Phytochemical analysis and ethnomedicinal uses of *Oroxylum indicum* in Nepal

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Research

Abstract

Background: Plant natural products have a long history of usage as a source of therapeutic agents. *Oroxylum indicum* (L.) Kurz is a prominent therapeutic plant in South Asia, comprising several phytochemicals with substantial medical significance, including the treatment of COVID-19.

Methods: Herein, we documented the medicinal importance of *O. indicum* throughout Nepal using a questionnaire-based survey and and validated the findings through biochemical analyses.

Results: All fractions (water, hexane and dichloromethane) obtained from different extraction solvents revealed a high abundance of alkaloids, flavonoids, phenolic compounds, saponins, and carbohydrates that is consistent with its vast ethnomedicinal uses generated through the questionnaire survey.

Conclusions: In a nutshell, *O. indicum* is a promising medicinal plant based on our current experiment, and more research on ethnomedicinal and plant biochemical capabilities might lead to new scientific avenues and novel drug discoveries. It further paves the scope of documentation of traditional knowledge for the benefit of local and national communities.

Keywords: antioxidant activity, COVID-19, drug discovery, *Oroxylum indicum*.

Background

Several natural pharmacological agents have been extracted and utilized from plants to cure different ailments (Subramani *et al.* 2017). This progress, practices, and prospect are solely based on the evidence provided by the indigenous communities for several generations (Malla *et al.* 2015, Rokaya *et al.* 2010, Singh *et al.* 2012). However, most often the traditional knowledge acquired from the rural and remote indigenous communities over the ages is gradually being lost because newer generations are reluctant in utilizing plants in the same way (Bhattarai *et al.* 2010, Joshi *et al.* 2020, Kunwar & Bussmann 2008, Uprety *et al.* 2010) and the measures for the preservation of such knowledge are under-emphasized in developing countries (Kutal *et al.* 2021).

Medicinal plants have been utilized as a foundation for traditional remedies since the dawn of humanity. They are generally ascribed because of their medicinal secondary metabolites. Among secondary metabolites, phenolic compounds possess multiple biological activities (Zhang *et al.* 2022), including cardiovascular diseases, some types of cancer, diabetes, cognitive dysfunction, and other aging-related disorders (Konczak & Zhang 2004, Scalbert *et al.* 2005).

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Plant parts such as leaves, roots, bark, and seeds are known to possess bioactive secondary metabolites, resulting in several activities such as antipyretic, laxative, analgesic, anti-fungal, antibacterial, etc (Dike *et al.* 2012, Samatha *et al.* 2012). Among different plants, woody plants, in general, offer a wide range of local therapeutic uses. In addition, woody trees are ecological drivers and govern a vital role in an ecosystem and provide several benefits to humans all over the world, including fuelwood, shade, agriculture instruments, furniture, house-making, ornament, war instruments, fodder, forage, pharmaceuticals, and fruits as nutrition (Mehwish *et al.* 2019). Furthermore, trees tend to have stronger antibacterial activity and a higher amount of quantitative and qualitative defensive chemicals than other lower plants (War *et al.* 2012).

Oroxylum indicum (L.) Kurz is a medium-sized tree that flourishes well in Asian tropical and subtropical low-altitude open forests (Dey *et al.* 1978). Because of its capability to cure various diseases, it is considered a vital herbal tree in many Asian countries (Biswas & Ghosh 1994). Despite the long history of plant usage in the Himalayan regions of Nepal, limited documentation of this plant has resulted in its constrained usage. We performed this study with two major aims (i) to assess how indigenous populations in different parts of Nepal employ *O. indicum* in their livelihood and primary health services (for instance, for the treatment of COVID-19), and (ii) to examine the phytochemical and biological aspects of the bark of *Oroxylum* since bark of this plant is frequently used in folklore medicine in Nepal.

Materials and Methods

Study area and sampling period:

Field observation and questionnaire surveys were carried out between 2019 and 2021 throughout Nepal. A questionnaire survey was conducted in all 77 districts of Nepal for this study. In each district, 5 people belonging to lower ethnic groups were selected as the key informants. Local healers were consulted for the pharmacological activities of the plant when suitable, which helped us to validate the results of the questionnaire survey. Thus, altogether 385 (5 from each district) indigenous people and local healers (if available) all of them being Dalits of the age range between 28 and 70 years were interviewed using a questionnaire survey.

Among the participants, the majority were illiterate and belonged to lower ethnic groups, with only a handful (2.72%) being teachers and small business owners (2.12%). The remaining significant respondents (88.48%) stated that they are confined to farming. The collected data was cross-checked with different plant users (local healers, 6.66%) in the study area.

