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PHYTOCHEMICAL PROFILING AND TOXICITY ASSESSMENT OF IRVINGIA GABONENSIS STEM BARK EXTRACTS

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Abstract: Traditional medicine remains integral to healthcare worldwide, particularly in developing regions where access to modern medical facilities is limited. The World Health Organization estimates that approximately 80% of the global population relies on traditional medicine as their primary healthcare source. This reliance is attributed to the accessibility, affordability, and cultural relevance of traditional medicinal practices. The therapeutic potential of bioactive compounds found in medicinal plants has sparked significant scientific interest in evaluating ethnomedicinal flora. Africa, with its rich biodiversity and deep-rooted plant-based healing traditions, plays a pivotal role in this context. Medicinal plants in Africa have historically been used to manage a wide range of health conditions, including gastrointestinal and infectious diseases. These plants are abundant in secondary metabolites such as alkaloids, flavonoids, saponins, tannins, and terpenoids, which exhibit diverse pharmacological properties. Understanding the phytochemical composition and safety profiles of these plants is crucial for validating their traditional use and for the development of novel therapeutics. This study focuses on the phytochemical characterization and toxicological evaluation of Irvingia gabonensis stem bark extracts, contributing to the scientific documentation and safety assessment of this important ethnomedicinal resource.

Keywords: Traditional Medicine, Ethnomedicine, Phytochemicals, Irvingia gabonensis, Toxicological Evaluation

Introduction

Traditional medicine continues to occupy a central position in healthcare systems across the globe, particularly in developing countries where modern medical facilities may be limited or inaccessible. According to the World Health Organization (WHO), approximately 80% of the world's population still relies on traditional medicine as their primary source of healthcare (Owolabi et al., 2007). This persistent reliance is largely due to the accessibility, affordability, and cultural compatibility of traditional medicinal practices. Moreover, the therapeutic promise of

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bioactive compounds derived from medicinal plants has catalyzed global interest in the scientific evaluation of ethnomedicinal flora.

Within the scope of ethnomedicine, Africa stands out due to its immense biodiversity and longstanding history of plant-based healing traditions. Medicinal plants have served as essential tools in African healing systems for centuries, treating ailments ranging from gastrointestinal disorders to infectious diseases. Many of these plants are rich sources of secondary metabolites such as alkaloids, flavonoids, saponins, tannins, and terpenoids—each possessing significant pharmacological effects.

As global pharmaceutical industries face diminishing returns from synthetic drug discovery pipelines, attention is increasingly shifting toward natural products for drug development. Several modern pharmaceuticals, including quinine, artemisinin, and aspirin, owe their origins to plant-derived compounds. As such, the systematic phytochemical and pharmacological evaluation of traditionally used plants is no longer of mere academic interest but has become a key strategic pursuit in the development of new and safer drugs.

One plant of considerable medicinal and economic interest in this regard is Irvingia gabonensis (commonly known as African mango or bush mango), a member of the Irvingiaceae family. Indigenous to the rainforests of West and Central Africa, I. gabonensis has become widely recognized not only for its nutritional value but also for its therapeutic applications. It is cultivated extensively in countries such as Nigeria, Ghana, Cameroon, and Côte d'Ivoire. Local communities refer to the plant by various names including "Apon," "Ogbono," and "Goron biri," depending on the dialect and region (Unaeze et al., 2017).

Botanically, the Irvingiaceae family comprises about ten species of medium to large tropical trees found predominantly in West Africa, Southeast Asia, and parts of Malaysia. These species typically thrive in regions with specific environmental conditions: altitudes between 200 and 500 meters, average annual rainfall of 1200 to 1500 mm, and temperatures ranging from 20 to 38°C (Raj et al., 2022). The genus Irvingia is especially distinguished by its glabrous branches, mucilage-producing secretory canals, and remarkably hard wood. Among the genus, I. gabonensis is the most extensively studied and utilized, primarily due to its edible fruits and medicinal seeds.

