

**PHYTO-PHARMACOGNOSTICAL AND PHYTOCHEMICAL ANALYSIS ON STEM BARK OF INDIGENOUS *Oroxylum indicum* (L.) VENT.: A FOLK MEDICINAL PLANT****M.P.V. Vikram Singh<sup>1</sup>, Vinay Kumar Prajapati<sup>2</sup> and Ranjana Singh<sup>\*3</sup>**<sup>1</sup>Department of Botany, Shri Jai Narain Mishra P.G. College, Lucknow, UP-India-226001.<sup>2</sup>Department of Botany, M.B.P. Govt. P. G. College, Ashiyana, Lucknow, UP-India-226012.<sup>3</sup>Department of Botany, Government Model Degree College, Arniya, Bulandshahr, UP-India-203131.**\*Corresponding Author: Ranjana Singh**

Department of Botany, Government Model Degree College, Arniya, Bulandshahr, UP-India-203131.

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**ABSTRACT**

Since times immemorial, medicinal plants have been used virtually in all cultures as a source of medicine. *Oroxylum indicum* Linn. (*O. indicum*) commonly known as-broken bones plant is traditionally used as a medicine in Ayurveda and other folk system of medicine. Stem bark of the plant is commonly used as analgesic, antioxidant and treat inflammatory diseases including rheumatoid arthritis and asthma. Presently, people's interest towards plants-based medicines or products has been increased worldwide which leads to the necessity of laboratory investigations of crude drugs or phytoconstituents for better and effective application in various systems of medicines. Present study is aimed to summarise the pharmacognostical profile of *O. indicum* (Linn.). This study comprises of preliminary phytochemical screening, examination of morphological and microscopic characters; determination of quality control parameters such as foreign matter, moisture content, ash values, extractive value and total sugar. The phytochemical screening of stem bark of *O. indicum* showed the presence of some important phytoconstituents like flavonoids, alkaloids and phenols. The information generated by this particular study provides relevant pharmacognostical data needed for proper identification and authentication of *O. indicum* stem bark.

**KEYWORDS:** *Oroxylum indicum* Linn., Sonapatha, Pharmacognosy, Microscopy, Physiochemical, Phytochemical.**INTRODUCTION**

Plant and plant-based products have played a crucial role in the healthcare of human since their evolution and continue to play an important role even in the most modern state of the art therapeutic era. India has a unique position in the world where a number of recognized indigenous systems of medicine viz., Ayurveda, Siddha, Unani, Homeopathy etc., are being employed for wellbeing of people. Medicinal herbs, the source materials for these remedies are readily available and are cost-effective. No doubt that the herbal drugs are popular among rural and urban community of India. Various drugs have entered in the international market through exploration of ethnopharmacology and traditional medicine. Although scientific studies are carried out on a large number of plants but smaller numbers of marketable drugs or phytochemical entities have entered the evidence-based therapeutics. Efforts are therefore needed to establish and validate evidence regarding safety and practice of Ayurvedic medicines.

Screening of medicinal plants on the basis of their

presence at various geographical locations and their presumptive folklore use indicate a huge number of plant species that can be worked upon to yield a wide range of plant derived metabolites of important therapeutic significance. During the past decade, the traditional systems have gained importance in the field of medicine. In many developing countries, a large proportion of the population relies heavily on traditional practitioners who are dependent on medicinal plants to meet the primary health care needs. Presently, both common consumers and healthcare professionals seek updated, authoritative information towards authenticity, safety and efficacy of any recommended medicinal plant as drug prior to its use.<sup>[1]</sup>

Sonapatha or *O. indicum* belonging to the family Bignoniaceae and it is characterised by brown bark and large pinnate leaves. It is medium sized, deciduous tree, distributed in India, Sri Lanka, Malaysia, China, Thailand, Philippines and Indonesia. In India *O. indicum* is found in Eastern and Western Ghats and also in the North East regions.<sup>[2]</sup> Owing to the indiscriminate

collection, over exploitation and uprooting of whole plants bearing roots, this valuable tree has become vulnerable in many states of India. Existence of *O. indicum* in natural population is highly threatened and has been categorized as vulnerable medicinal plants by the Government of India. The tree may become extinct in near future and would thus enter Red Data Book.

