

## Pharmacognostic and Phytochemical screening of *Oroxylum indicum*

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### Abstract

*Oroxylum indicum* plants and products derived from them provide a valuable source of drugs and medicines which can become accustomed to treat human illnesses as well as improve animal health and productivity, food safety and quality, and environmental preservation. India's Uttarakhand state is renowned for its breathtaking scenery and profusion of flowers. Many types of diseases are treated with thousands of substances that are present in plants. Herbal contraceptives are the best option available, and there is a great deal of desire for a safer yet equally effective substitute. Since the dawn of civilization, people have been using herbal remedies, and numerous plants have been recognized as effective fertility regulators in a variety of folklore and cultural documents. In conventional medicine, numerous healing substances have been found and employed from their natural sources. We extracted the drug using a solvent combination of chloroform and ethyl acetate after Soxhlet defatted it with petroleum ether. We then filtered the substance using Whatman filter paper. A rotatory vacuum concentrated the filtrate, which then evaporated at room temperature and 45 degrees Celsius. Physicochemical analysis included the moisture content, total ash value, acid insoluble ash value, soluble ash value, water soluble extractive value, alcohol soluble extractive value, and petroleum ether soluble extractive value. Phytochemical investigation deals with the recognition as well as unrefined medicines in respect to their phytochemical constituents. Petroleum ether: chloroform (8:2) and chloroform: methanol (8:2) was used as the mobile phases in thin layer chromatography of the chloroform extract, then spray with reagent.

**Keywords**-Environmental, Conventional, Physicochemical, Chromatography, Medicine

### Introduction

Since long ago, people have been utilizing herbs and plants with medicinal properties to treat a variety of illnesses. They've successfully been since the beginning of times because plants and products derived from them provide a valuable source of drugs and medicines which can become accustomed to treat human illnesses as well as improve animal health and productivity, food safety and quality, and environmental preservation [1]. Numerous contemporary pharmaceutical drugs have been extracted from their natural origins. Nature has served as the source of the concept of a novel medicinal agent for thousands of years. The natural origins of numerous therapeutic agents have been used to identify them, as has the application of some of these compounds in conventional medicine [2]. There have been rumors that a number of important drugs presently on the market come from the original roots of plants that Native Americans utilized. Around 60 percent of the world's population still gets their healthcare from traditional medicine and medicinal plant [3-4]. Herbal medicine is

now a crucial component of conventional medical therapy. According to estimates from the World Health Organization, 80% of people globally get part of their basic care from herbal remedies[5]. Anatomic integrity and function are restored as a result of the intricate, dynamic process known as wound healing. It is a well-planned series of overlapping events involving inflammation, proliferation, maturation, remodeling, and the vascular response phase/hemostasis [6, 7]. Nowadays, people use plants as a significant source of natural health products [8]. Many types of diseases are treated with thousands of substances that are present in plants. In conventional medicine, numerous healing substances have been found and employed from their natural sources [9]. Much research has been done on the potential therapeutic benefits of plant preparations and essential oils against the majority of microbiological illnesses[10]. Medicinal herbs have long been used as cures for wide range of illnesses, which include skin conditions, respiratory as well as urinary issues, gastrointestinal symptoms, asthma, hepatic and cardiovascular disease, and skin disorders [11]. There has been a significant increase in the human population, particularly in developing nations, and this increase has put extreme strain on natural, socioeconomic, and cultural resources. Herbal contraceptives are the best option available, and there is a great deal of desire for a safer yet equally effective substitute. Since the dawn of civilization, people have been using herbal remedies, and numerous plants have been recognized as effective fertility regulators in a variety of folklore and cultural documents [12-14].

Bioactive substances, like phenolic chemicals, are found naturally in plants and are very good for human health. Plants only have very small amounts of these chemicals, so we would have to eat a lot of plant matter to get the right amount. This might not be appropriate in real life. So, the best way to get health-develop things from plant matter is to remove beneficial chemicals from it. People have been extracting active pharmaceutical substances, which is thought to be one of the best ways to concentrate them, and adding these useful ingredients to food items since ancient times [15].