The fruits of I. gabonensis are consumed both fresh and processed. The seeds, which produce the popularly known "Ogbono" soup thickener, are rich in lipids—particularly medium-chain fatty acids like lauric (C12) and myristic (C14) acids—as well as vitamins and minerals (Sun & Jen, 2012). Beyond their nutritional applications, these seeds are increasingly being investigated for their pharmacological potential. Several studies have identified the presence of phytochemicals such as flavonoids, tannins, alkaloids, and sterols within different parts of the plant, suggesting a rich repository of biologically active substances (Mahunu et al., 2019; Olorundare et al., 2020).

One of the most compelling features of I. gabonensis is its antioxidant capacity, largely attributed to compounds such as lupeol, quercetin, and β-sitosterol (Ekpe et al., 2017). Antioxidants are crucial in neutralizing free radicals that contribute to oxidative stress, a pathological state implicated in chronic diseases including cancer, cardiovascular disease, and neurodegeneration. The presence of such compounds thus supports the ethnopharmacological claims of I. gabonensis being beneficial in treating inflammatory and metabolic conditions.

However, despite its popularity in traditional medicine and emerging scientific interest, much of the existing research has focused on the seeds and fruits of I. gabonensis, with relatively limited exploration of other anatomical parts such as the stem bark. Given that different plant parts can contain distinct phytochemical profiles,

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a comprehensive study of the stem bark is essential. The bark of many tropical trees is known to house bioactive molecules with therapeutic relevance. Therefore, evaluating the phytochemical and toxicological properties of I. gabonensis stem bark may unveil additional dimensions of its medicinal potential.

Furthermore, any therapeutic application of plant-derived extracts necessitates an assessment of safety. Traditional usage does not always equate to safety, and unregulated consumption of plant materials can lead to toxicological consequences. The OECD guidelines for testing chemicals underscore the importance of acute toxicity studies in animal models to estimate the potential harmful effects of herbal substances. Such studies not only determine the LD50 (lethal dose for 50% of the population) but also provide vital information on adverse clinical symptoms, behavioral changes, and possible organ damage following high-dose exposure.

In this context, the present study was designed to investigate the phytochemical composition and acute oral toxicity profile of Irvingia gabonensis stem bark. The plant samples were sourced from Iragbiji, Osun State, Nigeria—a region known for its botanical diversity and traditional knowledge. The stem bark was subjected to sequential solvent extraction, followed by qualitative and quantitative phytochemical screening. Additionally, an in vivo acute toxicity study was performed using Wistar rats to evaluate the safety margin of the extract at a limit dose of 5000 mg/kg, aligning with OECD recommendations.

Ultimately, this research aims to contribute to the pharmacognostic standardization of I. gabonensis and lay a foundation for its potential inclusion in future drug development initiatives. By bridging ethnobotanical knowledge with modern scientific methods, we seek to validate the traditional uses of I. gabonensis and provide credible data to support its safe and effective utilization in both traditional and contemporary medicine.

Material and Methods:

Ethanol (Analytical grade Sigma-Aldrich,
USA), Methanol (Analytical grade SigmaAldrich, USA), Ethyl acetate (Analytical grade Sigma-Aldrich, USA), N-Hexane (Analytical grade Sigma-Aldrich, USA), Aluminum Chloride powder (500g) (Reagent
Grade), Quercetin (Reagent grade), FolinCiocalteu reagent (FCR), Sodium Carbonate Powder (Reagent Grade), Dragendorff Reagent, N/50 iodine solution, Sudan Red Solution, Picric acid reagent, Lead subacetate, Mayer's reagent, Wagner's reagent, Libermann-Buchard reagent, Ferric chloride reagent, Nitric acid, Gallic Acid, Distilled water, Hydrogen tetraoxosulphate IV acid, Hydrochloric acid, Chloral hydrate (BDH Laboratory Chemicals Division, POOLE,
England), Rotary Evaporator (RE-210D-1L-
5L, 220V Rotavapor), UV-
Spectrophotometer (Model UV-2505
~ultraviolet and visible range of 195-1050 nm, Labomeds) ,UV Lamp (Short/LongWave UV lamp; 4 watts, 254/365nm wavelength, 220V/50Hz, COLE-
PARMER®), and Oven.