Various parts of this plant are utilized to cure many diseases.<sup>[3]</sup> The root bark, stem bark, leaves, flower, fruits and seeds have all been used to treat a great variety of human ailments for thousands of years. It has been used in Ayurveda and other traditional medicinal health systems since centuries.<sup>[4]</sup> It is one of the ingredients in many important Ayurvedic formulation, such as Dasmoolaristam, Dasmoola rasayanam, Amrutaristam, Chyavanaprash and others. The decoction of the bark is used to cure gastric ulcers and the bark paste is useful in treating mouth cancers, Scabies and other skin diseases.<sup>[5]</sup> The bark decoction of *O. indicum* is also a useful remedy to deworm cattle (NIF- India). Apart from this, *Oroxylum* species are reported to have a variety of medicinal properties like anticancer, antiulcer, anti-dysenteric, antimicrobial and anti-inflammatory.<sup>[6]</sup> It has been shown to be antibacterial, antioxidant, hepatoprotective and immunomodulatory.<sup>[7]</sup>

So, the present study has been carried out to provide a detailed account of the phytopharmacognostical analysis on *O. indicum* Linn. stem bark. Hence, the present work deals with the systematics, macro and microscopic characters, powder characteristics, physicochemical parameters, preliminary phytochemical screening etc. The information generated by this particular study provides relevant pharmacognostical data needed for proper identification and authentication of *O. indicum* Linn. stem bark, which could serve as a valuable source of information and provide suitable standards for the further quality assessment and standards of indigenous drug *O. indicum* Linn. stem bark.

**MATERIAL AND METHODS-** In the present investigation detailed phytopharmacognostical analysis of stem bark of *O. indicum* Linn. (Bignoniaceae) were undertaken and following methods were followed for investigation.

**Collection of Genuine Plants Material:** The stem bark of *O. indicum* Linn. were collected from natural habitats around Lucknow, UP, India. The plant was taxonomically authenticated at herbarium, National Botanical Research Institute (CSIR-NBRI), Lucknow, India.

**Processing of Plant Material for Study:** The plant stem bark was properly dried in shade at 40°C and powdered. The fresh material was preserved in FAA solution (formaldehyde: acetic acid: alcohol: water in a ratio of 10:5:50:35) for microscopic studies.

**Studies of Organoleptic Characters:** This study includes surface markings, texture, fracture, internal appearance, cut surface, odor and taste of the crude drug.

**Microscopic Study -** Microscopic study which deals with identification of the various characters of tissues, cells and cell contents was carried out by preparing specimens of crude material using compound microscope. Microscopic studies and specimen preparation may vary depending on the part used (entire material, cut or powdered).

**Disintegration of hard and woody tissues:** To observe microscopic characters hard and woody material was macerated with conc. Nitric acid (HNO<sub>3</sub>) along with a pinch of potassium chlorate (KClO<sub>4</sub>) and heat to boiling of the material. Tissue started disintegration and bleached completely. Allowed the macerated material to settle down and cool. Removed the macerating liquid and washed the material repeatedly with water to remove acid and bottled.

**Studies on powdered material (drug):** Organoleptic characteristics of drug powder were studied by observing various characters like color, odor, fitness, degree of uniformity of particles and sensation of smoothness.

**Physicochemical Parameters for the Standardization of Crude Drugs:** The physicochemical analysis which often plays an important role in herbal drug standardization were performed on stem bark powder of *O. indicum*. Following tests were performed which are simple and quick to perform and give valuable information about the nature and purity of a crude drug. The values given in the results are replicate of six samples. The tests include:

<b>Botanical name</b>	- <i>Oroxylum indicum</i> Linn.
<b>Family Vernacular name</b>	- Bignoniaceae
<b>Place of collection</b>	- Sonapatha - Natural habitats around Lucknow, U.P. India
<b>Voucher No.</b>	- 97372
<b>Part Used</b>	- Stem bark

**(A). Determination of foreign matter:** Drug should be entirely free from visible sign of contamination by moulds or insects and other animal contamination. No abnormal odor, discoloration, slime or sign of deterioration should be detected. It is seldom possible to obtain marketed plant materials that are entirely free from harmful foreign matter or residue. Thus, morphological examination can conveniently be employed for determining the presence of foreign matter in whole or cut plant materials. However, microscopy is indispensable.

