Exploration and selection of elite germplasm of *Oroxylum indicum* (L.) Vent. (Shyonak) in the forest divisions of Punjab, India

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***Oroxylum indicum* is a widely used multipurpose tree with its medicinal importance recognized by Ayurveda for centuries, as almost all parts of this tree possess medicinal value. Extracts of root and stem bark have antimicrobial, antifungal, anti-inflammatory and anti-cancerous properties. The high demand for this medicinal plant has caused a gradual depletion of the species from its natural habitat. The present study has been carried out to assess the distribution and mapping for the selection of elite germplasms on the basis of principal active constituents in different forest divisions of Punjab, India. The analysis led to the selection of three samples having a higher percentage of oroxylin-A, baicalein and chrysin. The three screened-out germplasms are recommended for conservation and multiplication to produce quality planting stock.**

Keywords: Conservation, elite germplasm, forest divisions, medicinal value, *Oroxylum indicum*.

OROXYLUM INDICUM (L.) Vent, commonly known as 'Shyonak', a member of the Bignoniaceae family, is a traditional herbal medicinal tree with immense importance in many Asian countries. It is a small- to medium-sized deciduous tree with soft wood and light-brown bark. The seeds are papery with broad wings arranged in layers inside flat, long, sword-shaped, capsular fruit[1]. It is indigenous to the Indian subcontinent with distribution in Southeast and South Asian countries. In India, it is found in the Himalayan foothills and the Eastern and Western Ghats up to 1200 m altitude[2], mainly in ravines and moist places[3]. Possessing a wide range of uses, traditionally, *O. indicum* has been utilized for centuries to treat different ailments[4]. It is used as fodder: its flowers, unripe fruits and young shoots are edible; non-drying oil is extracted from its seeds that is used in the perfumery industry; stem bark and fruits are used as mordant, with the former also yielding a 'khaki'-coloured dye and its wood is used to make matchboxes[5]. The phytochemical analysis estimated 111 compounds extracted from different parts of the plant, with flavonoids like chrysin, oroxylin and baicalein being the active principal components[6].

O. indicum has a high demand in the pharmaceutical, perfumery and incenses industries. Ellagic acid is present in its root bark; flavone colouring matter, chiefly baicalein, oroxylin-A and chrysin are present in the stem and root bark, while chrysin, oroxylin A, traces of alkaloid, scutellarein-7-rutinoside, tannic acid, galactose and sitosterol are present in the bark[7]. Prunetin and beta-sitosterol are present in the heartwood and baicalein in the leaves. Glucoside tetuin, flavonoids oroxin B, oroxindin and non-drying bright yellow oil, are present in the seeds[8]. *O. indicum* has diverse uses in Ayurvedic formulations[9]. The principal active constituent oroxylin A helps in the inhibition of adipogenesis and induces apoptosis in cells[10], while baicalein and chrysin possess antifungal, antibacterial, antiviral, antioxidant, anti-inflammatory, antiulcer, immunomodulatory and hepatoprotective properties[11]. Presence of ellagic acid in the root bark of the plant enables its use as an astringent in dysentery, diarrhoea, rheumatism and otorrhea[12]. It also possesses immunostimulant and antiarthritic properties[13,14]. Leaves contain baicalein-7-glucuronide and are used as a carminative, stomachic and its decoction is used in treating enlarged spleen, stomach ache and rheumatism[15]. Seeds of *O. indicum* are also extensively used in Chinese medicine for acute or chronic bronchitis, cough, pertussis, pharyngitis and other respiratory ailments[16].