The leaves, fruits, stem-bark, and roots of the plant were collected in October 2018 at Iragbiji community, Boripe Local Government Area of Osun State, Nigeria. The plant was identified, and a voucher number was obtained. The plant materials, after collection, were air-dried, powdered, and 3 kilograms of the powdered plant material were extracted sequentially at room temperature with n-hexane, ethyl acetate, and methanol by maceration for a period of 48 hours, 72 hours, and then 72 ho

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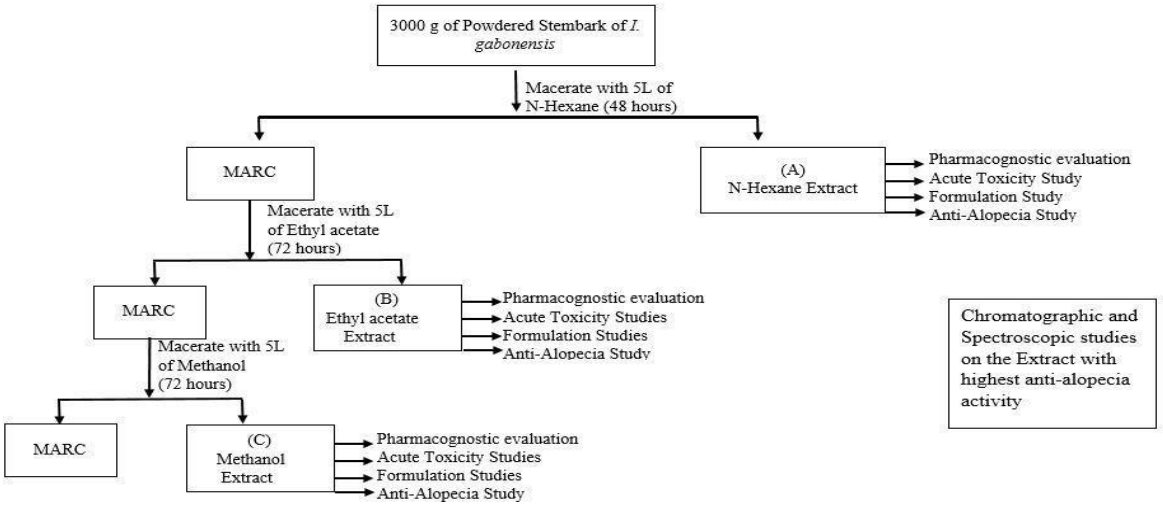

Chart 1: Schematics for the Extraction Process, Biological and Chemical studies of *I. gabonensis*

Respectively (Chart 1).

Qualitative Phytochemical Screening

The stem bark of Irvingia gabonensis was subjected to qualitative phytochemical screening to identify various chemical constituents present in the plant material. The screening involved tests for carbohydrates, anthracenes, cardiac glycosides, flavonoids, tannins, alkaloids, and steroids/triterpenes.

Tests for Carbohydrates: Three tests were conducted to detect carbohydrates in the stem bark extracts. The Molisch test, Fehling test, and Frothing test were performed on nHexane, ethyl acetate, and methanol extracts to identify the presence of carbohydrates based on specific chemical reactions.

Tests for Anthracenes: The presence of anthracenes was tested using the Bontrager test and Modified Bontrager's test. These tests involved the addition of specific reagents to the extracts and observing color changes indicative of the presence of anthracenes.

Tests for Cardiac Glycosides: Various tests such as Keller-Killiani test, Kedde test, and Baljet test were conducted to detect cardiac glycosides in the extracts. These tests involved the addition of specific reagents and observing characteristic color changes.

Tests for Flavonoids: Flavonoids were detected using the Shinoda test and Sodium hydroxide test. These tests involved the addition of specific reagents and observing color changes indicative of the presence of flavonoids.

