ORIGINAL ARTICLE

Pharmacognostic and Phytochemical Analysis on Bioactive Compounds Extracted from *Oroxylum indicum* Leaves

Asmaa' Mohd Satar[1], Farah Amna Othman[1], Badrul Hisham Yahaya[2], Mohd Zulkifli Mustafa[3], Firli Rahmah Primula Dewi[4], Suat Cheng Tan[1]

[1] School of Health Sciences, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.
[2] Department of Biomedical Sciences, Advanced Medical and Dental Institute, Universiti Sains Malaysia, Bertam, 13200 Kepala Batas, Penang, Malaysia
[3] Department of Neuroscience, School of Medical Sciences, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kelantan, Malaysia.
[4] Department of Biology, Faculty of Science and Technology, Universitas Airlangga, 60115 Surabaya, Indonesia.

ABSTRACT

Introduction: *Oroxylum indicum*, a medicinal plant native to Southeast Asia, is known for its broad therapeutic potential. This research aimed to establish the pharmacognostic profile and to identify the key phytochemicals of *O. indicum* leaves. **Material and Methods:** Thorough examinations of *O. indicum* leaves were conducted, including macroscopic, microscopic, physicochemical, fluorescence and phytochemical analysis. **Results and discussions:** Transverse section of the *O. indicum* leaf showed a clearly defined upper and lower epidermis, both covered by a cuticle and composed of thin-walled, rectangular cells. In powdered form, the *O. indicum* leaf showed distinctive features such as pitted vessels and stomata structures. Physicochemical analysis revealed that the total ash, water-soluble ash, and acid-insoluble ash values were 8.66%, 6.14%, and 0.059% w/w, respectively. Fluorescence analysis of the powdered leaf displayed versatile fluorescence characteristics which could be used as reference for the authentication of *O. indicum* leaves. Phytochemical analysis of crude extract isolated from the leaves showed the plant was rich in gallotannin. On the other hand, fractionated extract was found to contain tannin, phlobatannin, saponin and quinines which were absence in the crude extract. Fractionated extract also was found to be richer in phenol, flavonoid and glycosides, compared to the crude extract, indicating the fractionated extract contained more potent bioactive compounds. **Conclusions:** This preliminary analysis provides valuable reference standards that can facilitate the identification and authentication of *O. indicum* leaf for its future application in research and medicinal use.

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Corresponding Author:
Suat Cheng Tan, PhD
Email: tansc@usm.my
Tel : +609-7677776

INTRODUCTION

Oroxylum indicum, commonly known as the Indian trumpet tree or midnight horror, is a medicinal plant widely distribute across Southeast Asia. Botanically, *O. indicum* is classified under the Plantae kingdom, Magnoliophyta class, Lamiales order, Bignoniaceae family, Oroxylum genus, and indicum species. The tree is characterized by a soft, light to grayish-brown bark with prominent corky lenticels which can grow up to 12 meters in height (1)

Recently, this plant has garnered attention due to its diverse applications to treat various human diseases traditionally. It has been reported that the *O. indicum* has been used in Ayurveda, Traditional Chinese Medicine, and various folk medicines for asthma, bronchitis, coughs, as well as digestive disorders like dysentery, diarrhea, and stomach pain (2). The medicinal properties of this plant are largely attributed to the presence of bioactive compounds such as flavonoids, alkaloids and tannins in various parts of the plant, including the

leaves, bark, seeds and roots. These compounds have been found to be beneficial for anti-inflammatory, antioxidant and antimicrobial activities (3). This broad spectrum of phytoconstituents highlights the therapeutic potential of *O. indicum*, making it a significant subject of pharmacological research for its bioactive compounds.

Considering the extensive use of *O. indicum* in traditional medicine, as well as the new emerging evidences of its bioactive compounds as potential pharmaceutical drug, thus, it is important to expand the scientific understanding about this plant, particularly the scientifically validated pharmacognostic and phytochemical profiles of the plant. Such understanding not only facilitates proper identification and quality control of *O. indicum* in the natural medicine sector but also to support its application in the pharmaceutical industry. Furthermore, analyzing *O. indicum* phytochemical profiling could enable the identification of unique bioactive compounds that could have significant therapeutic applications. This preliminary analysis might uncover novel compounds or mechanisms, providing a scientific basis for future development of new drug derived from this plant.