About 80% of the population in developing countries and 60% globally rely on traditional medicine for their primary healthcare needs, deriving them mostly from plants[17]. According to a National Medicinal Plants Board report, the demand for *O. indicum* is 100 metric tonnes/year but the supply is much less, with increasing demand putting tremendous pressure on the plant. This has led to storage in the quality supply of raw material, indiscriminate collection of the plant and uprooting whole root-bearing plants. This overexploitation has led to a severe decline in the natural population of this valuable species. Consequently, it has been characterized as an endangered tree species in southern India[18] and as an International Union for Conservation of Nature (IUCN) vulnerable medicinal plant[19]. To bridge the present gap between

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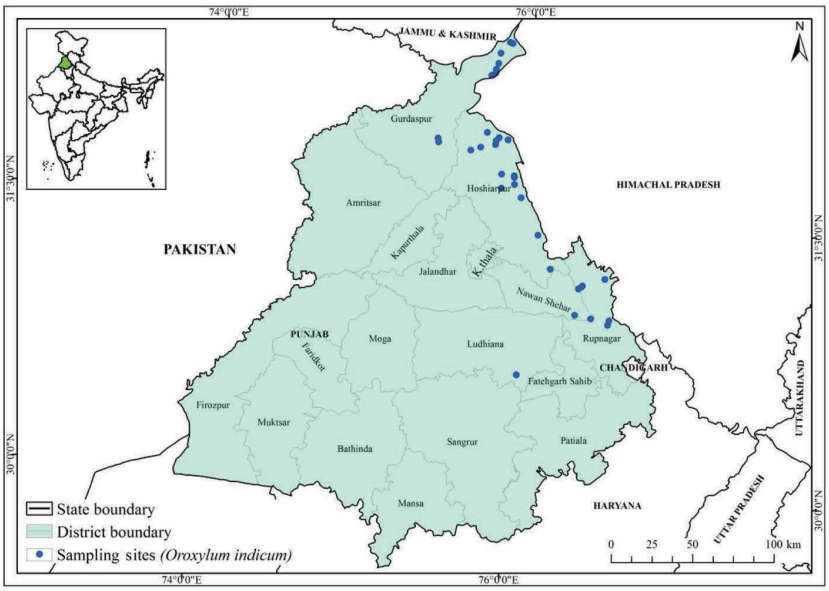

Figure 1. Survey sites in different forest divisions of Punjab, India.

demand and supply, it is important to conserve the germplasm. Hence there is an urgent need for its conservation and multiplication.

Materials and methods

The surveys were conducted in different forest divisions of Punjab, India during April 2016 to March 2018. The state is situated in the northwest of India between 29°33′–32°32′N lat. and 73°53′E–76°56′E long., comprising 1.53% of the geographical area of the country with an area of 50,362 sq. km. Fertile alluvial plains form a major part of Punjab and the belt of the low Shivalik hills runs along its northeastern border with Himachal Pradesh. It has a tropical, semiarid, hot and subtropical monsoon-type climate with cold winters and hot summers. The state receives 480–960 mm average annual rainfall, with average annual temperature ranging from 0°C to 47°C. The forests in Punjab are grouped as subtropical pine, tropical dry deciduous and tropical thorn according to the Champion and Seth classification of forest types (1968). The recorded forest area is 3084 sq. km, of which 1137 sq. km is Protected Forest, 44 sq. km is Reserved Forest and 1903 sq. km is unclassified[20]. Figure 1 shows different forest types and survey location maps.

A total of 32 locations were surveyed in six forest divisions of Punjab to estimate the occurrence of *O. indicum* along with frequency, density and abundance. The date, locality, habitat and brief identification features were also noted. Valuable information on its uses was obtained according to Curtis and McIntosh[21]. During field surveys, the collected plant samples were labelled and parts used by local forest-dwellers were collected. Latitude, longitude and altitude of the location were recorded using GPS. Transects and quadrats were laid randomly in different forest divisions of Punjab depending upon the geographical coverage of the habitats. At every 100 m interval, a 10 m × 10 m plot was laid, resulting in 10 quadrats in 1 km of the transect. The number of surveyed quadrats in each kilometre of the transect per forest division was counted, where every kilometre of the transect corresponds to a surveyed site. Thus, we calculated the total length of the transects covered. The total surveyed area was subsequently calculated, since 1 km of the transect has 10 quadrats of 100 sq. m each.