Sampling, phytochemicals extraction, and screening:

For biochemical analysis, two field visits were made in Arghakhanchi district (27°56′05.0″ N, 83°06′18.7″ E), Nepal during the flowering (July - August) and fruiting (December - March) seasons to check the ground reality and to harvest the samples. Harvesting of the flowers, fruits, and stems was performed, and their herbarium was prepared following the protocol of Martin (1995). The samples were identified by comparing them with the herbarium specimens housed in the Central Department of Botany, Tribhuvan University (TUCH), Nepal. A copy of the herbarium sample has been housed in the Institute of Biological Resources (IBr) under an accession number UKIB_46.

The stem bark of *O. indicum* for phytochemical analysis was collected from Arghakhanchi district and was air-dried for 2 weeks. The dried pieces were ground to powder and 100 g of it was processed for extraction using methanol. Hundred grams of the pulverized material was extracted with one liter of 80% methanol by soaking it for 3 days, followed by agitation and filtering. The filtrate was dried with a Rotavapor (Rotavapor® R-100, Germany) at 40°C before being resuspended in 400 ml of water (to get the working solution). The extract was fractionated into hexane, dichloromethane (DCM), and water phases through liquid-liquid extraction. After extracting with an appropriate solvent system, each extract phase was loaded to a thin layer chromatogram (TLC) after being treated with an appropriate solvent system. In precoated aluminum TLC sheets, hexane and water fractions were chromatographed with 10% and 30% methanol in DCM as mobile phases, respectively, while the DCM fraction was chromatographed with a 1:1 ratio of EtoAc (Ethyl acetate) and hexane as mobile phase. The spot bands were visualized under UV light (CAMAG) at 254 nm.

Qualitative phytochemical profiling

Experimental reagents of analytical grade were purchased from Sigma Aldrich, Germany. The phytochemical screening for alkaloids, carbohydrates, saponins, tannins, phenols, flavonoids, proteins, fatty acids, steroids, and glycosides was performed by conventional techniques as described in Maharjan *et al.* (2011). In brief, procedures as described below have been followed for the individual tests:

Test for alkaloids

Alkaloid test was performed by two major qualitative test methods, Mayer's test and Dragendroff's test. For Mayer´s test method, 1 mg extract was treated with 0.5 ml of Mayer´s reagent. The formation of a yellow precipitation indicated the presence of alkaloids. For Dragendroff's test method, 1 mg extract was treated with 0.5 ml of Dragendroff's reagent. The formation of red precipitation indicated the presence of alkaloids.

Test for carbohydrates

Molisc's test (α-naphthol addition), Benedict's test, and Fehling's test were used to acquire the carbohydrate test. One mg of extract was treated with 0.4 ml of alcoholic-naphthol solution, 0.5 ml of Benedict's reagent, and 0.5 ml of 0.5M HCl for each test. The presence of carbohydrates was shown by the development of a violet ring at the junction. In Benedict's test, the addition of benedict reagent to the extract resulted in an orange red precipitate in the presence of reducing sugars. The extract was hydrolyzed with 0.5 ml of 0.5 M HCl for Fehling's test, and the solution was neutralized with 0.1M NaOH till pH 7. The neutral solution was heated with 0.5 ml Fehling's A and 0.5 ml Fehling's B solutions, which formed red precipitate in the presence of reducing sugars.

Test for saponins

A foam test was used to detect saponins, in which 1 mg of extract was combined with 1 ml of water. The presence of saponins was established by the formation of foam and its persistence for at least 10 minutes.

Test for tannins

For tannins test, 1 mg of extract was mixed with 0.5 ml of 1% lead acetate. The formation of a yellowish precipitate indicated the presence of tannins.

Test for phenols

Phenol test was performed by qualitative test method called ferric chloride test. For ferric chloride test, 1 mg extracts were treated with 0.4 ml of ferric chloride solution. The formation of bluish black color indicated the presence of phenols.

Test for flavonoids

Flavonoid test was acquired using one of the qualitative methods called lead acetate test. For lead acetate test 1mg extract was treated with 0.4 ml of 10% lead acetate solution. The formation of a yellow precipitate indicated the presence of flavonoids.

Test for proteins

Protein test was performed using xanthoproteic test. For xanthoproteic test, 1 mg extract was treated with 0.4 ml of concentrated nitric acid, which upon the formation of yellow color indicated the presence of proteins.

Test for fatty acids

For fatty acid test, 1 mg extract was mixed with 0.5 ml of diethylether. These ether solution was poured on a filter paper and dried. The appearance of transparency on filter paper indicated the presence of fatty acids

Test for steroids

Steroid test was performed using the Salkowski test. For Salkowski test, 1 mg of extract was mixed with 2 ml of chloroform and 2 ml of concentrated H_2SO_4, followed by vigorous shaking. The turning of chloroform layer to a red color and acid layer to a greenish yellow fluorescence indicated the presence of steroids.

Test for glycosides

Glycoside test was performed with bromine water test, for which 1 mg extract was treated with bromine water test solution. The formation of a yellow precipitate indicated the presence of glycosides.

