



Phytochemical and Acute Toxicity studies of the Stem bark of *Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) Baill. (IRVINGIACEAE)**T. S. Malgwi¹, K. Y. Musa², I. M. Maje³, H. M. Mshelia⁴, Cletus A. Ukwubile¹, M. Y. Dibal¹**¹Department of Pharmacognosy, Faculty of Pharmacy, University of Maiduguri, Maiduguri, Nigeria²Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria.³Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria⁴Department of Pharmacognosy and Ethno Pharmacy,, Faculty of Pharmaceutical Sciences, Usman Dan Fodiyo University, Sokoto**Corresponding Author Phone:** +234 (80) 63738523 **E-mail:** troysm2013@unimaid.edu.ngDOI No.: <http://doi.org.10.55639/607.phar.101201.0017>

Abstract

Phytochemical analysis of plant extracts plays a crucial role in discovering bioactive compounds with potential pharmacological activities. Understanding the phytochemical profile of *Irvingia gabonensis* could provide insights into its medicinal properties and facilitate the development of novel therapeutics. The conducted research aimed to assess the phytochemical composition and acute toxicity profile of *Irvingia gabonensis* stem bark with the goal of establishing pharmacognostic standards for the plant.

Samples of leaves, fruits, stem bark, and roots were collected from the Iragbiji community, Osun State, Nigeria, and a voucher number was obtained after identification. The stem bark was powdered and sequentially extracted using n-hexane, ethyl acetate, and methanol through maceration. Results revealed the presence of saponins, glycosides, flavonoids, tannins, and steroids in the stem bark extracts. The total phenolic content was found to be 122 mg/mL and 79.47 mg/mL ($\pm$ SEM) in the ethyl acetate and methanol extracts, respectively. Similarly, the total flavonoid content was measured as 73.42 mg/mL and 36.61 mg/mL ($\pm$ SEM) in the ethyl acetate and methanol extracts, respectively. The acute oral toxicity studies conducted on rats revealed no signs of toxicity at a dosage of 5000 mg/kg for all experimental animals. These findings suggest that *Irvingia gabonensis* stem bark possesses phytochemical compounds with potential medicinal benefits and a favorable safety profile.

Keywords: *Irvingia gabonensis*, Phytochemical, Test, Acute, and Toxicity

Introduction

Traditional medicine remains a cornerstone in global healthcare, serving as the primary source of medical treatment for approximately 80% of the world's population

(Owolabi *et al.*, 2007). This reliance on traditional medicine underscores the enduring significance of natural remedies, particularly those derived from plants, in managing various ailments. In African

traditional medicine, the utilization of higher plants and their extracts has been well-documented for treating numerous diseases, contributing to the development of new drugs, and enriching the drug discovery process (Farnsworth and Fabricant, 2001; Cragg *et al.*, 1997).

The resurgence of interest in natural remedies stems from their widespread availability, accessibility, and perceived minimal side effects (Chanda, 2014). Additionally, there is a growing inclination towards exploring the correlation between the phytochemical composition of medicinal plants and their pharmacological properties (Sharma *et al.*, 2017). Amidst this backdrop, the *Irvingiaceae* family emerges as a significant botanical entity within traditional African medicine.

The *Irvingiaceae* family comprises ten species of tropical trees primarily found across Africa, Southeast Asia, and western Malaysia. These trees thrive in regions characterized by specific environmental conditions, including altitudes ranging from 200 to 500 meters, annual rainfall between 1200 to 1500 mm, and temperatures spanning from 20 to 38 °C (Raj *et al.*, 2022). Morphologically, members of the *Irvingiaceae* family exhibit distinct features such as glabrousness, extremely hard wood, and unique leaf structures with mucilage-containing secretory canals.

Among the genera within the *Irvingiaceae* family, *Irvingia* stands out as a particularly noteworthy one. *Irvingia* species, commonly referred to as wild African mango or bush mango, are valued for their edible fruits and fat/protein-rich nuts. The genus comprises several species, including *Irvingia*

gabonensis, *Irvingia smithii*, *Irvingia grandifolia*, and others (Harris, 1996; Leakey, 1999).

Irvingia gabonensis, also known as African Mango, is a prominent member of the *Irvingiaceae* family. Indigenous to West Africa, including countries like Nigeria, Cameroon, and Ghana, *Irvingia gabonensis* bears edible fruits and seeds that hold significant cultural and medicinal importance. The tree is referred to by various local names such as Apon, Ogbono, and Goron biri in different Nigerian dialects (Unaeye *et al.*, 2017).

Culturally, *Irvingia gabonensis* is cultivated across West African regions for its fruit, which is used in traditional cuisines. The seeds of *Irvingia gabonensis* yield dika fat, rich in C12 and C14 fatty acids, alongside essential vitamins, minerals, and flavonoids (Sun and Jen, 2012). Phytochemical analyses have revealed the presence of various bioactive compounds in *Irvingia gabonensis* seeds, including tannins, alkaloids, flavonoids, and terpenoids (Mahunu *et al.*, 2019; Olorundare *et al.*, 2020).

Furthermore, *Irvingia gabonensis* seed extract has demonstrated notable antioxidant properties attributed to compounds like lupeol (Ekpe *et al.*, 2017). The medicinal potential of *Irvingia gabonensis* extends beyond its nutritional value, with ongoing research exploring its therapeutic applications in diverse health contexts.