Preparation of bark samples

The root bark samples of equal diameter were collected from different forest divisions of Punjab during September 2016 to January 2018. The collected root plant materials were brought to the laboratory of the non-wood Forest Products Division, Forest Research Institute, Dehradun. The barks were separated from the root bark samples; the size was further reduced and root barks were shade-dried, ground to a fine powder and stored in airtight containers for chemical analysis.

Chemical analysis of bark samples

Standards of oroxylin-A, chrysin and baicalein (99% HPLC-grade) were procured as marker compounds and used during physico-chemical analysis. All the solvents used for analysis were HPLC-grade. They were utilized for chromatographic separation, whereas trifluroacetic acid (TFA) was used for adjusting the pH of the eluent. A 0.45 µm membrane (Durapore, PVDF Merck) was used to filter all solvents before use. After running different mobile solvents with a reverse-phase C18 column, optimal chromatographic conditions were established. To further

Table 1. Distribution of *Oroxylum indicum* in different forest divisions of Punjab, India

Forest division	Range	Location	Elevation (m)	Presence (+) absence (–)
Dasuya	Talwara–II	31°54′29.1″N, 075°47′48.3″E	461	+
	Badla	31°53′17.8″N, 075°42′04.1″E	276	+
	Dasuya	31°52′06.0″N, 075°38′21.3″E	293	–
	Mukeriyan	31°58′19.1″N, 075°44′17.4″E	300	–
	Talwara–I	31°56′18.6″N, 075°52′31.4″E	373	+
	Talwara–II	31°56′48.7″N, 075°49′02.6″E	330	–
	Talwara–I	31°55′45.2″N, 075°47′57.2″E	376	–
Gurdaspur	Gurdaspur	31°55′16.3″N, 075°25′19.4″E	250	–
	Qadian	31°54′00.2″N, 075°25′36.5″E	250	–
Hoshiarpur	Dolbaha	31°44′55.2″N, 075°50′56.3″E	348	+
	Dolbaha	31°44′04.9″N, 075°56′01.5″E	469	–
	Dolbaha	31°44′37.8″N, 075°55′54.9″E	430	+
	Mangrowal	31°41′45.5″N, 075°56′13.7″E	493	–
	Hariana	31°40′16.8″N, 075°51′14.4″E	331	–
	Hoshiarpur	31°37′32.7″N, 075°59′07.4″E	419	+
Nawansher	Garhshankar	31°14′40.9″N, 076°12′15.4″E	363	–
	Mahilpur	31°25′37.1″N, 076°06′39.5″E	362	–
	Balachaur	31°08′47.0″N, 076°23′36.0″E	449	–
	Balachaur	31°00′01.7″N, 076°22′48.0″E	407	–
	Kathgarh	30°59′11.7″N, 076°29′06.2″E	269	–
Pathankot	Dhar	32°24′41.4″N, 075°47′22.2″E	724	–
	Pathankot	32°17′11.9″N, 075°44′21.5″E	389	+
	Pathankot	32°18′06.7″N, 075°45′56.3″E	410	+
	Dhar	32°19′15.5″N, 075°46′02.6″E	528	+
	Dhar	32°21′14.3″N, 075°46′47.9″E	586	+
	Dhunera	32°28′30.2″N, 075°50′45.5″E	613	–
	Dhunera	32°28′06.3″N, 075°51′47.3″E	626	+
Roopnagar	Rupnagar	30°57′27.0″N, 076°35′42.9″E	293	–
	Rupnagar	30°58′54.2″N, 076°36′08.4″E	324	–
	Anandpursahib	31°12′28.3″N, 076°33′38.2″E	329	+
	Nurpurbedi	31°09′53.0″N, 076°25′05.1″E	345	–
	Nurpurbedi	31°09′31.1″N, 076°24′45.5″E	400	–

improve the peak symmetry and response, acid additives, particularly acetic acid, formic acid and TFA, were used in varying concentrations. Acetonitrile and water (0.1% TFA) as the solvent mixture allowed for more efficient HPLC separation of flavonoids than using methanol and water (0.1% TFA). The above-mentioned chromatographic conditions were thus selected for separating and quantifying marker compounds in *O. indicum* test samples. The HPLC set-up consisting of an analytical (C18) column and PDA detector was used for analysis. For separating marker compounds, the binary mobile phase was used consisting of the solvents acetonitrile and water (0.1% TFA; 34 : 66 v/v). Throughout the analysis, column temperature was maintained at 30°C with a flow rate of 1.0 ml min^{-1}. Spectra from 200 to 400 nm were measured using a photodiode array detector. Analysis was done at 270 nm wavelength with 15–45 min run time.