MATERIALS AND METHODS

Plant material

Fresh leaves of *O. indicum* were collected from Kampung Pasir Parit, Pasir Mas (LPGS coordinate: latitude 5.905471, longitude 102.1884469). The collected plant material was validated and deposited in the Universiti Sains Malaysia Herbarium (voucher specimens: USM Herbarium 11751) (4). The verified fresh leaves were collected for pharmacognostic study which consisted of macroscopic, microscopic, physicochemical and fluorescence analysis based on standard procedures described previously (5-9).

Macroscopic and microscopic analysis

The macroscopic analysis was performed based on observation of the morphological characteristics of the fresh leaves of *O. indicum*, including the size, shape and fracture, with naked eyes. On the other hand, microscopic study involved staining the transverse sections of the leaves with methylene blue and observed the internal structures of the leaves, including the midrib, lamina, nuclei and stomata, using microscope (5-6).

Powder microscopy

Fresh leaves were dried in oven at 50 °C and grinded into fine powder to study the powder microscopical characters. Firstly, one drop of glycerine was added to 1 mg of the fine powdered leaves and mounted on a glass slide. Without any staining reagents, the glass slide containing the fine powdered leaves was placed under microscope for observation (7).

Physicochemical analysis

The physicochemical analysis of *O. indicum* leaves included the determination of moisture content, total-ash value, water-soluble ash value and acid-insoluble ash value were performed based on protocols described in previous study (8).

Fluorescence analysis

10 g of the *O. indicum* leaves powder was treated with 5 ml of various chemical reagents including distilled water, methanol, ethanol, hydrochloric acid, nitric acid, ferum (III) chloride, chloroform, and ethyl acetate, and observed under visible and ultraviolet (UV) rays at 254 nm (short) and 366 nm (long) wavelength to assess the fluorescence activity of the leaves. The evaluation was based on characteristic colour changes of *O. indicum* powdered leaves in different reagents, as previously described (9).

Phytochemical analysis

Phytochemical analysis was performed on the crude and fractionated extracts obtained from the leaves of *O. indicum* using binary solvent Soxhlet extract system as described previously (4). In brief, 25 g of *O. indicum* leaf powder was weighed and placed in cellulose thimble loaded into a Soxhlet extractor. Then, 300 ml of petroleum ether was added and heated to 42-62 °C for 1 hour. After 1 hour, the solvent was discarded and another 300 ml of fresh petroleum ether was added. The same extraction procedure was continued at 42-62 °C for 1 hour. Then, the solvent was discarded again and the cellulose thimble was taken out from the Soxhlet extractor to air dry. After completely air dried, the cellulose thimble was loaded back into the same Soxhlet extractor and added with 500 ml of fresh methanol. Then, the solvent was heated to 62-65 °C until the solvent turned clear. The solvent was collected, concentrated and dried using rotary evaporator (Buchi AG, Flavil, Switzerland). Subsequently, 5 g of the dried crude extract powder was dissolved in 5 ml of methanol until a slurry suspension was formed and carefully loaded into a Diaion HP-20 column (Sigma Aldrich). The column was eluted with 750 mL of methanol at an increasing concentration from 0-100%. The fractionated extract was collected as the last fraction and was dried using a rotary evaporator. Both the dried crude and fractionated extracts were subjected for phytochemical tests based on the previous study to identify the presence of important active compounds (3).

RESULTS

Macroscopic characteristics

The macroscopic characteristics of fresh leaves of *O. indicum* were observed and recorded. Based on the

observations, *O. indicum* leaves were green in colour with an ovate shape and an acuminate apex, typically measuring 3–15 cm in length. The leaves exhibited pinnately compound venation with 2–4 pairs of leaflets and were evergreen with a wavy texture. The margin of the leaves was entire and serrate with a narrow base. The fresh leaves had a rigid structure and were relatively thinner and flaccid.