Researchers have focused especially on green extraction techniques most of these methods are effective but time-consuming because they require the use of solvents. Earlier studies claim that even though typical extraction includes maceration, percolation, and Soxhlet extraction, to extract the leaves, leaves are utilized in the extraction process. However, while sonication with methanol proved to be a successful extraction technique, the yield percentage was not particularly encouraging. The plant material was crushed into a powder and allowed to air dry. It was then weighed at fifteen grams and subjected to extensive normal temperature maceration with a solvent (80% ethanol v/v, 2 × 150 ml). Furthermore, calculating the % yield required additional time in each of these situations. The goal of this work was to create a practical plan for using the MAE approach to extract pharmacologically active components [16–17]. The majority of Himalayan plants—some of which have medicine but are scarce in their natural habitats—are in danger of going extinct. India's Uttarakhand state is renowned for its breathtaking scenery and profusion of flowers. The state's outstanding floral diversity is gradually disappearing due to man's growing contact with the natural environment. Only 432 plants remain in Uttarakhand due to the harsh environment, and many of them are in danger of going extinct[18].

**Taxonomy**

Kingdom- Plantae, Division- Magnoliophyta, Family- Bignoniaceae, Class-Magnoliopsida

| Plant name        | Plant part       | Medicinal uses   |
|-------------------|------------------|--|
| <i>O. indicum</i> | Root             | Astringent, Asthma, Leucoderma, Dysentery, Vomiting        |
|                   | Bark             | Increase appetite, Tonic, Aphrodisiac, Cooling, Bronchitis |
|                   | Powder of leaves | Digestive tonic  |
|                   | Seeds            | Hypertension, Throat infection                             |
|                   | Fruits           | Anti-helmintic, Stomachic, Acrid, leucoderma               |
|                   | Leaves           | Ulcer, Enlarge spleen, Snake bite                          |

**Material and Methods**

We gathered the plant leaves in the Indian state of Uttarakhand between September and October. B.S.I. India has certified the plant parts.

**Processing of plant material and preparation of extract:**

We first shade-dried the leaves at room temperature and then dried them for two weeks at 40°C in a hot air oven to remove all the moisture. Prior to extraction, we sieved the plant material, crushed it into a coarse powder, and stored it securely in an airtight container. We extracted the drug using a solvent combination of chloroform and ethyl acetate after Soxhlet defatted it with petroleum ether. We then filtered the substance using Whatman filter paper. A rotatory vacuum concentrated the filtrate, which then evaporated at room temperature and 45 degrees Celsius. We freeze-dried the crude extract after completely removing it from the solvent [19-20].



**Figure. 1 Plant leaves and Soxhlet Extraction**

### **Physicochemical analysis**

The measurements of the air-dried powdered pharmaceutical included the following physical and chemical characteristics: The measurements included the moisture content, total ash value, acid insoluble ash value, soluble ash value, water soluble extractive value, alcohol soluble extractive value, and petroleum ether soluble extractive value (Table-2).

### **Phytochemical screening**

According to protocol, preliminary phytochemical analyses were performed on each extract [21-25].

**Detection of alkaloid:** The extracts were separated and filtered after being dissolved in diluted HCL:

**Mayer's Test:** We applied potassium mercuric iodide, also known as Mayer's reagent, to the filtrates. When a yellow-colored precipitate forms, alkaloids are present.

**Detection of carbohydrates:** We dissolved each extract in five milliliters of distilled water before filtering. We used the filtrates to calculate the carbohydrate amount.

**Molisch's Test:** Two drops of an alcoholic naphthol solution was added to the test tube's filtrates. There are violet rings.

**Benedict's test:** The screens were treated with Benedict's solution, and heated up. An orange-red residue shows that reducing sugar is present.

**Fehling's Test:** We boiled the filtrates with Fehling's A and B solutions, neutralized them with an alkali, and broke them down with hydrochloric acid that had been dampened. Red color are present.

**Detection of glycosides:** We tested the glycosides after hydrolyzing extracts with diluted hydrochloric acid.

**Modified Borntrager test:** We treated the extracts with a solution of ferric chloride and then submerged them in boiling water for approximately five minutes. The extract mixture use amount of benzene after it cooled. We

divided the benzene layer and then treated with an ammonia solution. The ammonical layer indicates the rose-pink color shows the presence of anthranol glycosides.