**Procedure:** 100-500g of the drug sample to be examined was spread out in a thin layer and detected the foreign matter by inspection with the unaided eye or by

the use of a lens (6xs). Separated the other material, weigh it and calculated the percentage present. The amount of foreign matter shall not be more than the percentage prescribed in the pharmacopoeia (2%).

**B. Determination of moisture content (loss on drying):** Determination of the amount of volatile matter in the drug is measure of loss on drying for substances. **Procedure:** 10 g of drug sample were kept in oven at 100°C for 3h and made it moisture free, weigh till constant weight was attained and calculated the percentage of moisture by the following formula-

$$\text{Moisture percentage} = \frac{P_w - F_w}{W} \times 100$$

Where's,

Fw = Final constant weight of the sample

Pw = Pre weight of sample

W = Total weight of sample

**C. Ash value:** Ash value is determined to estimate the total amount of the inorganic salts present in the drug. This includes total ash and acid insoluble ash.

(a) **Total ash:** Method is designed to measure the total amount of material remaining after ignition. This includes both-physiological ash which is derived from the plant tissue itself, and non-physiological ash which is the residue of the extraneous matter (e.g. sand and soil) adhering to the plant surface.

**Procedure:** To observed total ash content, 2g of ground air-dried material was evenly spread as a thin layer in a previously ignited and tarred crucible. To obtain a white carbon-free residue the crucible was ignited by gradually increasing the temperature not exceeding 450°C. then, residue was allowed to cool in suitable desiccators for 30 minutes and weight. Total ash content of air-dried material was calculated using the following formula-

$$\text{Total ash percentage} = \frac{P_w - F_w}{W} \times 100$$

Where's,

Pw = Pre weight of crucible

Fw = Final weight of crucible

W = Total weight of powdered plant material

(b) **Acid-insoluble ash:** To observe the acid-insoluble ash in air – dried material, total ash obtained from above procedure was boiled in 25 ml of dilute hydrochloric acid in a crucible for 5 minutes. Rinsed the watch glass with 5ml of hot water and add this liquid in the crucible. The insoluble matter was collected on an ash-less filter paper and washed with hot water until the filtrate neutral. Finally, the ash-less filter paper containing the insoluble matter was transferred in the crucible kept on hot plate and ignite to constant weight. Allowed the residue to

cool in suitable desiccators for 30 minutes and weight. The content of acid -insoluble ash in air-dried material was calculated using the following formula-

$$\text{Acid- insoluble ash percentage} = \frac{FW_b - FW_a}{W} \times 100$$

Where's,

FWa = Final weight of crucible with acid insoluble ash

FWb = Final weight of crucible with total ash

W = Total weight of powdered plant material

**D. Determination of extractive values:** It is the quantity of soluble constituents (active or otherwise) extracted with solvents like alcohol, water, hexane and other solvents from a given amount of medicinal plant material. These are used to determine the amount of the matter, which is soluble in the solvents used; it includes alcohol soluble extractive, water soluble extractive, and hexane soluble extractive etc. To determine extractive value, 5g of air-dried drug powder is macerated with 100 ml of solvents like ethyl alcohol and chloroform water (0.1%) for 24 h with frequent shaking. Filtrates were then transferred to flat-bottomed shallow dish to evaporate and dried at 105°C until constant weight and the percentage of alcohol and chloroform-water soluble extractive with reference to the air -dried drug was calculated.<sup>[8]</sup>

(E). **Total sugar:** Total amount of sugar present in the drug can be calculated.<sup>[9]</sup>