Microscopic characteristics

Detail microscopic features of *O. indicum* fresh leaves (Figure 1a-c) and powdered leaves (Figure 1d) were presented in this study. Transverse section through the midrib of *O. indicum* fresh leaf revealed the main cells of dicot stem anatomy (Figure 1a). At the center, vascular bundle which composed of cambium, phloem and xylem, was clearly visible and well-organized. The xylem which responsible for water conduction appeared as large, circular vessels, while the phloem which involved in the transport of organic nutrients appeared as a layer of smaller, densely packed cells situated just outside the xylem. The cambium was located between xylem and phloem. It plays role for formation of new xylem and phloem cells. Surrounding the vascular bundle was the pith, which consisted of parenchymatous cells. The outer region of the leaf cross-section consisted of the cortex which could differentiate into collenchyma cells which provide mechanical support, and parenchyma cells which serve in storage and basic metabolic functions. The outermost layer was the epidermis, which protects internal tissues from mechanical damage and desiccation.

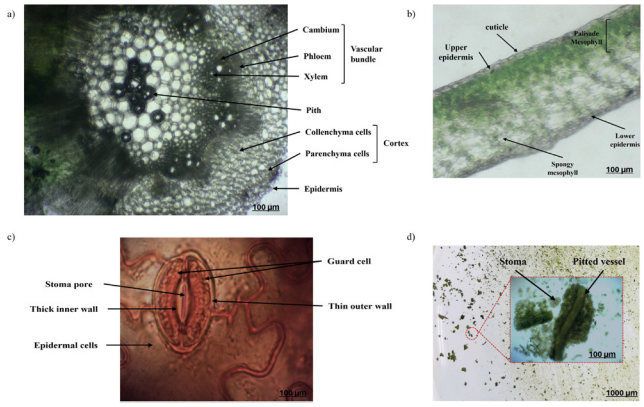


Figure 1: Transverse section of *O. indicum* leaf (a) midrib, (b) lamina, (c) stomata structures and (d) powdered *O. indicum* leaf.

Meanwhile, Figure 1b illustrates a transverse section through the leaf lamina. The upper epidermis and lower epidermis were both covered by a protective cuticle layer that minimizes water loss. Beneath the upper epidermis was the palisade mesophyll which composed of tightly packed chlorenchyma cells optimized for photosynthesis. Below these cells was spongy mesophyll which consisted of loosely arranged cells with air spaces that facilitate gas exchange. This structural arrangement supports both photosynthesis and transpiration processes.

On the other hand, the surface of the leaf epidermis (Figure 1c) revealed the presence of anisocytic stomata, characterized by a stoma pore flanked by two guard cells, each with a thick inner wall and thin outer wall. Besides, the stomata was surrounded by epidermal cells which form the outer layer of the leaf and provide additional protection. The surrounding epidermal cells also exhibited sinuous walls, contributing to the identification of leaf surface morphology.

Lastly, the microscopic analysis of powdered *O. indicum* leaf also was conducted and showed the presence of vascular and epidermal components such as stoma and pitted vessels (Figure 1d). The stomata are openings that enable gaseous exchange, while the pitted vessels represent lignified xylem elements with pits that facilitate lateral water movement between vessels, a key feature in vascular transport.

Together, these micrographs offer a thorough view of the anatomical features of *O. indicum* fresh and powdered leaves. These structural features could be an essential reference for the botanical identification and pharmacognostic standardization of *O. indicum*.

Physicochemical parameters

Physicochemical parameters of *O. indicum* leaves such as moisture content, total ash, water-soluble ash, and acid-insoluble ash values were reported in Table I. The data indicated that the leaves of *O. indicum* were highly moist (71.68%) with low contamination of inorganic matter, as indicated by only 8.66% total-ash value, 6.14% of water-soluble ash and 0.059% of acid-insoluble ash value. These values provide valuable information about the inorganic content of plant samples which are crucial for quality control and standardization of herbal medicines.