Different solvents (methanol, hexane and chloroform) were used to optimize extraction conditions along with different techniques (sonication, cold, hot and microwave assisted) for different temperatures and time intervals. Room-temperature cold percolation with methanol for 24 h was identified as optimum and was used for extracting markers from plant matrix due to comparatively higher extraction efficiency. After 24 h, the extracts were filtered using a filter paper (Whatman No. 1) and made up 25 ml in a volumetric flask. Then they were stored at 4°C till further analysis. Before injecting into the HPLC column, the extracts were filtered through a 0.45 µm membrane filter and after filtration 20 µl extracts were injected.

The percentage of active constituents, i.e. baicalein, chrysin and oroxylin-A in the samples was estimated. A total of nine root bark samples (marked 1–9) collected from different Forest divisions of Punjab were analysed. Parameters such as ash content, water-soluble ash, acid-insoluble ash, moisture content and alcohol-/water-soluble extractive values were determined (in percentage) according to the Ayurvedic Pharmacopoeia of India[22].

Results and discussion

Distribution in Punjab

The survey of *O. indicum* plants was carried out from 250–724 m amsl. A total of 32 locations were surveyed, covering six forest divisions of Punjab and occurrence of the species was observed in 12 locations. During the survey, no population of *O. indicum* was reported in two forest

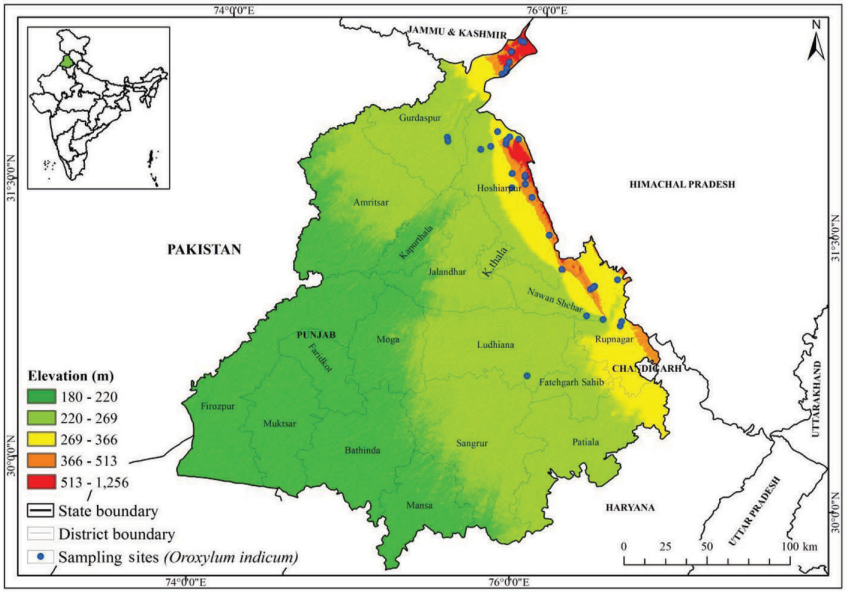

Figure 2. Survey site elevation (m) in different forest divisions of Punjab.

divisions, namely Gurdaspur and Nawansher. Table 1 and Figure 2 provide the details of distribution of *O. indicum* in different forest divisions of Punjab.

A detailed review of *O. indicum* has been done by Preety and Sharma[23], who have presented an overview of the growth, propagation, taxonomy, phytochemical characteristics, medicinal applications and conservation needs. Important information such as local name, botanical description and biological activity of individual parts, ethnomedicinal uses and current status of research with the scope for further studies has been provided by Deka *et al.*[24].