**Leal's Test:** Sodium nitroprusside was used to treat samples of sodium hydroxide and pyridine. Heart glycosides are present because the color changes from pink to blood red.

#### **Detection of spooning**

**Froth Test:** We shook the extracts for 15 minutes in a graduated cylinder after adding of distilled water to thin them out. A one-centimeter layer of foam forms.

**Foam Test:** We mixed 0.5 grams of extract with two milliliters of water. If the foam continues to form after 10 minutes, saponin is present.

#### **Detection of phytosterol**

**Salkowski test-** We filtered the extracts after chloroform treatment, 1-2ml concentrated sulfuric acid to the filtrates, agitated them, and left them to stand. Golden yellows appear.

**Libermann Burchard's test:** We filtered the extracts after chloroform treatment. We added acetic anhydride to the filtrates, heated them, and allowed them to cool. Conc. added sulfuric acid. Brown rings are present.

#### **Detection of phenol**

**Ferric Chloride Test:** We applied three to four drops of a ferric chloride solution to the extracts. A bluish-black color is formed.

#### **Detection of tannins**

**Gelatin Test:** We added a 1% sodium chloride-containing gelatin solution to the extract. White precipitates are formed.

#### **Detection of flavonoids**

**Alkaline Reagent Test:** We added sodium hydroxide solution to the extracts. The addition diluted acid results in a bright yellow color.

**Lead acetate Test:** Lead acetate solution was added to extracts. A yellow precipitate is present.

#### **Detection of proteins and amino acids:**

**Xanthoproteic Test:** We added a small amount of concentrated nitric acid to the extracts. The yellow tint visible.

**Ninhydrin Test:** We added a 0.25% w/v reagent to the extract and heated it for a brief period. Blue colour is present.

## Chromatographic studies

In chloroform chromatography, the R<sub>f</sub> value was calculated using the mobile phase chloroform:pet ether (8:2) and chloroform:methanol (8:2). The figure below (Fig. 3) displays the picture of the TLC plate. We dissolved the sample in a small quantity of hexane for TLC examination. We used a 0.2 mm-thick silica gel 60 as the stationary phase on a TLC plate. We saturated the TLC chamber's mobile phase with Pet. ether: chloroform (8:2) and chloroform: methanol (8:2) for thirty minutes. We turned on the TLC plate and placed a sample on it using a capillary tube. We then allowed the plate to dry for a brief period. Next, we placed the plates into the TLC chamber. We filled the mobile phase to its full capacity and allowed it to penetrate the plate up to 75% of its length. After development, we removed the plate, placed it in an iodine room, and allowed it to dry in the air. We measured the distance that the moving and still stages traveled to determine the R<sub>f</sub> number [12-15].

## Results

**Extraction-** Medication (250g) was dried at room temperature after being defatted with petroleum ether using Soxhlet. The medication was subsequently extracted over a 15-day period utilizing the Soxhlet technique and 1200 milliliters of chloroform as a solvent. Table 1 displays color, yield, and yield % of the dried and concentrated final extracts.

**Table.1 Solvent, Color, Yield and Percentage yield of leaves**

| S.no | Solvent       | Color      | Yield (in grams) | %yield |
|------|---------------|------------|------------------|--------|
| 1    | Pet. Ether    | Dark green | 0.647            | 0.369% |
| 2    | Chloroform    | Dark green | 0.24             | 0.13%  |
| 3    | Ethyl acetate | Green      | 0.391            | 0.22%  |

**Table.2 Physical Parameters**

| Physical parameters                 | %(Air dried drug) |
|-------------------------------------|-------------------|
| Moisture content                    | 9.6               |
| Total Ash                           | 8.8               |
| Watersoluble ash                    | 4.6               |
| Acid Insoluble Ash                  | 0.8               |
| Extractive value of ethanol-soluble | 11.3              |
| Extractive value of Water –soluble  | 21.5              |
| Foaming Index                       | 1.3               |
| Swelling Index                      | 0.7               |

## Phytochemistry

Phytochemical investigation deals with the recognition as well as unrefined medicines in respect to their phytochemical constituents. The plant's chemical composition was evaluated. In Table 3, you can see the data for each group of phytochemicals.