Table I: Physicochemical evaluation of *O. indicum* leaves.

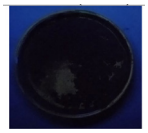
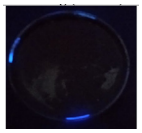
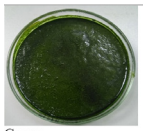
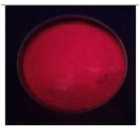
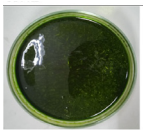
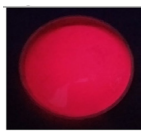
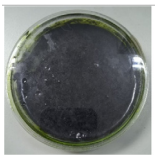
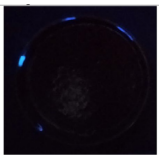
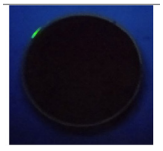
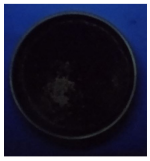
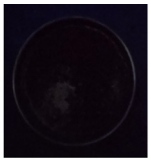
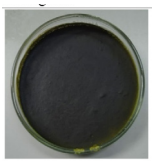
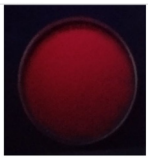
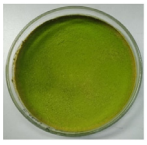
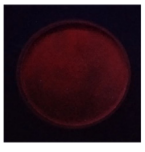
Parameters	Dry weight (%)
Moisture content	71.68 ± 0.18
Total – ash value	8.66 ± 0.20
Water soluble ash	6.14 ± 0.003
Acid insoluble ash value	0.059 ± 0.04

*Values are expressed as mean ± SD (standard deviation) of three replicates.

Fluorescence analysis

Many plant materials showed fluorescence signal when mixed with different reagents. Therefore, the fluorescence analysis could be used to qualitatively observe quality and purity of chemical constituent presence in certain plant material. In this study, the *O. indicum* leaf powder exhibited versatile fluorescence characteristics with different colours in different reagents as presented in Table II. These information could be used as reference for the authentication of *O. indicum* leaves.

Table II: Fluorescence analysis of *O. indicum* leaf powder.

Treatment	Visible light	UV Light	
		At short (254 nm)	At long (365 nm)
Distilled water	 Green	 Black	 Black
Methanol	 Green	 Red	 Bright red
Ethanol	 Green	 Red	 Bright Red
1N Hydrochloric acid, HCl	 Dark green	 Black	 Black
50% Nitric acid, HNO_3	 Brown	 Black	 Black
Ferum (III) Chloride, $FeCl_3$	 Dark green	 Black	 Black
Chloroform, $CHCl_3$	 Dark green	 Black	 Red
Ethyl acetate, $C_4H_8O_2$	 Light green	 Black	 Red

Phytochemical analysis

The presence of important bioactive compounds in crude extract and fractionated extract of *O. indicum* leaves were determined using phytochemical tests. The observations and results for each of the tests were presented in Table III. Based on the result obtained, the crude extract contained phenol, gallotannin, flavonoid and glycosides. It also was found to be richer in gallotannin (also known as tannic acid) compared to the fractionated extract. On the other hand, fractionated extract was found to contain tannin, phlobatannin, saponin and quinines which were absence in crude extract. Fractionated extract also was found to be richer in phenol, flavonoid and glycosides, compared to the crude extract, indicating the fractionated extract contained more potent bioactive compounds.

Table III: Phytochemical evaluation of *O. indicum* leaf crude and fractionated extracts.

Phytochemical test	Observations	
	Crude extract	Fractionated extract
Phenol Crude extract + Fractionated extract ++		
Tannins Crude extract - Fractionated extract ++		
Phlobatannin Crude extract – Fractionated extract +++		
Gallotannin Crude extract +++ Fractionated extract ++		
Flavonoid Crude extract ++ Fractionated extract +++		
Saponin Crude extract - Fractionated extract +++		
Quinines Crude extract - Fractionated extract +++		
Glycosides Crude extract + Fractionated extract ++		

DISCUSSION

The findings of this study contribute significantly to the pharmacognostic and phytochemical characterization of *O. indicum* leaves, providing a valuable baseline reference to authenticate the plant material prior to its application for medicinal or industrial usage.

