

CHEMICAL COMPOSITION AND SAFETY EVALUATION OF STEM BARK EXTRACTS FROM *IRVINGIA GABONENSIS*

Adewale Samuel Olufemi

Department of Botany, Faculty of Science, Obafemi Awolowo University, Ile-Ife, Nigeria

DOI: <https://doi.org/10.5281/zenodo.19218452>

Abstract: Phytochemical investigations of medicinal plants have emerged as pivotal in identifying natural compounds with therapeutic potential. *Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) Baill., a member of the Irvingiaceae family, is traditionally used in West African ethnomedicine for its wide-ranging health benefits. This study aimed to analyze the phytochemical constituents and assess the acute oral toxicity of *I. gabonensis* stem bark, thereby contributing to the pharmacognostic and toxicological profiling of the species. Plant materials (leaves, fruits, stem bark, and roots) were harvested from Iragbiji, Osun State, Nigeria, and identified with voucher authentication. The stem bark was air-dried, pulverized, and sequentially extracted with n-hexane, ethyl acetate, and methanol via maceration. Qualitative screening revealed the presence of flavonoids, tannins, saponins, steroids, and glycosides, suggesting rich bioactive potential. Quantitative analysis indicated that the ethyl acetate extract contained the highest total phenolic (122 mg/mL) and flavonoid (73.42 mg/mL) content, while methanol extracts showed 79.47 mg/mL and 36.61 mg/mL for phenolic and flavonoid content, respectively. Acute toxicity studies conducted in vivo with Wistar rats showed no signs of toxicity at a limit dose of 5000 mg/kg, indicating a favorable safety margin. These findings affirm the medicinal value of *I. gabonensis* stem bark and provide foundational data for future pharmacological and clinical investigations.

Keywords: *Irvingia gabonensis*, Phytochemicals, Acute toxicity, Traditional medicine, bioactive compounds

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 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, 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, Labomed), 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 hours respectively (Chart 1).

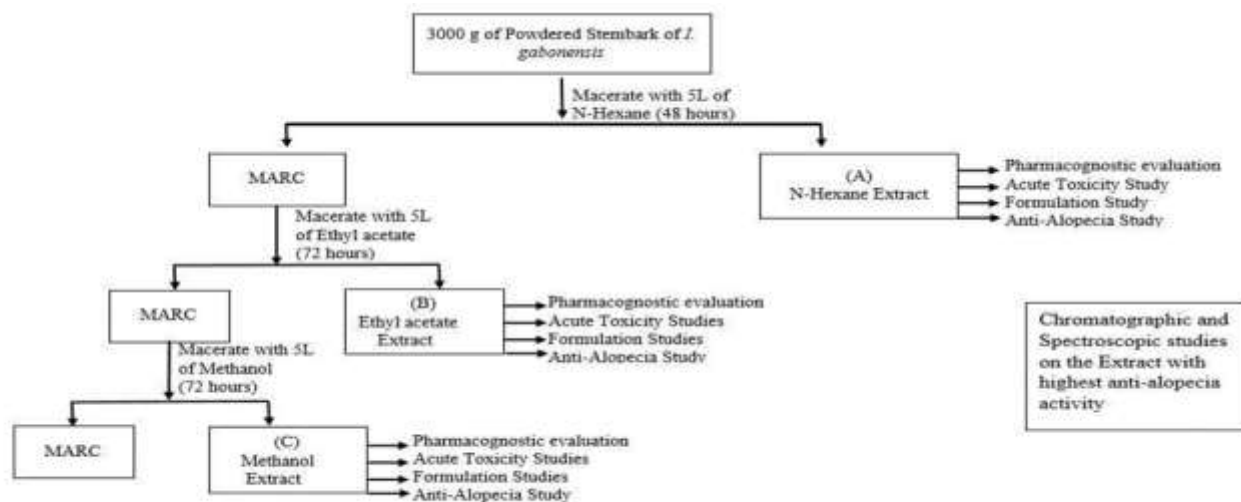


Chart 1: Schematics for the Extraction Process, Biological and Chemical studies of *I. gabonensis*

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:

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.

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.

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 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

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/QEg, 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.

References:

- Aliyu, N., Abdurahman, E., Danmalam, U., Kawu, M. U., Zakariya, A. M., & Ayeni, A. E. (2020). Safety profile of *Irvingia gabonensis* (Aubry-Lecomte ex O'Rorke) Baill. root bark extract: Acute and sub-acute toxicity studies in Wistar rats. *Journal of Medicinal Plants for Economic Development*, 4(1), 1–8.
- Ashutosh, K. (2007). *Pharmacognosy and pharmacobiotechnology* (2nd ed.). New Age International Publishers.
- Atanu, F. O., Ikeojukwu, A., Owolabi, P. A., & Avwioroko, O. J. (2022). Evaluation of chemical composition, in vitro antioxidant, and antidiabetic activities of solvent extracts of *Irvingia*. [Journal details needed].
- Burkill, H. M. (1985). *The useful plants of West Tropical Africa* (Vol. 2). Royal Botanic Gardens, Kew.
- Burkill, H. M. (1995). *The useful plants of West Tropical Africa* (2nd ed., Vol. 3, Families J–L, pp. 489–490). Royal Botanic Gardens, Kew.
- Chanda, S. (2014). Importance of pharmacognostic study of medicinal plants: An overview. *Journal of Pharmacognosy and Phytochemistry*, 2(5), 69–73.
- Cragg, G. M., Newman, D. J., & Snader, K. M. (1997). Natural products in drug discovery and development. *Journal of Natural Products*, 60(1), 52–60.
- Ekpe, O. O., Nwaehujor, C. O., Ejiofor, C. E., Arikpo, P. W., Woruji, E. E., & Amor, T. E. (2019). *Irvingia gabonensis* seeds extract fractionation, its antioxidant analyses, and effects on red blood cell membrane stability. *Pharmacologyonline*, 1, 337–353.
- Evans, W. C. (2011). *Trease and Evans pharmacognosy* (16th ed.). W. B. Saunders.
- Evans, W. C. (2009). *Trease and Evans pharmacognosy* (16th ed.). W. B. Saunders.
- Ewere, E. G., & Okolie, N. P. (2018). Phytochemicals, antioxidant activity and acute toxicity of ethanol stem bark extract of *Irvingia gabonensis* O'Rorke Baill. *EC Pharmacology and Toxicology*, 6(6), 390–399.
- Farnsworth, N. R., & Fabricant, D. S. (2001). The value of plants used in traditional medicine for drug discovery. *Environmental Health Perspectives*, 109(Suppl. 1), 69–75. <https://doi.org/10.1289/ehp.01109s169>
- Ghafar, F., Nazrin, T. T. N. N., Salleh, M., Hadi, N. N., Ahmad, N., Hamzah, A. A., & Azman, I. N. (2017). Total phenolic content and total flavonoid content in *Moringa oleifera* seed. *Galeri Warisan Sains*, 1(1), 23–25.



Harris, D. J. (1996). A revision of the Irvingiaceae in Africa. *Bulletin du Jardin Botanique National de Belgique*, 65(1–2), 143–196.

Kubitzki, K. (2014). Irvingiaceae. In *Flowering Plants. Eudicots* (pp. 229–232). Springer