Antioxidant assay

Antioxidant properties of each fraction were measured by 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay following protocol as devised by Blois (1958) with minor modifications. Briefly, 0.1 mM DPPH solution was prepared by dissolving 1.7 mg of DPPH in 45 ml methanol. Each extract fraction includes 250 µl of 0.1 mM DPPH in each and was serially diluted in methanol to obtain working concentrations (µg/ml): 32, 16, 8, 4, 2, 1, 0.5, and 0.25. During the dilution, 250 µl 0.1 mM DPPH solution was added to each diluted 750 µl sample solution. The final 1 ml diluted solutions of each fraction were left to stand for 30 min and their absorbance was measured using a spectrophotometer (λ_{max}) at 517 nm.

IC_{50} values were determined by calculating percentage of radical scavenging of each fraction. The percentage of radical scavenging is calculated using the following formula:

$$\text{Radical scavenging activity (\%)} = \frac{\text{Control}_{\text{Abs}} - \text{Solution}_{\text{Abs}}}{\text{Control}_{\text{Abs}}} \times 100$$

Antimicrobial assay

Antimicrobial tests of each fraction were performed using two human pathogenic bacterial strains, *Staphylococcus aureus* ATCC 25923 and *Klebsiella pneumoniae* ATCC 25955. The bacterial specimens were obtained from the Central Department of Microbiology, Tribhuvan University, Kirtipur, Nepal. Each bacterial strain was grown in an agar plate and different concentration of each sample fraction was analyzed to observe the Minimum Inhibition Concentration (MIC). The concentration of the sample fractions was measured in micrograms (µg) and ceftriaxone disc (10 µg) was used as a positive control.

Result and Discussion

Plant profile and uses

Oroxylum indicum (L.) Kurz (Trumpet tree in English, Tatelo in Nepali) belongs to Bignoniaceae and is found mostly in temperate to tropical climates. The vernacular name for this species includes Tatelo, Sanna, Shyonaka, Saunetata, Faltate, Sonapatha, Syonaka, midnight horror, and Indian trumpet tree (Ahad *et al.* 2012, Harminder *et al.* 2011, Luitel *et al.* 1970).

For ages, the fruit, seed, stem barks, and root of this plant have been exploited by people for curing different ailments. It is used for wood, tannins, and dyes. This plant has also been used in traditional medicine and is known to have anti-inflammatory, diuretic, anti-arthritic, antibacterial, anti-tussive, and anti-fungal qualities (Samatha *et al.* 2012). The bark comprises medicinal components for Chinese medicine and Sri Lankan medicines that have been used to relieve joint discomfort (Kumar *et al.* 2010, Veeresham *et al.* 2013). Various ethnic groups in the region utilize different parts of the plant for different therapeutic reasons and as a nutritional supplement. Moreover, the bark may be used to heal stomach ulcers, and a paste made from the bark powder can be used to treat oral cancer, scabies, and other ailments (Mao 2002). A decoction of fresh bark offered to animals for deworming has also been demonstrated to be useful when applied to wounds for killing maggots. The plant leaves contain therapeutic agents such as Chrysin (5, 7-dihydroxyflavone) and baicalein (5, 6, 7-trihydroxyflavone) (Rojsanga *et al.* 2020), and have antioxidant, antianaphylaxis and anticancer, anti-diabetes, anti-anxiolytic effect and anti-inflammation properties. The plant's seed and root include tetuin and 6-glucosides of baicalein, as well as oroxindin (Harminder *et al.* 2011, Jagetia 2021, Lodh and Swamy 2020, Nair *et al.* 1979). The plant's stem bark extract contains alkaloids, flavonoids, phenolic chemicals, saponins, and carbohydrates (Chetry and Bharali 2018, Pathak *et al.* 2020, Samatha *et al.* 2012).

Ethnomedicinal usage in Nepal

Our analysis led us to the finding that people living in different geographical regions, viz., Arghakachi, Baitadi, Bajhang, Dadeldhura, Darchula, Dhading, Gulmi, Kaski, Ilam, Makawanpur, and Terhathum use *O. indicum* as a source of medicinal purposes in their daily life, including for the cure of COVID-19 in the latter days. Among these districts, people from Baitadi, Darchula, and Ilam use the plant for several purposes apart from therapeutic implications (Fig. 1). However, people inhabiting other areas of Nepal were unaware of the plant's medicinal value. Thus, we were not able to record any information on the plant's usage from other districts of Nepal.

Indigenous people from these areas mostly use plant bark, seeds, fruits, and roots, which respondents indicated as being used for a variety of purposes. People, on the other hand, use stem bark for medicinal purposes. Plant components have traditionally been used to treat diarrhea, urinary troubles, indigestion, cuts and wounds, ulcers, rheumatism, typhoid, jaundice, and other ailments. Thus, based on the plant usage (as respondents mentioned), we performed a phytochemical analysis for the presence of different chemicals present on the stem bark.