Establishing detailed pharmacognostic standards is crucial to ensure the accurate identification and quality control of the plant prior to its use. This study was the first to describe the macroscopic and microscopic features of *O. indicum* leaves in detail, offering critical information for their correct identification and quality control. Based on the microscopic and macroscopic analysis, several key anatomical features were identified to support the identification and standardization of *O. indicum* leaves. These include well-organized vascular bundles composed of xylem, phloem, and cambium (Figure 1a), a distinct leaf lamina structure with upper and lower epidermis, cuticle, palisade, and spongy mesophyll layers (Figure 1b), and the presence of stomata with characteristic guard cells, thick inner walls, thin outer walls, and surrounding epidermal cells (Figure 1c). Additionally, the appearance of stomata alongside pitted xylem vessels in powdered leaves (Figure 1d) also could further supports species-level identification. These distinctive microscopic features are valuable for the authentication of *O. indicum* leaves and can significantly reduce the risk of adulteration in herbal preparations (10).

The physicochemical parameters also could serve as an important basis for evaluating the purity and quality of the plant material used in a study. The acid-insoluble ash, primarily composed of silica, indicates potential contamination with earthy matter. In this study, it was found that the *O. indicum* leaves contained high moisture level, therefore, maintaining minimal moisture content in the leaves is important to prevent the growth of microorganisms such as bacteria, yeast or fungi during storage. Fluorescence analysis suggested that the *O. indicum* leaves likely to contain active agents which may underlie their therapeutic properties. Fluorescence analysis techniques are frequently used to assess identity and authenticity, quality assessment, detection of adulteration of the plant materials and making them an important aspect of pharmacognostic evaluation.

Additionally, the phytochemical analysis also revealed distinct differences between the crude and fractionated extracts, with specific compounds, including tannin, phlobatannin, saponin and quinines, found only in the fractionated extract but not in the crude extract. Fractionated extract also was found to be richer in phenol, flavonoid and glycosides, compared to the crude extract, indicating the fractionated extract contained more potent bioactive compounds. This is probably because the fractionation process selectively separates and concentrates specific phytochemicals based on their chemical properties, such as polarity, solubility, or molecular size. This helps to remove less active or non-polar components present in the crude extract, thereby enriching the extract with higher concentrations of bioactive compounds like phenols, flavonoids, and glycosides. As a result, the fractionated extract could exhibit enhanced pharmacological activities due to the increased availability and purity of these therapeutic agents.

Nonetheless, despite the usefulness of preliminary phytochemical screening in identifying the major classes of bioactive compounds present in *O. indicum*, such as flavonoids, alkaloids, and tannins, this approach has inherent limitations. This is mainly because phytochemical test is primarily qualitative analysis, as a consequence, it does not provide specific information on the concentration or exact identity of individual compounds, which is critical for standardization, therapeutic efficacy, and safety assessment. Moreover, the results can be influenced by subjective interpretation, solvent selection, and variations in extraction methods, potentially leading to inconsistent outcomes (11). To address these limitations, future research should incorporate quantitative analytical techniques such as high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) which offer higher sensitivity and specificity to identify and quantify phytoconstituents precisely. Additionally, integrating spectrophotometric assays and nuclear magnetic resonance (NMR) spectroscopy can further validate compound structures and concentrations. The adoption of these advanced methodologies will strengthen the reliability of phytochemical data and support the development of standardized herbal formulations derived from *O. indicum* in the development of natural and medicinal products (12).

