Research Article

PHYSICO-CHEMICAL ANALYSIS AND PRELIMINARY PHYTOCHEMICAL SCREENING OF OROXYLUM INDICUM ROOT

Renu Chaudhary¹, Sanjeev Kumar¹, Vartika Saini², Aarti Bhatti¹ and Sarvesh Kumar*¹,³

¹Mahaveer College of Pharmacy, Meerut (UP)
²Ramanand Institute of Pharmacy and management Jawalapur Haridwar, Uttarakhnd
³Department of Pharmaceutical Sciences, HNB Garhwal (A Central University), Srinagar Garhwal, Uttarakhnd, India

ABSTRACT

Oroxyllum indicum is a plant that is used as an important ingredient in Indian Ayurvedic medicine for the treatment of many diseases. Proper identification of the drug to optimize its organizational assessment, physical evaluation (loss on drying, ash value, acid insoluble ash, extract ash value, phytochemical screening), chemical evaluation (qualitative refining of metals) to achieve its full therapeutic effects. In this study, we investigate the phytochemical screening of the Oroxyllum indicum plant, which belongs to the family Bignoniaceae, which are extracted by the use of soxhlation in ethanolic, Chloroform, Petroleum ether, methanol and aqueous solutions. Studies the phsyico-chemical analysis results such as extracts drying after drying, total ash value, acid insoluble ash, water soluble ash, and Phytochemical screening was done to find out the chemical constituents. The extract of Oroxyllum indicum showed the presence of alkaloids, flavonoids, phenols, tannins, resins, glycosides, steroids, fixed oils respectively. Result showed that O. indicum root was found complying LOD (126 mg/gm), Total ash (87.5 mg/gm), Acid insoluble ash (37.5mg/gm), water soluble ash (15 mg/gm), swelling index (1.2). Phytochemical screening showed alkaloids, flavonoids etc was present. Ethanolic extractive value 6.6% was found. In this study evaluate the standards for commonly used herbal formulation, which can be utilized in quality control of the formulation.

INTRODUCTION

Oroxyllum indicum has been used as an herbal medicine for years as a food in many Asian countries, including Thailand, with the genus Oroxyllum and the family Bignoniaceae. In the vernacular of many countries, it is called Bigonia indica, Spathodea indica, Calosanthes indica, Hippoxylon indica. Researchers have always been widely used as a crude medicine to make many Ayurvedic medicines in traditional medicine. Oroxyllum indicum has been used with an active ingredient in many Ayurvedic medicines thus the need for standardization has arisen. For safety and efficacy, pharmacognosy has been employed as a tool to properly test raw drugs and detect adulteration / replacement of these plant materials. Despite many modern techniques, QC of raw drugs still rely on pharmacokinetik studies³. According to the WHO, the quality and process of purification of a medicinal plant should be standardized through various parameters such as morphological, micro, physical, chemical, and biological optimization and must be completed before beginning any test⁴. There are many examples in which the wrong raw drugs /containing compounds are selected with other plants due to their similar morphological characteristics therefore; a comprehensive microscopic and phytochemical screening is required for the raw drug to avoid any uncertainty⁵.

Oroxyllum is an herbal medicine contains a broad range of bioactive compounds such as lipids, carbohydrates, phenolics, terpenoids, carotenoids, anthocyanin, flavors and fragrances⁶. Plant investigations have estimated that only a few percent of compounds can be isolated from biological sources, while

*Corresponding author: Sarvesh Kumar
Department of Pharmaceutical Sciences, HNB Garhwal (A Central University), Srinagar Garhwal, Uttarakhnd, India
nearly the best-selling pharmaceuticals are closely related to natural or natural products, standardizing pharmacologically important bioactive compounds are standardized by process. Has tremendous ability to recognize [7]. Preparation of standardization standard for the plant adhering to the World Health Organization Various studies has been carried out for Pharmacognostic evaluation of leaves, flower, stem bark and seeds of Oroxylum indicum. But the roots of the plant are not of valuable medicinal value for the purpose of medicinal importance. Therefore the present study was carried out to evaluate the roots of Oroxylum indicum. Oroxylum indicum is a medicinal plant of the family Bignoniaceae. The Bignoniaceae is a family of flowering plants with twine climbers or tendril climbers found in hilly areas in the form of shrub about 650–750 species in 116–120 genera. It was named after Jean-Paul Bignon by Joseph Pitton de Tournefort in 1694 in the Bignoniaceae family and its genus Bignonia. This plant is mainly grown in North America and Eastern temperate regions of Asia. 13 species (including 2 natural) in 8 genera of these are found mainly in southern Africa, and 35 species in 12 genera are present in China. In mainland states in Australia, 17 species of 10 genera are found and its 15 genera and 40 species have been studied in India too, which are found mainly in western and southern India and some species are also found in the Himalayan hills. In the local language, it is a native tree called a broken bone tree. It is often grown as an ornamental for its strange appearance. Oroxylum indicum has long been used as conventional herbal medicine in China and Japan [8].