Plant distribution and use

The current questionnaire survey reveals the geographical distribution of the plant *O. indicum* as well as its pharmacological implications. Our investigation found that the plant has a limited range compared to the results in the published literature. However, we discovered the plant in the Kaski district, which prior observations had missed. This gap in the plant distribution data might be explained by local ethnic populations being unaware of the plant or not using it for medical purposes. The plant was found across the country, from low altitude to high altitude hilly regions typified by moderate temperatures (Fig. 2, Table 1).

Distribution of knowledge

Based on the questionnaire survey of plant medicinal usage, *O. indicum* is one of the prominent traditional medicines in Nepal. The plant has been widely utilized as an herbal remedy in Nepal's far east and far west, although it is underutilized in the mid-region. For the chemical profiling of this plant in pursuit of potent bioactivities, we extracted the stem bark of the plant sorted and analyzed its phytochemical and biological properties against different pathogens.

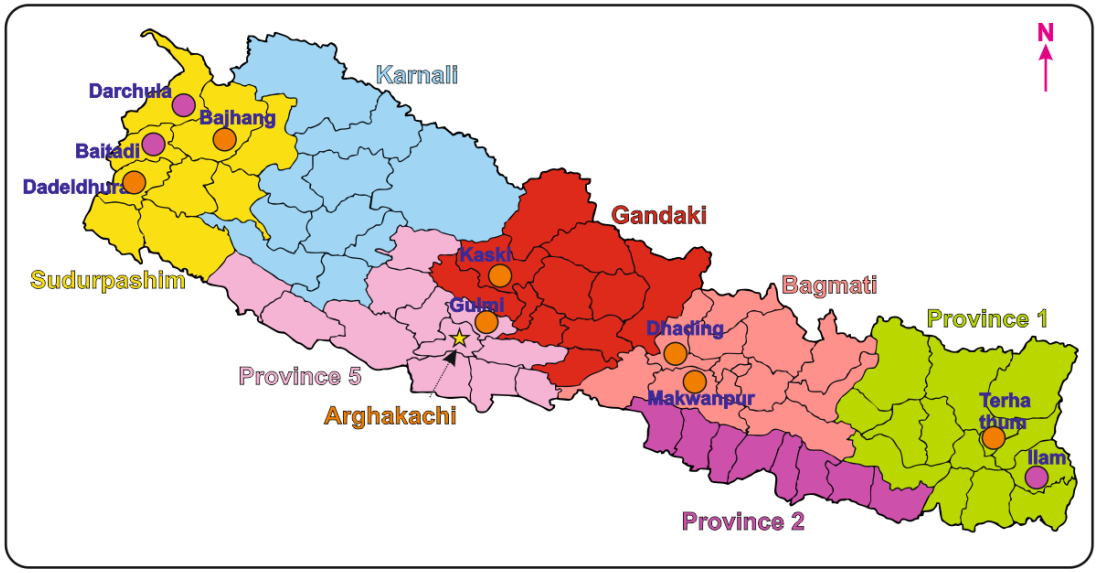

Figure 1. Political map of Nepal showing seven different provinces. Based on our questionnaire survey, the bubble in the graphic indicates the area where *O. indicum* was utilized for medical reasons. The violet-colored bubble suggests that *O. indicum* is widely used, whereas the orange-colored bubble shows that it is less widely used. An asterisk denotes the location of the specimen's collection and study. All other locations that are not highlighted with either a bubble or an asterisk are determined to be devoid of plant medicinal use.