**Procedure:** Plant tissue homogenate (10% w/v) is prepared in 80% ethanol. Homogenate was centrifugated at 2000 rpm for 50 minutes. The supernatant obtained is made upto 10 ml and 0.1 ml of aliquot was added in the reaction mixture of 0.1 ml of 80% phenol and 5 ml of conc. H<sub>2</sub>SO<sub>4</sub>. The absorbance of colored reaction mixture was read at 490 nm using spectrophotometer. The percentage of total sugar was calculated according to the absorbance with the help of following formula-  
Total amount of sugar percentage =  $\frac{3.1 \times \text{Absorbance}}{\text{Sample amount}}$

**Phytochemical Screening (Qualitative Analysis):** The preliminary phytochemical studies are used for testing the different chemical groups present in plant extracts. Preliminary quantitative tests for screening primary (carbohydrates, lipids, proteins, etc.) and secondary secondary (alkaloids, glycosides, saponins, flavanoids, terpenoids, tannins etc.) were performed in alcoholic and aqueous extracts of dried -drug powder used earlier in this study for quantitative analysis. 10% (w/v) solution of extract is taken unless otherwise mentioned in the respective individual test.

**A. Alkaloids- Dragendorff's test:** Dissolved few mg of alcoholic or aq. extract of the drug in 5 ml of distilled water. Added 2M hydrochloric acid until an

acidic reaction occur, then added 1 ml of Dragendorff's reagent, an orange or orange - red ppt produced immediately indicate the presence of alkaloid.

**B. Carbohydrates- Anthrone test:** To 2 ml of anthrone solution, added 0.5 ml of aq. extract of the drug. A green or blue color indicates the presence of carbohydrates.

**C. Flavonoids: Schinoda test:** In a test tube containing 0.5 ml of alcoholic extract of the drug, added 5-10 drops of dil. hydrochloric acid followed by a small piece of magnesium. In the presence of flavonoids a pink, reddish pink or brown color is produced.

**D. Saponins:** In a test tube containing about 5 ml of an aqueous of the drug added a drop of sodium bicarbonate solution, shaken the mixture vigorously and left it for 3 minutes. Honeycomb like forth formed indicates saponins.

**E. Steroids: Liebermann-Burchard's test:** Added 2 ml of acetic anhydride solution to 1 ml petroleum ether extract of the drug in chloroform followed by 1 ml of conc. sulphuric acid. A greenish color is developed which turnsto blue.

**F. Tannins:** To 1-2 ml of extract of the drug added a few drops of 5% FeCl<sub>3</sub> solution. A green color indicates the presence of Gallo tannins while brown color indicates tannins.

**G. Glycoside:** Small amount of extract was mixed with 1ml water and was shaken well. Then aq. solution of NaOH was added. Yellow color appeared that indicates the presence of glycosides.

**H. Phenol: Ellagic Acid test-** The test solution was treated with few drops of 50% (w/v) glacial acetic acid and 5% (w/v) NaNO<sub>2</sub> solution. The solution turned muddy or Niger brown precipitate occurred in the extract indicated the presence of phenols solution.<sup>[10]</sup>

## RESULTS

### Systematics

Botanical name – *Oroxylum indicum* Linn.  
Family – Bignoniaceae

### Vernacular names

Hindi English –  
Assamese – Sonapatha, Shyonak,  
Bengal – Broken bones plant  
English – Bhatghila Naorichilana  
Gujrati – Tree of DamoclesTendu  
Kannada – Tigadu Vashrppathiri  
Malayalam – Tetu Archangkawn  
Marathi Oriya – Tatpaling Venga maram  
Punjabi Tamil – Chettu Sonapatha  
TeluguUrdu –

**Part used:** Stem bark

### Identification

Plants with seeds, ovules enclosed within the ovary Two cotyledons, pentamerous flowers, reticulate venation and vascular bundles in aring Ovary superior, stamens in one whorl, carpels 2 Zygomorphic pentamerous flowers, stamens less than 5, bicarpellate superior ovary and fruit a capsule Leaflets ovate, 7.5-15 cm long, 5-6 cm broad; bark smooth (blaze yellowish)	- Angiosperms - Dicotyledenae - Magnoliopsida - Lamiales - Bignoniaceae - <i>Oroxylum indicum</i>
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**Taxonomic Description of the Plant:** A brief taxonomic description of the plant is as follows (fig. 1)

**Habit:** Evergreen and semievergreen forests. medium sized trees.

**Habitat:** *Oroxylum indicum* is native to the Indian

subcontinent, in the Himalayan foothills with a part extending to Bhutan and southern China, in Indo-China and the Malaysia ecozone. It is native to Indian subcontinent and is mainly spread over the Himalayan foothills up to an elevation of 1000-1200 m above mean sea level.