The unsustainable management of this species and uprooting of whole root-bearing plants have led to categorization of *O. indicum* as vulnerable in Andhra Pradesh and Karnataka, and endangered in Maharashtra, Kerala, Madhya Pradesh and Chhattisgarh. There is an apprehension that it may become endangered in other states of India[25]. Mishra[26] reported that besides over-harvesting (unscientific collection of underground parts for medicinal use), the other threats include habitat destruction, over-grazing, deforestation and fire that are causing a gradual depletion of this important plant species from its natural habitat. Yasodha *et al.*[9] have reported that destructive collection practices together with low regeneration and destruction of habitat have posed grave danger to this species.

Quantitative analysis of O. indicum

The maximum average population of *O. indicum* with respect to frequency, density and abundance was observed in the Pathankot Forest Division of Punjab (24% frequency, 0.42 sq. m density and 1.63 abundance), and minimum in Hoshiarpur Forest Division (10% frequency, 0.13 sq. m density and 1.0 abundance; Table 2).

Population status of O. indicum

As indicated in Table 1, out of the six forest divisions of Punjab, the *O. indicum* populations were observed only in four. The Pathankot Forest Division (3.0 km) recorded the maximum average population per kilometer, whereas the minimum average population per kilometre was recorded in Roopnagar Forest Division of Punjab (0.4/km; Figure 3).

Physico-chemical analysis of O. indicum

According to physico-chemical analysis, a total of nine root bark samples were collected from different forest divisions of Punjab. The parameters like ash value, water-soluble ash, acid-insoluble ash, moisture content, alcohol and water-soluble extractive value were determined (in percentage). Three of the six parameters, i.e. moisture content, ash value and water-soluble ash, showed a narrow range of differences in the analysed samples (Table 3). The remaining three parameters, i.e. water-soluble extractive value, alcohol-soluble extractive value and acid-insoluble ash, were found optimum in three samples (numbered 1, 5 and 8), whereas based on water-soluble extractive value, which ranged from 19.74% to 64.00%, five samples (numbered 1, 4, 5, 8 and 9) were found optimum.

Physico-chemical analysis of active constituents of O. indicum: Active constituents of *O. indicum*, namely chrysin, baicalein and oroxylin-A were analysed using the samples collected from different forest divisions of Punjab. Based on the percentage of active constituents, three samples numbered 5, 3 and 2 (in ascending order) were screened out from the six samples which were collected from two forest divisions. The percentage of oroxylin-A ranged from

Table 2. Quantitative analysis of *O. indicum* in different forest divisions of Punjab

Forest divisions	Average no. of individuals per species	Quadrat of occurrences	Frequency (%)	Density (sq. m)	Abundance
Dasuya	2.67	2.0	20	0.27	1.33
Hoshiarpur	1.3	1.0	10	0.13	1.0
Pathankot	4.2	2.4	24	0.42	1.63
Roopnagar	2.0	2.0	20	0.2	1.0

Table 3. Physico-chemical parameters of *O. indicum* in different forest divisions of Punjab

Sample no.	Moisture content (%)	Ash value (%)	Water-soluble ash (%)	Acid-insoluble ash (%)	Alcohol-soluble extractive value (%)	Water-soluble extractive value (%)
1	12.83	11.58	97.23	1.83	8.82	37.60
2	9.22	17.30	94.62	3.10	9.02	22.66
3	9.02	12.23	97.26	1.90	10.22	19.74
4	6.48	12.28	97.53	4.69	7.92	34.40
5	8.68	16.45	94.66	2.53	8.42	64.00
6	12.91	12.64	97.13	2.86	7.02	26.82
7	12.15	14.19	95.75	4.15	8.72	21.32
8	9.60	11.72	96.12	2.63	8.42	41.60
9	10.15	8.80	96.66	4.47	7.96	55.00