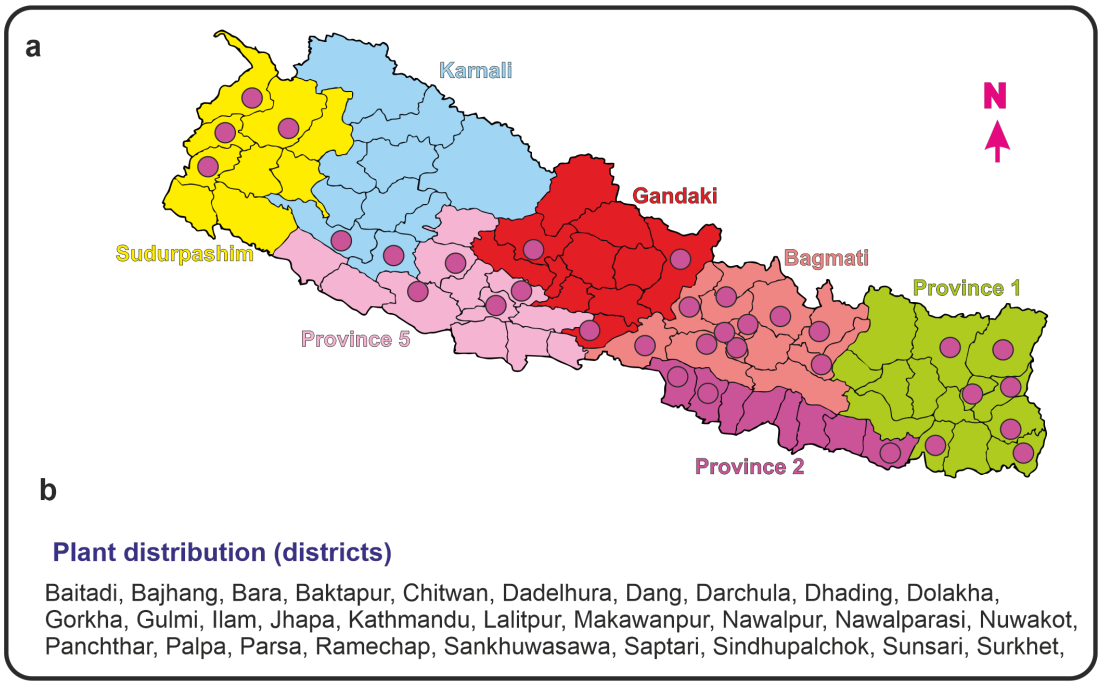

Figure 2. Political map of Nepal revealing the sites of distribution of *O. indicum* according to the available literatures. a) Small violet-colored bubbles show the districts for the presence of *O. indicum*. b) Name of the districts where *O. indicum* are found.

Table 1. Distribution of *O. indicum* across Nepal and their therapeutic uses

Plant distribution	Use and references
Baitadi, Bajhang, Bara, Baktapur, Chitwan, Dadelhura, Dang, Darchula, Dhading, Dolakha, Gorkha, Gulmi, Ilam, Jhapa, Kathmandu, Lalitpur, Makawanpur, Nawalpur, Nawalparasi, Nuwakot, Panchthar, Palpa, Parsa, Ramechap, Sankhuwasawa, Saptari, Sindhupalchok, Sunsari, Surkhet, Tehrathum	Appetizer, digestive, dyspepsia, colic, diarrhea, dysentery, indigestion, stomachic and (Burlakoti *et al.* 2008, Kilham & Manandhar 2002, Pradhan *et al.* 2020, Singh 2017), seed powder chest pain, pneumonia, measles (Bhattarai 1991), sore throat, body pain, asthma (Parajuli 2000, Rai 2004, Turin 2003), dropsy, sprains, urinary problems (Parajuli 2000), bark, roasted seeds for cuts and wounds (Manandhar 2002, 1993, Rai & Singh, Shrestha 1998), ulcer, rheumatism (Rai 2003, Tamang & Singh 2015), typhoid (Acharya and Acharya 2009), hepatitis, jaundice (Ranjitkar & Rajbhandary 2008), carminative (Malla 1994), religious purposes, control menstrual and blood stool (Mandar & Chaudhary 1993).

Bioactive compounds and phytochemical analyses

The hexane fraction, DCM fraction, and the water fraction obtained after extraction of 100 g SBOI were found to be 2.3694 g, 1.0023 g, and 4.8488 g respectively (Fig 3a). All fractions were studied separately.

Thin Layer Chromatography

The TLC sheets of all three fractions of the extract revealed the hexane fraction to have four distinct bands, which implies the presence of at least four different UV active chemical constituents (Fig. 3i), while the water fraction has a smear of bands in both polar and non-polar mobile phases (Fig. 3ii, iii). The smear obtained on the water phase might be due to the higher content of sugary components. The DCM fraction possesses three distinct bands referring to the abundance of at least three different UV active chemical components (Fig. 3iv).