Fig. 1: Plant of *Oroxylum indicum* Linn.



Fig.-2: A trunk of *O. indicum* Linn.



Fig.-3: Outer and inner surface of stem bark of *O. indicum* Linn.

**Bark:** 5-6 mm thick, surface brownish grey, blaze yellowish green.

**Leaves:** Opposite, 1-5 m long, 2-3 pinnate, imparipinnate, pinnae numerous, pinnules 3-5 foliolate, leaflets 7.5-15 x 5-6 cm, ovate, tip acuminate, base asymmetric, margin entire.

**Flowers:** Bisexual, in terminal racemes, 6-7.5 cm long, reddish purple without, pinkish yellow within.

**Fruit:** Capsule, 80-95 mm long, 8-10 cm wide.

**Seeds:** 7.5 x 3.7 cm, rectangular, flat.

**Flowering & Fruiting:** Flowering starts in the cold season, from January to March and fruits are developed in April to July.

**Macroscopic Study of the Stem Bark-** The trunk is around 40 cm in diameter and the stem bark is light brown or greyish-brown color. Mature dried stem bark of *Oroxylum indicum* Linn. showed following morphological standards of the dried crude drugs (fig.2&3) –

Size	-	Varies, 0.5-1 cm in thickness
Shape	-	Curved
Outer surface	-	Rough, buff to blackish in color
Inner surface	-	Longitudinally striated, yellowish to yellowish-green in color
Texture	-	Rough, twisted, wrinkled often crowned with stem
Fracture	-	Coarse
Appearance	-	Uneven fibrous
Odor	-	Odorless
Taste	-	Slightly astringent

**Microscopic Study of the Stem Bark-** The stem of *O. indicum* is soft and spongy with numerous corky lenticels. Cork is composed of 15-20 layers of elongated rectangular cells. Parenchyma and stone cells are present

in phelloderm.

Fibers are arranged in concentric rings and in groups.

#### Studies in Stem Bark Powder

**A. Organoleptic characters:** Following are the organoleptic characters of stem bark crude drug.

Color	-	Pale pinkish-brown
Taste	-	Bitter, astringent
Odor	-	Characteristic leathery odor

**B. Microscopic study-** Stem bark powder examined under microscope shows cork cells in sectional view, thick-walled cork cells in surface view, parenchyma cells thick walled with wide lumen tetragonal to hexagonal group of stone cells.

**Physicochemical Evaluation:** Physicochemical evaluation of the powdered bark of *O. indicum* have been recorded for standardization and to get valuable information about the nature, purity and strength of the drug (table 1).

**Table 1: Physicochemical parameters of stem bark powder of *O. indicum*.**

S. No.	Parameters	Results (% w/w)
1.	Foreign organic matter	0.5 ± 0.002
2.	Moisture content (Loss on drying)	14.20 ± 0.68
3.	Ash values	
	a. Total ash	18.34 ± 0.85
	b. Acid insoluble ash	02.73 ± 1.20
4.	Solvent extractive values	
	a. Water soluble extractive	13.9 ± 0.74
	b. Alcohol soluble extractive	3.87 ± 0.90
5.	Total sugar	0.56 ± 0.008

**Phytochemical Screening:** The preliminary phytochemical screening of stem bark drug recorded for different primary and secondary phytoconstituents present in different extractives are shown in table 2.