Its extracts have been used by the tribal communities of Manipur (India) to treat various ailments in various beans with normal doses such as anal, kuki, mao, marram, tanghakul and zeliangrong [9]. Oroxylum Indicum is one of the ayurvedic herbs called Dashamula herbal products. Traditionally, it is used for the treatment of many disorders such as abdominal pain, arthritis, jaundice, cough, pertussis, pharyngitis and acute and chronic bronchitis. Oroxylum indicum has recently been researched for anticancer activities [10]. The plant stem, bark, leaves, fruits, and seeds are valued for their medicinal properties but major medicinal properties are attributed to the root bark. Most of in-vivo and in-vitro studies have indicated its anti-inflammatory [11], anti-ulcer [12], immunomodulatory [13], anticancer [14], anti-oxidant [15], photocytotoxic [16], anti-arthritic [17], anti-microbial [18], Antihyperlipidemic activity [19], Nephroprotective activity [20], hypoglycemic activity [21] and anti-fungal and anti-bacterial activity [22]. Different parts of the plant are used in Ayurveda and common medicine for the treatment of various diseases like, diarrhea, fever, ulcers jaundice and cancer [23].

**MATERIAL AND METHODS**

**Collection and identification of crude drug**

The Oroxylum indicum root was collected from Rajasthan, India. Herbarium was prepared for certification by collecting plant roots and drying at room temperature. The plant was certified by authentic taxonomy Laboratory; Department of Botany, Chaudhary Charan Singh University, Meerut (UP).

**Physico-chemical analysis** [24-28]

There are three different methods for determining the remaining ash after ignition of medicinal plant material called total ash, acid insoluble ash and water-soluble ash.

**Total Ash value**

Accurately weighed 2 grams of powder, dry was taken in a crucible. The material was spread in a uniform layer in crucible and then the temperature gradually increasing the heat to 500–600°C, indicating the absence of carbon. It was cooled in desiccators for 30 minutes and weighed without delay. A content of Total ash was calculated in milligrams per gm of the air dried materials.

**Acid insoluble Ash**

To the ash obtained in the crucible or described as total ash, 25 mL hydrochloric acid was added and heats the material to boiling. The insoluble material was collected on filter paper and the ash was washed with hot water and ignited and weighed continuously. The weight of the residue was reduced to milligrams by the weight of total ash and the water soluble ash content was calculated in milligrams per air-dry material.

**Water soluble Ash**

25 mL of distilled water and total ash was mixed in a crucible and boiled the content gently for 5 min. The insoluble substance was collected on ash less filter paper and washed with hot water until the filter was neutral and ignited in a crucible for 15 minutes at a temperature not exceeding 450 C. The content of water-soluble ash with reference to dried drug was calculated by weight of residue was subtracted from the weight of total ash.

**Loss on drying**

Place about 2-5 grams of prepared air-dried material, or the quantity specified in the procedure for the plant material concerned, accurately weighed in a previously dried and tarred flat weighing bottle. Dry the sample by one of following techniques, In an oven at 100-105 °C. In desiccators over phosphorus pentoxide R under atmospheric or reduce and at room temperature. Dry until two consecutive weighing do not differ by more than 5 mg unless otherwise specified in the test procedure. Calculated the loss of weight in mg/gm of air dried material.

**Determination of Swelling Index**

The plant material particle size was minimizes to fineness passing from sieve no. 22 and was accurately weighed 4 gm into a 100 ml glass stoppered measuring cylinder. Water (100 ml) was added and shaken thoroughly after every 10 min. for 1 hour. Then the mixture was allowed to stand for 3 hours at room temperature. The mean value of individual reading was determined and calculated related to 4 gm of plant material.