CONCLUSION

Ideally, the present study is a novel attempt to identify the pharmacology and phytochemical characteristics of *O. indicum* leaves. The findings presented in this study will be useful for the further application of this plant for medicinal and industrial applications. The other parameters of moisture content ash value and fluorescence analysis would add value to its quality control and assurance. In addition, *O. indicum* pharmacognostic and phytochemical profiles could assist in confirming the quality, purity and identification of this plant for future applications and perhaps the adulteration of *O. indicum* can be prevented.

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REFERENCES

1 Rajak V, Pathak V, Tripathi M. Comparative Phytochemical Screening and Pharmacognostic Study on Stem Bark and Root bark of Endangered Medicinal Plant Oroxylum indicum (L.) Vent. International Journal of Scientific Research in Biological Sciences 2018; 5. (DOI: 10.26438/ijsrbs/v5i3.8084)

2 Jagetia GC. A Review on the Medicinal and Pharmacological Properties of Traditional Ethnomedicinal Plant Sonapatha, Oroxylum indicum. Chemical and Pharmaceutical Bulletin 2021; 69: 421–431. (DOI: https://doi.org/10.3390/sinusitis5010009)

3 Souhoka FA, Kapelle IBD, Sihasale E. Phytochemical and Antioxidant Test of Binahong (Anredera cordifolia (Tenore) Steenis) Leaves Ethanol Extract. Fullerene Journal of Chemistry 2021; 6. (DOI: 10.37033/fjc.v6i1.248)

4 Kang IN, Salleh NNHN, Chung WJ, Lee CY, Tan SC. Baicalein-enriched fraction extracted from Oroxylum indicum (L.) Benth. ex kurz leaves exerts antioxidant and inhibitory effects against glioblastoma multiforme. Processes 2019; 7: 963. (DOI: 10.3390/PR7120963)

5 Gupta PC, Sharma N, Rao C V. Pharmacognostic studies of the leaves and stem of Careya arborea Roxb . 2012; 2: 404–408. (DOI: 10.1016/S2221-1691(12)60065-3)

6 Tabasum S, Khare S, Jain K. Macroscopic and Microscopic Evaluation of Abrus Precatorius L. (Gunja), Seeds. International Journal of Pharmaceutical Sciences and Research 2017; 8: 2631–2635. (DOI: 10.13040/IJPSR.0975-8232.8(6).2631-35)

7 Sakti AS, Saputri FC, Munim A. Microscopic characters, phytochemical screening focus on alkaloid and total phenolic content of Uncaria gambir Roxb. and uncaria sclerophylla Roxb. Leaves. Pharmacognosy Journal 2019; 11: 119–123. (DOI: 10.5530/pj.2019.1.20)

8 Prasanth D, Rao AL. Pharmacognostic Study of Eranthemum nigrum Stem. 2018; 15. (DOI: 10.19080/CTBEB.2018.15.555916)

9 J. D, Hiremath RS. Fluorescence analysis and extractive values of some ethno-medicinal plants of the Western Ghats of India. Advance Pharmaceutical Journal 2023; 8. (DOI: 10.31024/apj.2023.8.3.4)

10 Pondugula SR, Salamat JM, Abbott KL, Flannery PC, Majrashi M, Almaghrabi M, Govindarajulu M, Ramesh S, Onteru SK, Sandey M, Haung CCJ, Gill K, Narayanan N, McElroy E, Desai D, Nadar R, Moore T, Kalyanam N, Majeed M, Dhanasekaran M. Oroxylum indicum Extract, at a Physiologically Relevant Dosage, Does Not Induce Hepatotoxicity in C57BL/6J Mice. Natural Product Communications 2021; 16. (DOI: 10.1177/1934578X211016966)

11 Rao A, Kumari S, Laura JS, Dhania G. Qualitative Phytochemical Screening of Medicinal Plants Using Different Solvent Extracts. Oriental Journal Of Chemistry 2023; 39. (DOI: 10.13005/ojc/390312)

12 Santos EL, Maia BHLNS, Ferriani AP, Teixeira SD. Flavonoids: Classification, Biosynthesis and Chemical Ecology. Flavonoids - From Biosynthesis to Human Health (DOI:10.5772/67861)