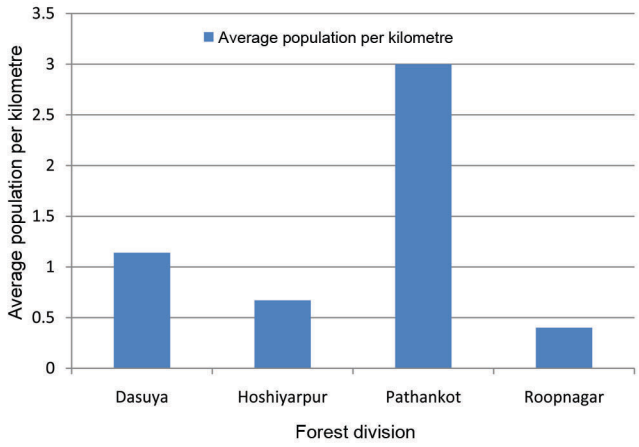

Figure 3. Population status of *Oroxylum indicum* in different forest divisions of Punjab.

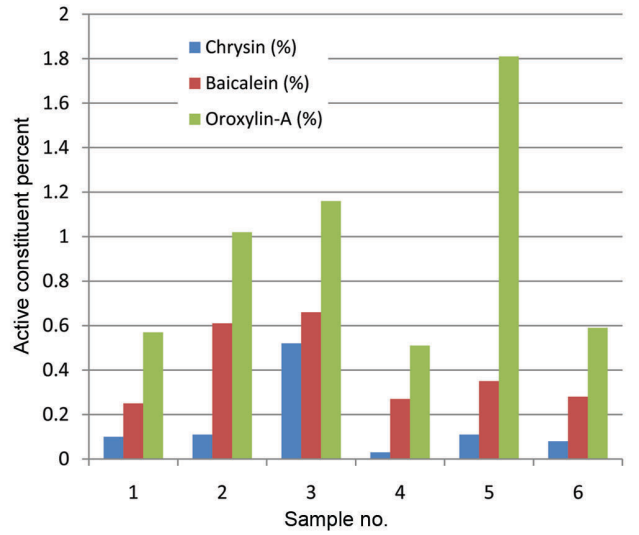

Figure 4. Physico-chemical parameters of *O. indicum* in different forest divisions of Punjab.

Table 4. Physico-chemical parameters of active constituents of *O. indicum* in different forest divisions of Punjab

Sample no.	Chrysin (%)	Baicalein (%)	Oroxylin-A (%)
1	0.10	0.25	0.57
2	0.11	0.61	1.02
3	0.52	0.66	1.16
4	0.03	0.27	0.51
5	0.11	0.35	1.81
6	0.08	0.28	0.59

0.51 to 1.81, whereas that of baicalein and chrysin ranged from 0.25 to 0.66 and 0.03 to 0.52 respectively (Table 4 and Figure 4). Three samples, viz. nos. 5, 3 and 2 were recommended for future conservation and multiplication as they had a higher percentage of principal active constituents.

Physico-chemical studies and HPTLC fingerprinting of *O. indicum* were done by Saraf *et al.*[27], and used as diagnostic methods for identification, standardization and quality control. Anatomical studies were carried out along with phytochemical screening. The root bark was evaluated for pharmacognostical parameters. Phytochemical screening indicated that the root bark of *O. indicum* was rich in flavonoids[28]. Similar studies were conducted by Tamta *et al.*[29,30] on the selection of superior quality germplasms of *O. indicum* covering forest divisions of Uttarakhand and Uttar Pradesh. On the basis of physico-chemical traits, the screened-out germplasms were recommended for the development of a germplasm repository.

Conclusion

O. indicum is a valuable medicinal plant both in national and international markets. Results of the present study may lead to the selection of the best clone or germplasm

on the basis of the principal active constituent for commercial production. As a result of this survey, the various Forest Departments now have information regarding the distribution and population size of this species. Therefore, more emphasis should be given for enriching the population in areas with fewer occurrences. This will also encourage further initiatives towards quantitative assessment in other areas to enhance our understanding of the need for the conservation and cultivation of this species. The superior germplasm screened out from the present study can be used to develop a germplasm repository of superior clones of *O. indicum*. This will not only augment the number of planting materials, but will also enhance their availability for the concerned stakeholders.

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