Material and Methods:

Ethanol (Analytical grade Sigma-Aldrich, USA), Methanol (Analytical grade Sigma-Aldrich, USA), Ethyl acetate (Analytical grade Sigma-Aldrich, USA), N-Hexane

(Analytical grade Sigma-Aldrich, USA), Aluminum Chloride powder (500g) (Reagent Grade), Quercetin (Reagent grade), Folin-Ciocalteu 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, Labomed) ,UV Lamp (Short/Long-

Wave 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, Boriipe 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 hours, respectively (Chart 1).

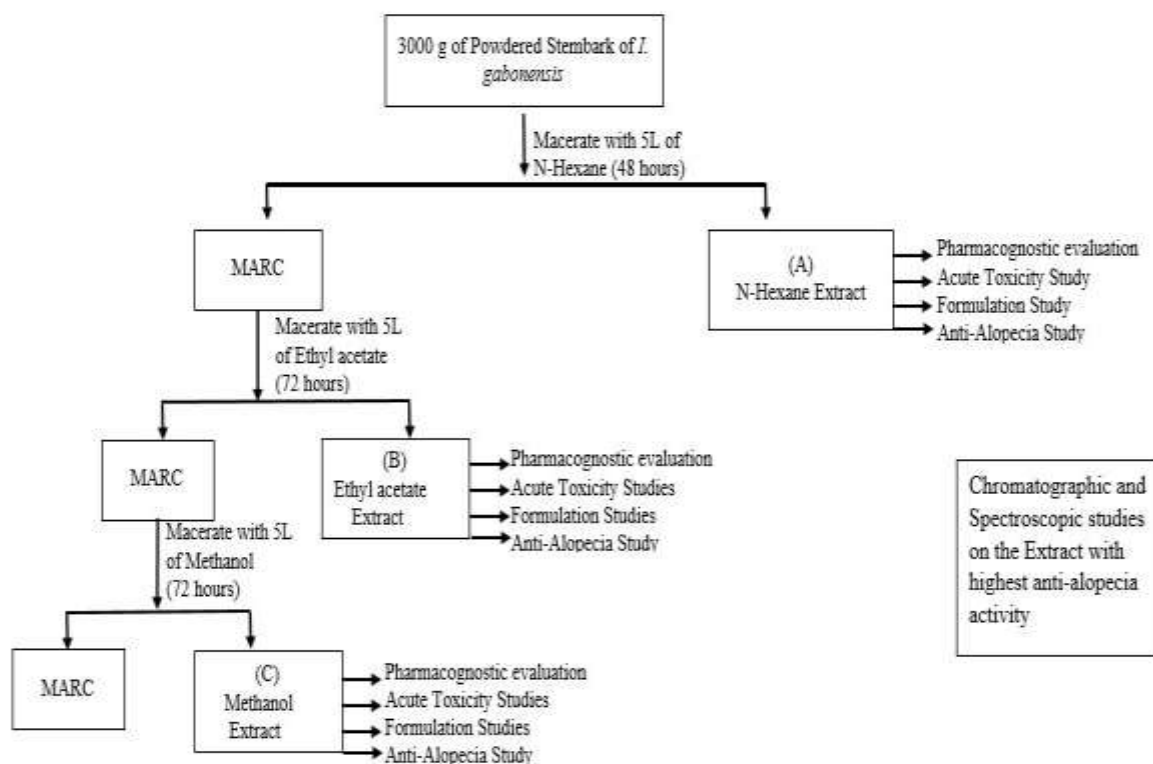


Chart 1: Schematics for the Extraction Process, Biological and Chemical studies of *I. gabonensis*

All the extracts were filtered, and the filtrates were concentrated and stored properly in a desiccator for further analysis. The

percentage yield was also calculated as well with reference to the weight of the powdered plant material (Table 1).

$$\text{Percentage yield of extracts} = \frac{\text{Weight of total } \text{[extracted material]} / \text{Weight of powdered material}}{\text{Weight of powdered material}} \times 100$$

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 n-Hexane, 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:

Total Phenolic Content (TPC): The TPC of the extracts was determined using the Folin-Ciocalteu 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 Folin-Ciocalteu 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

were employed to identify the presence of different phytochemical compounds in the extracts, as represented in Table 2

Table 2: Phytochemical Screening of Constituents of *Irvingia gabonensis* Stem Bark

Tests	nH-E	Et-E	Me-E
Carbohydrate			
Molisch	Absent	Present	Present
Fehling	Absent	Present	Present
Saponin			
Frothing	Absent	Absent	Present
Anthraquinones			
Borntrager	Absent	Absent	Absent
Modified Borntrager	Absent	Absent	Absent
Cardiac glycosides			
Keller- kiliani	Present	Present	Present
Baljet	Present	Present	Present
Flavonoids			
Shinoda	Present	Present	Present
Sodium Hydroxide	Present	Present	Present
Tannins			
Ferric chloride	Present	Present	Present
Lead sub acetate	Present	Present	Present
Alkaloids			
Dragendorff	Present	Present	Present
Mayer	Present	Present	Present
Wagner's Test	Present	Present	Present
Picric acid	Present	Present	Present
Steroids/Triterpenes			
Salkowski	Present	Present	Present
Lieberman-Burchard	Present	Present	Present

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.

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.

Table 3: Quantitative Phytochemical Constituents of *Irvingia gabonensis* Stem Bark

Metabolites	Et-E(mg/g)	Me-E (mg/g)
Total Phenols	122.18 ± 0.76	79.47 ± 0.83
Total Flavonoids	73.42 ± 1.02	36.61 ± 0.49
Total Tannins	164.81 ± 0.82	159.37 ± 0.81

Toxicological Profile of *Irvingia. gabonensis* stem bark.

Acute oral median lethal dose (LD₅₀) in Rats:

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

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 non-polar 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.

that the LD₅₀ 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

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. 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 LD₅₀ 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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