**Table. 3** Examination of Chemical Compounds in Qualitative

| S.no. | Chemical constituent        | Tests                      | Chloroform extract | Ethyl acetate extract |
|-------|-----------------------------|----------------------------|--------------------|-----------------------|
| 1     | Alkaloids                   | 1.Mayers test              | Present            | Present               |
| 2     | Carbohydrates               | 1.Molisch test             | Present            | Present               |
|       |                             | 2.Benedict test            | Present            | Absent                |
|       |                             | 3.Fehlings test            | Present            | Absent                |
| 3     | Glycosides                  | 1.Modified borntreger test | Present            | Absent                |
|       |                             | 2. Legal test              | Absent             | Absent                |
| 4     | Saponins                    | 1. Froth test              | Absent             | Absent                |
|       |                             | 2. Foam test               | Absent             | Absent                |
| 5     | Phenols                     | 1. Ferric chloride test    | Absent             | Absent                |
| 6     | Tannins                     | 1. Gelatin test            | Absent             | Absent                |
|       |                             | 2. Ferric chloride test    | Absent             | Absent                |
| 7     | Flavonoids                  | 1. Alkaline reagent test   | Present            | Present               |
|       |                             | 2.Lead acetate test        | Present            | Present               |
| 8     | An amino acid and a protein | 1.Xanthoprotectic test     | Present            | Present               |
|       |                             | 2. Ninhydrin test          | Present            | Present               |

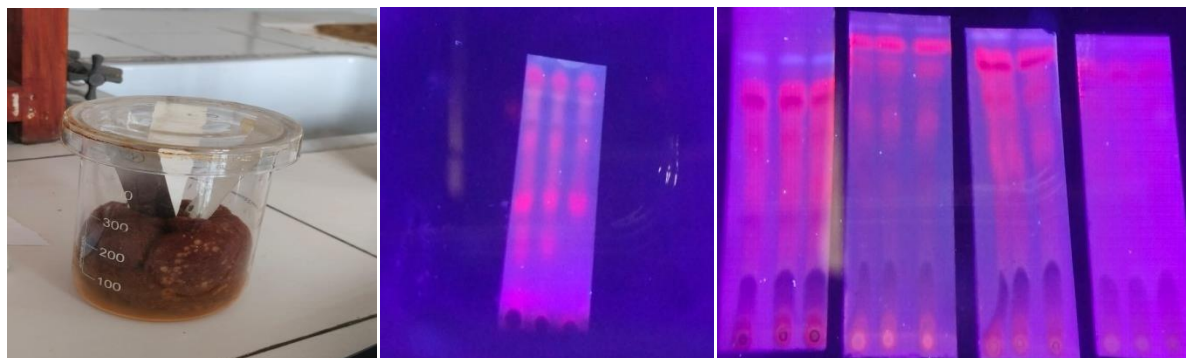
**Figure-2 Phytochemical screening**



**TLC of Chloroform Extract**

Petroleum ether: chloroform (8:2) and chloroform: methanol (8:2) was used as the mobile phases in thin layer chromatography of the chloroform extract, then spray with reagent. Table 4 has the recorded Rf value.

**Figure.3 TLC slide of Chloroform Extract**



**Table4 Thin layer chromatography of Chloroform Extract**

| S. no. | Distance travelled by solvent | Distance travelled by solute | Rf value | No. of spot |
|--------|-------------------------------|------------------------------|----------|-------------|
| 1      | 6                             | 3.8                          | 0.6      | 4           |
| 2      | 7                             | 3.9                          | 0.5      | 2           |

## Discussion

The current study set out to look at the pharmacognostic properties of leaves. The physical inspection of ash values that may be used to identify the plant, like total ash, acid insoluble ash, and water soluble ash, as well as extractive values that are dissolvable in water, alcohol, as well as ether. Extractive values, ash values, and drying loss percentages were calculated with respect to the medication that had been allowed to air dry. The components of leaves were extracted thoroughly and repeatedly using common solvents in increasing order of polarity in order to segregate them according to polarity. The percentage of extractives in various solvents indicates the amount and kind of components present in the extracts. Deciding which components are soluble in which solvent may also be done with the use of extractive values.

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