Tests for Tannins: Two tests, the Ferric chloride test and the Lead sub-acetate test, were conducted to detect tannins in the extracts. These tests involved the addition of specific reagents and observing precipitate formation.

Tests for Alkaloids: Several tests, including Dragendorff's reagent test, Mayer's reagent test, and Wagner's reagent test, were conducted to detect alkaloids. These tests involved specific chemical reactions leading to the formation of characteristic precipitates.

Tests for Steroids/Triterpenes: The Lieberman-Burchard test and the Salkowski test were conducted to detect steroids and triterpenes. These tests involved the addition of specific reagents and observing color changes indicative of the presence of these compounds.

Quantitative Phytochemical Determination:

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Total Phenolic Content (TPC): The TPC of the extracts was determined using the FolinCiocalteu method. Absorbance readings were taken at 765 nm, and the concentration of phenolic compounds was calculated using a calibration curve prepared with gallic acid solutions.

Total Flavonoid Content (TFC): TFC was determined using the aluminum chloride colorimetric assay. Absorbance readings were taken at 533 nm, and the concentration of flavonoids was calculated using a calibration curve prepared with quercetin solutions.

Total Tannin Content (TTC): The tannin content was determined using the FolinCiocalteu method. Absorbance readings were taken at 725 nm, and the concentration of tannins was calculated using a calibration curve prepared with gallic acid solutions.

Acute Toxicity Studies:

Acute oral toxicity studies were conducted according to OECD guidelines in rats (OECD Test Guideline 425, 2001). Rats were orally dosed with different concentrations of extracts and observed for signs of toxicity and mortality over a 14-day period.

Results

The yield obtained from n-Hexane was 110.16g, with a calculated percentage yield was calculated as 3.67%. Ethyl acetate gave a total yield of 160.86g, which was 5.36%. The methanol extract had a weight of 280.22 with a percentage yield of 9.34%, as presented in Table 1.

Table 1: Percentage Yield for the Extract of *Irvingia gabonensis*.

Extract	Weight (g)	Percentage Yield (%w/w)
n-Hexane	110.16	3.67
Ethyl acetate	160.86	5.36
Methanol	280.22	9.34

The preliminary phytochemical screening of Irvingia gabonensis stem bark was conducted using crude extracts in n-Hexane, ethyl acetate, and methanol. Various standard tests re employed to identify the presence of different phytochemical compounds in the extracts, as represented in Table 2

Molisch's test showed a reddish-colored interfacial ring, indicating the presence of carbohydrates. Fehling's test resulted in a brick-red precipitate, confirming the presence of reducing sugars.

The frothing test displayed a persistent honeycomb froth for more than 15 minutes in all extracts, suggesting the presence of saponins.

Anthracenes: Borntrager's test and modified Borntrager's test showed no formation of pinkish-red coloration in any of the extracts, indicating the absence of free and combined anthraquinones.

The Keller-Killiani test revealed a brown ring at the liquid junction and a pale green upper acetic acid layer, indicating the presence of deoxy sugar. The Baljet test resulted in an immediate red coloration, confirming the presence of cardiac glycosides.

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Shinoda test and sodium hydroxide test showed red and yellow solutions respectively, indicating the presence of flavonoids in all extracts.

Ferric chloride test and lead sub-acetate test produced greenish-black and brownish-white precipitates respectively in all extracts, confirming the presence of condensed tannins.

Mayer's test, Dragendorff test, and Wagner's test all yielded precipitates (cream-colored, red, and reddish-brown respectively), indicating the presence of alkaloids in all extracts.

The Lieberman-Burchard test resulted in a brownish-red ring at the interface of the organic and acid layers, along with a pale green upper organic layer, suggesting the presence of steroids and/or triterpenoids. The Salkowski test produced a reddish-brown ring at the interphase of the two liquids, confirming the presence of steroids.

