’s test, Wagner’s test), saponins, glycosides (Baljet’s test, Kellar-Killiani test), carbohydrates (Molisch’s test, Fehling’s test), proteins (Biuret test, Xanthoprotein test, Millon’s test), tests for tannins, amino acids, flavonoids, steroids (Liebermann-burchard test), phenols, terpenoids were performed using specific reagents. Preliminary phytochemical screening of *Plectranthus amboinicus* leaves revealed the
presence of bioactive compounds such as alkaloids, aminoacids, tannins, phenols, terpenoids, flavonoids, glycosides, carbohydrates and saponins in different extracts (Table IV).

Table IV: Phytochemical analysis of extracts *Plectranthus amboinicus* young Leaves.

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Petroleum ether</th>
<th>Chloroform</th>
<th>80% Ethanol</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Aminoaacids</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Proteins</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Sterols</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Phenols</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

“+” present, “-” absent

CONCLUSION

Medicinal plants are used by 80% of the world population as the only available medicines especially in developing countries. A wide range of medicinal plant parts extracts is used as raw drugs and they possess varied medicinal properties. The use of different parts of several medicinal parts to cure specific diseases has been in vogue from ancient times. Phytochemicals are non-nutritive plant chemicals that have protective or disease preventive properties. Plant produces these chemicals to protect itself, but recent research demonstrates that many phytochemicals can protect humans against diseases. Young leaves of *Plectranthus amboinicus* contain a number of phytoconstituents, which are the key factors in the medicinal value of this plant. The millenarian use of *Plectranthus amboinicus* in folk medicine suggests that they represent an economic and safe alternative to treat various diseases. As the pharmacologists are looking forward to develop new drugs from natural sources, development of modern drugs from *Plectranthus amboinicus* can be intended for their better monetary and therapeutic utilization.
ACKNOWLEDGEMENTS

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