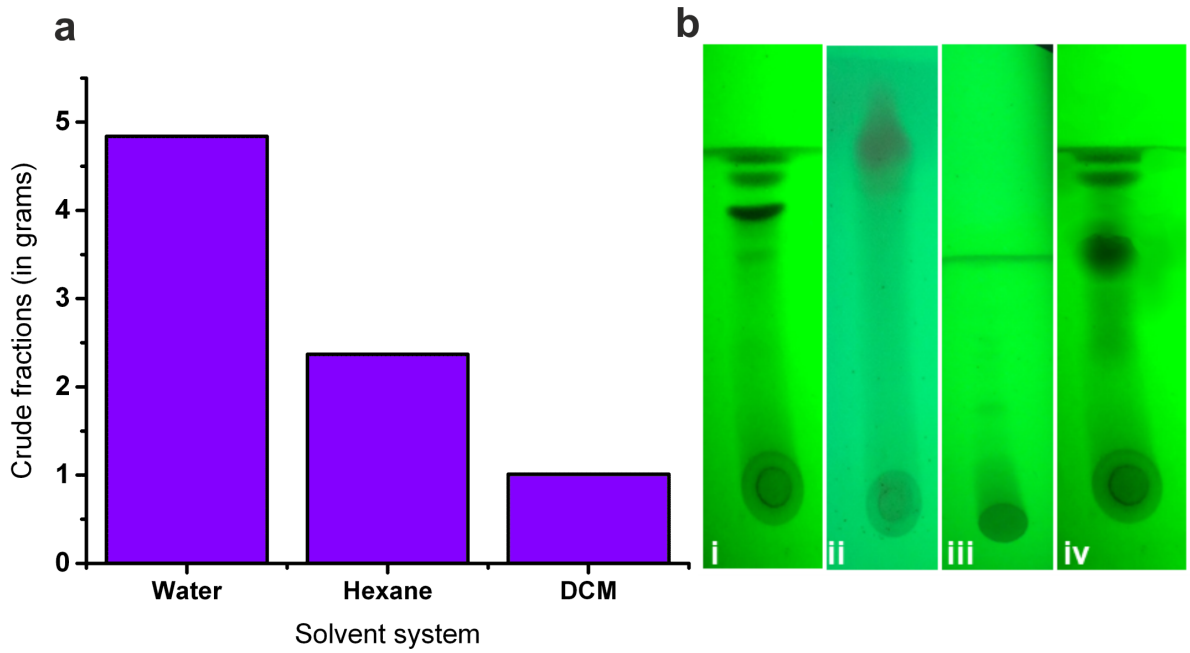

Figure 3. Extraction of crude extract and their analysis using TLC. a) Extraction of phytochemicals from stem bark by using different solvents, viz., water, hexane, and dichloromethane (DCM). b) Thin-layer chromatography of *O. indicum* extract.

Qualitative phytochemical profiling

Phytochemical screening revealed various chemical components present in different fractions of *O. indicum* bark extract. Proteins, reducing sugar, and glycosides were found to be absent in the bark extract. All fractions of the extract were found to have significant levels of alkaloids, flavonoids, phenols, saponins, and carbohydrates (Table 2).

Table 2. Phytochemical screening of the hexane, DCM, and water fractions of extract from the bark of *O. indicum* L.

Phytochemicals	Hexane phase	DCM phase	Water phase
Alkaloids	++	+++	-
Flavonoids	+	+	+
Proteins	-	-	-
Phenols	+	+	+
Saponins	+	+	+
Tannins	-	-	+
Fatty acids	+	-	-
Reducing sugar	-	-	-
Steroids/Terpenoids	+	-	-
Carbohydrates	++	+	+++
Glycosides	-	-	-

Note: '+', '++ 'and '+++' signs represent a mild, moderate, and high abundance of the compound respectively while the '- 'sign represents the absence of the compound.

Antioxidant properties

In the DPPH experiment, all three fractions revealed antioxidant characteristics, with the water fraction having more antioxidant activity than DCM and the hexane fraction having the least antioxidant qualities (Table 3).

Table 3. Absorbance and % radical scavenging capacity of different fractions

Fractions conc. (μg/ml)	Hexane fraction (IC_{50} = 2.08 μg/ml)		DCM fraction (IC_{50} = 1.15 μg/ml)		Aqueous fraction (IC_{50} = 1.00 μg/ml)	
	Abs	% RSC	Abs	% RSC	Abs	% RSC
32	0.008	87.8	0.004	93.9	0.004	93.9
16	0.014	78.8	0.008	87.8	0.008	87.9
8	0.018	72.7	0.012	81.8	0.010	84.8
4	0.024	63.6	0.014	78.8	0.012	81.8
2	0.030	54.5	0.018	72.7	0.016	75.8
1	0.044	33.3	0.035	46.9	0.033	50.0
0.5	0.060	9.0	0.053	19.6	0.048	27.3
0.25	0.064	3.0	0.059	10.6	0.054	18.2
Control	0.066	0.0	0.066	0.0	0.066	0.0

Abs: Absorbance, % RSC: Percentage radical scavenging activity

Antimicrobial properties

All three fractions of bark extract were evaluated on two human pathogenic bacterial strains, Gram-negative *Klebsiella pneumoniae* ATCC 25955 and Gram-positive *Staphylococcus aureus* ATCC 25923. Ceftriaxone disk (10 g) was used as a positive control (Fig 4). Antimicrobial assay of all three fractions on *K. pneumoniae* revealed DCM fraction to have comparatively effective antimicrobial among all three fractions. Water fraction, however, does not affect the growth of the bacterial strain, while DCM fraction showed a zone of inhibition (ZOI) of 9 mm diameter at 500 μg concentration and hexane fraction showed ZOI at 1 mg with the same area of a clear zone as shown in the figure (Fig 4).

Antimicrobial assays of all three fractions on *S. aureus* strain revealed that only DCM fraction was comparably efficient among all three fractions, although still less effective than *K. pneumoniae*. The water fraction had no

impact on the bacterial strain, but the DCM fraction displayed a zone of inhibition at 1 mg concentration and the hexane fraction showed a modest zone of inhibition in the same concentration range.