**Determination of Foaming Index**

The content of 1 gram of crude drug was reduced to coarse powder, which was accurately weighed and transferred into a 500 mL conical flask with 100 mL boiling water. It was maintained at a moderate temperature for 30 minutes. The boiling water was cooled and filtered into a 100 ml volumetric flask and added sufficient water. The decoction was collected.
into 10 Stopped test tubes in consecutive portions of 1 ml, 2 ml, 3 ml, etc. up to 10 ml. The tubes were Stopped and shake in along the length motion two shakes per second for 15 seconds, hold for 15 min and the height of foam were measured Foaming index is calculated as per the following formula: - Foaming index = 1000/a

Where The decoction volume in ml used for preparing the dilution in the tube where foaming to a height of 1 cm is observed

The water and extract of plant material was put in ten tubes in ratio as shown in table then test tubes was shacked for few min, foams were formed and measured from the scale.

MATERIAL AND METHODS

Preparation of plant Extract

Air dried root of *Oroxylum indicum* approximately 500 gm was powdered to coarse particle size no.(#) 40 and subjected to continuous hot extraction with 90 % ethanol and Chloroform water in soxhlet extractor for 48 hours and total extracts were filtered and concentrated to dryness under reduced pressure.[29][31]

Preliminary phytochemical analysis[12-16]

Preliminary phytochemical analysis were done for screening of deferent kinds of secondary metabolites. Phytochemical investigation was performed by doing deferent qualitative chemical tests including tests for alkaloids, carbohydrates, glycosides, tannins, saponins, resins and flavonoids in deferent root extracts of *O. indicum*.

Detection of alkaloids

Plant root extracts were freely dissolved in dilute hydrochloric acid and filters. Filtrate was then used to test for the presence of alkaloids

**Mayer’s test:** Mayer reagent gives positive result when filtrates were treat with Mayer’s reagent (Potassium mercuric iodide). The formation of a yellow precipitate in the solution indicates the presence of alkaloids.

**Hager’s test:** Hager’s reagent (saturated picric acid solution) adds in filtrates and treated if a yellow colored precipitate observed this indicates alkaloids are present in extract.

**Carbohydrate detection:** Plant root extracts are mixed with distilled water in separate test tubes, after which the solution is filtered with filter paper. The following tests were carried out to detect the presence of carbohydrates with the help of different reagent in extracts from the filtered solution.

**Benedict’s test:** Benedict’s reagent adds into filtrate and heated on a water bath till formation of an orange red precipitate occurred this indicates the presence of reducing sugars.

**Molisch’s test:** Take filtrates in a test tube and treated with 2 drops of alcoholic α-napthol solution then sulfuric acid was carefully mixed with the sides of the test tube. The development of a violet ring at the junction in the test tube establishes the presence of carbohydrates.

**Flavonoids detection**

**Zinc hydrochloric acid reduction test:** Take alcoholic solution of extract in a test tube, add a piece of Zinc dust and conc. HCl. Magenta color appearance occurred after few minutes which gives the information about flavonoids present in extract.

**Alkaline reagent test:** This test was done by mixing a few drops of sodium hydroxide solution with plant extracts when the solution showed intense yellow formation and became colorless to the dilute acid, indicating that the extract contained flavonoids.

**Detection of proteins and amino acids**

**Xanthoproteic acid test:** extracts and few drops of nitric acid were mixed together. Proteins are present if yellow color is formed in the solution.

**Ninhydrin test:** extracts and 0.25% ninhydrin reagent was added together and boiled the content for few minutes. Reaction is formed and change blue color gives the identification of amino acids.

**Detection of phenols**

**Ferric chloride test:** In this test, root extracts of plants were mixed with a few drops of ferric chloride solution. If blue-black color is formed in the solution, it indicates the presence of phenols.

**Detection of Tannins**

**Gelatin test:** A 1% gelatin solution with sodium chloride content was mixed with plant extracts. Tannins are identified during the formation of white precipitate in solution

**Resins**

One ml test solution was broken down in acetone (1 ml) and the solution was have poured in distilled water (2 ml). Turbidity demonstrated the confirmation of resin.