**Table 2: Phytochemical screening of stem bark powder of *O. indicum*.**

S. No.	Phytochemicals	Aqueous extract	Alcoholic extract
1.	Carbohydrates	+	+
2.	Saponins	-	-
3.	Glycosides	+	+
4.	Flavonoids	+	+
5.	Alkaloids	+	+
6.	Steroids	-	-
7.	Phenols	+	+
8.	Tannins	-	-

## DISCUSSION

Any medicinal plant requires detailed systematic study prior to its use because the therapeutic efficacy is absolutely dependent on the quality of the plant material used. The original and basic approach towards Pharmacognosy includes study of morphological system, study of the cell structures and organization and study of tissue system, which still holds a key in the identification of the correct species of the plant and also to help us to differentiate between closely related species of the same genus. It is also the first step to standardize a drug, which is the need of the day. A detailed pharmacognostical investigation of the stem bark of the plant the *O. indicum*, was carried out to establish its correct pharmacognostical identity through morphological, microscopic and chemical methods (fig. 1, 2, 3). The trunk of Sonapatha is around 40 cm in diameter and the stem bark is light brown or greyish brown coloured, which is soft and spongy with numerous corky lenticels. The inner bark layers of Sonapatha is a golden yellow coloured and hence, the name Sonapatha (fig.3).

Systematics and morphological assessment of crude drug helps in identification of plant as well as detection of substitution and adulteration.<sup>[11]</sup> The quality of crude drug can be checked only on the basis of morphology.<sup>[12]</sup> Morphological profile of the plant is a qualitative evolution depend on organoleptic and macroscopic characters. The powder and powder microscopy of crude drug allows more information of a drug and it can be used to identify the unorganized drugs by their known historical characters.<sup>[13]</sup> The powder microscopy as

standard for authentication of this valuable powder form of crude drug. Physicochemical parameters can be used as standard to ensure the quality of crude drugs. Foreign matter should not be more than 2% as per pharmacopeia standards. Moisture content of drug should be at minimum level to discourage the growth of bacteria, yeast or fungi during storage. Low moisture content indicates the appropriate standard, quality and stability of plant material and can be considered in future study or application.

The degradation of phytoconstituents of the drug during storage dependent on the presence of water quantity in plant material.<sup>[14]</sup> High water content may support the growth of fungal colonies and lead to interfere with the quality of drug easily.<sup>[15]</sup>

The ash value of plant was determined by two different forms, viz. total ash and acid-insoluble ash (table 1). The ash values of the drug are also a significant parameter for the detection of nature of material, adulteration, impurities, authenticity of drug, quality and purity of the test sample. The total ash value indicates the impurities like carbonate, oxalate and silicate etc. The acid-insoluble ash is used to estimate the amount of silica present, especially sand which is the indication of contamination with earthy material.<sup>[16]</sup> Relatively less amount of these two parameters indicate low inorganic matter and silica were detected in stem bark of *O. indicum*.

The extractive values of stem bark of *O. indicum* are

manifested and the highest extractive value was found in water followed by alcohol (table 1). The above results indicate the presence of higher amount of polar compounds than nonpolar. The amount of extract yield in a solvent system is often an approximate measure of the amount of certain constituents that drug contains. High water-soluble extractive value indicates the presence of acids, sugars and inorganic compounds, and high alcohol soluble extractive value indicates the presence of polar constituents such as steroids, phenolics, flavonoids and glycosides.<sup>[17]</sup> The qualitative phytochemical analysis was done in crude powder is an important parameter of quality control. It reveals the presence of bioactive molecules which were known to possess presence of various types of phytoconstituents in different amounts that help in the selection of specific extract for isolating the active principle.<sup>[18]</sup> So, it is a necessary step in the study of pharmacognostic attribute of the plant that may be used in the protection against chronic diseases, pharmaceutical formulation and further research.<sup>[19]</sup>

Phytochemical studies performed on different plant extracts reveal the presence of bioactive molecules which were known to possess medicinal and physiological activities. Extraction in different solvent gives information about availability of soluble phytoconstituents in particular solvent.<sup>[20]</sup> Generally, water soluble extractive is more as compared to alcohol soluble extractive value suggesting the use of aqueous

#### ABBREVIATION

%	percent	v/v	volume per volume
°C	degree centigrade	w	weight
aq.	aqueous	w/v	weight per volume
conc.	concentrated	nm	nanometer
ml	milliliter	cm	centimeter
ppt	precipitate	mm	millimeter

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