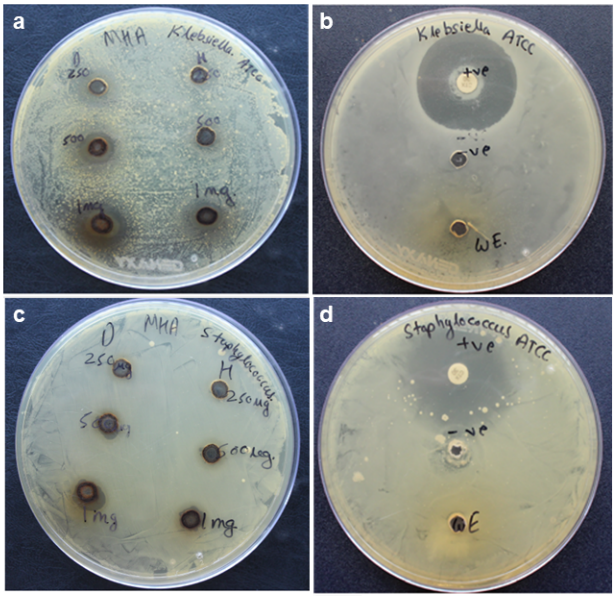

Figure. 4. Antibacterial properties of the bark extract against different pathogens. a) Agar plate showing zone of inhibition of DCM and hexane fraction of the extract as D and H respectively in the concentration of 250 µg, 500 µg and 1 mg over *Klebsiella* ATCC 25955 bacterial strain. Here diameters of zone of inhibition were found to be 9 mm and 4 mm for 500 µg and 1 mg DCM fractions respectively while 7 mm for 1 mg hexane fraction. b) Agar plate showing antimicrobial activity of water fraction of the extract (WE), antibiotic, ceftriaxone and negative control of methanol over *Klebsiella* ATCC 25955 bacterial strain. Here diameter of zone of inhibition was found to be 3 cm for 10 µg ceftriaxone. c) Agar plate showing zone of inhibition of DCM and hexane fraction of the extract as D and H respectively in the concentration of 250 µg, 500 µg and 1 mg over *Staphylococcus* ATCC 25923 bacterial strains. d) Agar plate showing antimicrobial activity of water fraction of the extract (WE), antibiotic, ceftriaxone and negative control of methanol over *Staphylococcus* ATCC 25923 bacterial strains.

Chemical constituents and biological properties of SBOI

The research concerning the biological activities and phytochemical screening of this plant is required to identify the scientifically approved verification of the lead component present in the stem bark of *O. indicum* (hereafter referred to as SBOI), Fig. 5. Scientific research has progressively accumulated knowledge on the biological potential of extracts of SBOI as antioxidants (Mishra *et al.* 2010) and antimicrobials (Kaisarul Islam *et al.* 2010) but thorough studies on the plant's whole phytochemical potential are lacking.

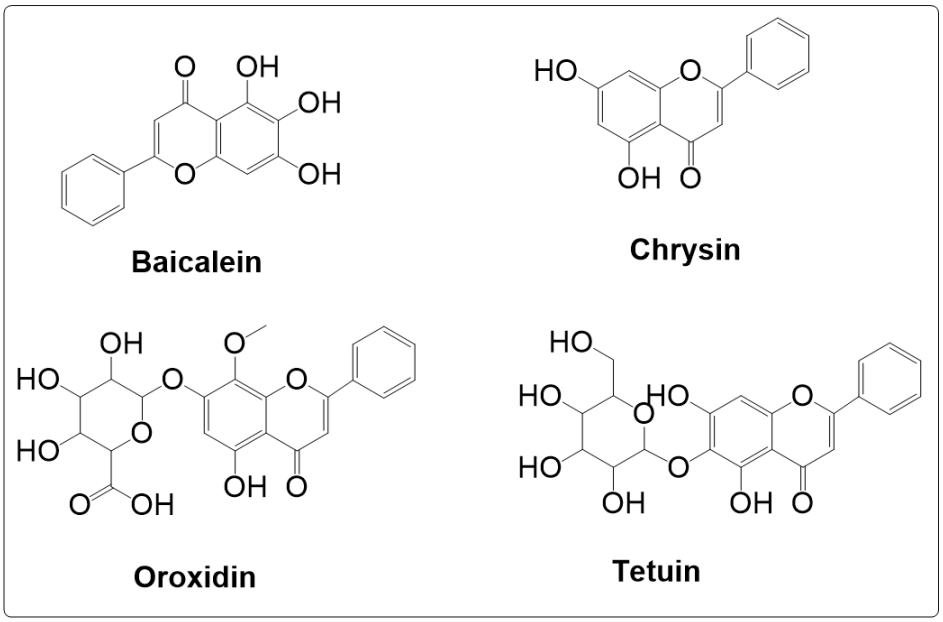

Fig. 5. Phytochemicals present in the bark of *O. indicum*

The polar extract of the SBOI showed potent antioxidant properties, even 1.5-5 times higher than ascorbic acid. Antioxidant compounds are known for inhibition of inflammation based on rat arthritis model and mouse model experiments (Yoshino *et al.* 2006). This suggests the plant might bear greater anti-inflammation activity which makes sense that people have been using it as a traditional medicine against inflammation. There are several