**Glycosides**

a. In the solution of 2 ml extracts, 3 ml chloroform and 10% ammonia solution was added to the plant for its investigation, the pink color of the solution in the result indicated the presence of glycosides.

b. A few drops of ferric chloride and 2 ml glacial acetic acid were added to the extract 0.5 ml extract. Then sulfuric acid mixed with the edge of the test tube formed a brown ring at the junction in the test tube indicating the presence of cardiac glycoside

**Tests for steroids and terpenoids**

**Salkowski test:** About 100 mg of extract was shaken separately with chloroform (2mL) in which the concentrate H2SO4 (2mL) was carefully mixed with the edges of the test tube; a reddish-brown appearance of the interface gives the presence of terpenoid.

**Liebermann-Burchard test:** Chloroform was mixed with extracts in each test tube after which a few drops of acetic anhydride were added to the test tube and boiled in a water bath and the mixture was rapidly cooled in iced water. The 2mL concentrate H2SO4 was carefully met in the solution from the edges of the test tube. The formation of a brown ring at the junction of two layers and the upper layer turned green
indicates the presence of steroids while the formation of dark red indicates the presence of triterpinoid.

RESULTS

Physico-chemical analysis

Dry powder of root of Oroxylum indicum was used as a sample for physico-chemical analysis. Results of these physicochemical tests Table 1 shows the loss values, total ash value, water soluble, acid insoluble ash, swelling index and O on drying in fresh plant and Foaming index of Oroxylum indicum root dry powder.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Test</th>
<th>Value (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Total ash value</td>
<td>12.5%</td>
</tr>
<tr>
<td>2</td>
<td>Acid insoluble ash value</td>
<td>36.925</td>
</tr>
<tr>
<td>3</td>
<td>Water soluble ash value</td>
<td>15.532</td>
</tr>
<tr>
<td>4</td>
<td>Loss on drying</td>
<td>87.4</td>
</tr>
<tr>
<td>5</td>
<td>Swelling index</td>
<td>1.2</td>
</tr>
<tr>
<td>6</td>
<td>Foaming index</td>
<td>100</td>
</tr>
</tbody>
</table>

Extractive values: Table No. 2 showed that ethanol and aqueous soluble extractive values were calculated in percentage for which O. Indicum root dry powder was used for extractive value.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Extracts</th>
<th>Extractive value (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ethanol extract</td>
<td>6.6%</td>
</tr>
<tr>
<td>2</td>
<td>Aqueous extract</td>
<td>5.4%</td>
</tr>
</tbody>
</table>

Phytochemical Evaluation of O. indicum- Differential physicochemical screening tests was carried out using plant root extracts. O. indicum ethanol and aqueous extracts were used in phytochemical tests for alkaloids, flavonoids, terpenoids, glycosides, carbohydrates, and saponins. Table no. 3 present results for the presence and absence of phytochemicals.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Chemical constituents</th>
<th>Test</th>
<th>Root of extracts Oroxylum indicum</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ethanol</td>
</tr>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>Mayer’s test</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>Carbohydrates</td>
<td>Molisch’s test</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Glycosides</td>
<td>Benedict’s test</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Steroids</td>
<td>Borntrager’s test</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Resins</td>
<td>Legal test</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Phenols</td>
<td>Saikowski’s test</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Tannins</td>
<td>Burchard test</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Flavonoids</td>
<td>Acetone-Water test</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Proteins</td>
<td>Alkaline test</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Zn-HCl acid reduction</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Merion’s test</td>
<td>-</td>
</tr>
</tbody>
</table>

CONCLUSION

Analyzing medicinal plants is a quality control parameter whereby the total ash content detected is revealed with physiologic and non-physiologic ash, which was found more in O. Indicum. The findings of the studies help to examine the quality and purity in plant species and also contribute to future studies related to plant character, effects of environmental changes of plant species. These findings were helpful in herbal formulation studies.

Acknowledgement

The author thankful our deepest core of heart to director Translem Institute Meerut and MIT Institute of Technology for his valuable and laboratories.

References

An evaluation of the activity related to inflammation of Oroxylum indicum


Wang L, Weller CL. Recent advances in extraction of nutraceuticals from plants. Trends Food Sci and Tech. 2006; 17:300-312.


How to cite this article:

******