Quantitative phytochemical determination of Irvingia gabonensis stem bark using the spectrophotometric method: Table 3 shows the results for the quantitative phytochemical content of n-hexane, ethyl acetate, and methanol extracts of I. gabonensis stem bark. The table shows a total phenolic, flavonoid, and tannin content of the n-hexane extract as 41.76 mg/g, 43.19 mg/g, and 67.23 mg/g respectively that of ethyl acetate as 122.18 mg/g, 73.42 mg/g, and 164.81 mg/g, and for the methanol extract as 79.47 mg/mL, 36.61 mg/g, and 159.37 mg/g respectively.

The limit test results revealed that a single oral dose of n-hexane ethyl-acetate, and methanol extracts of I. gabonensis stem bark did not cause mortality or any signs of toxicity in rats within 24 hours or during the 14-day observation period. It was observed that the LD_{50} was greater than 2000 mg/kg body weight in rats. When the dose was increased to 5000 mg/kg, no mortality or signs of toxicity were observed with the test within the first 24 hours or over the 14-day observation period

Discussion

The research conducted on Irvingia gabonensis stem bark revealed significant findings regarding the extraction process, chemical composition, and potential biological activities of the plant extracts. The extraction process involved using n-hexane, ethyl acetate, and methanol, resulting in extracts with varying yields and compositions. The n-hexane extraction yielded 3.67% (w/v) of extract, while the subsequent extractions with ethyl acetate and methanol produced higher yields of 5.30% (w/v) and 9.34% (w/v), respectively. This indicates that Irvingia gabonensis stem bark contains more polar constituents than nonpolar ones, consistent with previous research. The total tannin content of the stem bark extracts was determined, with the ethyl acetate and methanol extracts showing tannin content of 164.81 and 159.37 mg GAE/GAE g, respectively. Other studies reported varying tannin content, with values ranging from 85.5 mg to 773.3 mg GAE/g, indicating a high concentration of tannins in polar solvents.

Similarly, the total phenolic content of the extracts was analyzed, and higher concentrations were found in polar extracts compared to non-polar ones. The phenolic content ranged from 79.47 to 253.39 mg/GAE g, indicating the presence of potent antioxidants.

Flavonoid content analysis showed higher levels in ethyl acetate extracts compared to methanol extracts, with values of 73.42 and 36.61 mg/QE g, respectively. This discrepancy may be attributed to differences in extraction techniques and environmental factors.

Phytochemical screening of the extracts revealed the presence of carbohydrates, alkaloids, saponins, and flavonoids, while tannins, glycoside proteins, fats/oils, steroids, terpenes, and cardiac glycosides were absent.

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These secondary metabolites contribute to the biological activity of the plant, including antioxidant and anticancer properties.

Previous research has demonstrated the potential health benefits of Irvingia gabonensis extracts, including prophylactic effects against cadmium-induced toxicity, anti-diabetic, antihyperlipidemic, and antioxidant effects. Phytochemical analysis consistently identified alkaloids, glycosides, flavonoids, and tannins as predominant compounds in Irvingia gabonensis extracts. Acute toxicity studies indicate that Irvingia gabonensis extracts are well-tolerated by experimental animals, with no signs of morbidity or mortality observed at high doses. This suggests the safety of the extracts for potential therapeutic use.

Conclusion

Research on Irvingia gabonensis stem bark has revealed promising findings regarding its phytochemical composition and toxicology profile. Through several analyses, a number of bioactive compounds, including alkaloids, flavonoids, tannins, and glycosides, were identified within the n-hexane, ethyl acetate, and methanol extracts of the plant material. Moreover, the ethyl acetate and methanol extracts exhibited notably high levels of total phenolic, flavonoid, and tannin contents, underscoring the potential therapeutic value of Irvingia gabonensis in traditional medicine and pharmaceutical applications. Furthermore, the acute toxicity study demonstrated a reassuring safety profile, with an LD_{50} exceeding 5000 mg/kg, suggesting a wide margin of safety for therapeutic use. These findings contribute significantly to the growing body of research on Irvingia gabonensis and highlight its potential as a source of bioactive compounds with diverse pharmacological activities.

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