antioxidant compounds reported from *O. indicum* such as ellagic acid (Bisht *et al*. 2011, Harminder *et al*. 2011, Rojsanga *et al*. 2020). Two compounds without structure elucidation from SBOI one of them showing strong antioxidant properties have also been reported (Rajkumar *et al*. 2012). The purification and structure elucidation of the lead antioxidant compound is still necessary to propose this plant in the pharmaceutical field.

The medium polar extract showed antimicrobial activities against *Klebsiella pneumoniae* ATCC 25955 and *Staphylococcus aureus* ATCC 25923. This leads to the assurance of this plant has antibiotic compounds that might be interesting to pharmaceutical industries. There are some antimicrobial compounds already reported from the stem and root extract of *O. indicum*. Lapachol, for example, had been reported as an antibiotic against *E. coli* and *Pseudomonas aeruginosa*, while chrysin was active against *P. aeruginosa* and *Candida albicans* (Mat Ali *et al*. 1998). Not all the isolated compounds from this plant have tested for the antimicrobial activity so it has been indeed necessary to perform bioactivity of all the isolated chemical components to verify its thorough antimicrobial potential.

The SBOI extract also seems to contain abundant flavonoids, alkaloids, phenolic compounds, saponins, and carbohydrates based on our preliminary phytochemical screenings. Alkaloids and phenolic compounds from plant natural products have been found to have antioxidant properties (Barbosa-Filho *et al*. 2006, Kao *et al*. 2005, Shaheen *et al*. 2005, Souto *et al*. 2011). Flavonoids such as baicalein-7-O-caffeate, 8,8''-bisbaicalein, baicalein, chrysin, scutellarein, 6-hydroxyluteolin, 6-methoxyluteolin, baicalein-7-O-glucoside, and beta-sitosterol have been reported from SBOI extract (Fig. 4) (Dinda *et al*. 2007). Several compounds are reported from stem bark and seed extract of *O. indicum* among them scutellarein 7-O-*β*-D-glucopyranosyl-(1 → 6)-*β*-D-glucopyranoside, baicalein-7-O-diglucoside, baicalein-7-O-glucoside, scutellarein-7-O-glucopyranoside, baicalin, and baicalein have shown potent antioxidant activity in DPPH assay, while additional compounds such as chrysin-7-O-gentiobioside, chrysin-7-O-glucuronide, chrysin-6-C-*β*-D-glucopyranosyl-8-C-*α*-L-arabinopyranoside, chrysin, oroxylin A, pinocembrin, and pinobanksin have shown antioxidant activity in oxygen radical absorbance capacity assay (Yan *et al*. 2011).

These preliminary findings and review research in such an underexplored region can somehow justify the traditional medicinal values of the plant. Our current research provided detailed ethnomedicinal insights regarding the usage of plants and the compounds therein along with their different biological aspects.

Conclusions

Our ethnomedicinal questionnaire survey revealed that the plant *O. indicum* is being used by the tribes for various therapeutic implications, which has also been validated through our biochemical approaches in the laboratory. The chromatographic analysis of the bark extract revealed the hexane fraction to possess higher phytochemicals compared to the DCM fraction. However, a smear of water fraction was also observed on the TLC sheet. The water extract revealed stronger antioxidant activity than DCM and hexane fractions, while all fractions have elevated radical scavenging activity. Even ascorbic acid is outperformed by the water component in terms of antioxidant activity. Antimicrobial activity of non-polar to polar fractions of bark extract against two clinical pathogens revealed DCM fraction to have higher antibacterial activity. The phytochemical study revealed to have a higher number of alkaloids, flavonoids, phenolic chemicals, saponins, and carbohydrates. In addition, DPPH analysis indicated bark extract of *O. indicum* possesses greater antioxidant activity than normal ascorbic acid. However, the indication of antioxidant activity and antimicrobial activity at the crude or fraction level also supports the ethnomedicinal uses of this plant in the community. This preliminary research opens the space and direction for the pharmaceutical institution as a research resource. As a result, more research should be performed to isolate the specific lead component responsible for such potent antioxidant properties. Finally, using biochemical analysis, the ethnomedicinal implications of the plant were documented and the presence of bioactive components was established. This signifies the plant has significant therapeutic value and should be conserved either *in-situ* or *ex-situ*.

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