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PHYTOCHEMICAL CHARACTERIZATION AND ACUTE TOXICITY EVALUATION OF AQUEOUS AND HYDROALCOHOLIC EXTRACTS OF DANIELLIA OLIVERI (FABACEAE) LEAVES

Coulibaly Seydou Ouolouho*, Coulibaly Tialafolo Alassane, Gboko Abiba Ouattara, Toure Abdoulaye

Environment Climate Health Engineering and Sustainable Development Laboratory (LECSI2D), Peleforo GON COULIBALY University, BP 1328 Korhogo, Côte d'Ivoire.

***Corresponding Author: Coulibaly Seydou Ouolouho**

Environment Climate Health Engineering and Sustainable Development Laboratory (LECSI2D), Peleforo GON

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ABSTRACT

In African traditional medicine, *Daniellia oliveri*, a species belonging to the Fabaceae family, holds a prominent place due to its use in the therapeutic management of numerous pathologies, particularly fever, diarrhea, diabetes, inflammation, and urinary tract or uterine infections. Despite its widespread use in traditional medicine, scientific information regarding its phytochemical composition and toxicological profile remains insufficient. In this perspective, the present study aimed to characterize the phytochemical profile of aqueous and hydroethanolic extracts of *Daniellia oliveri* leaves and to evaluate their acute toxicity in order to provide scientific evidence on their safety and therapeutic potential. The phytochemical profile of both types of extracts was established using standard qualitative methods, whereas the acute oral toxicity analysis of *Daniellia oliveri* leaf extracts was performed in accordance with OECD Guideline 423 to estimate the median lethal dose (LD50) and determine its acute toxicity class. Phytochemical analyses revealed the presence of alkaloids, polyphenols, flavonoids, polyterpenes, and sterols in both extracts. These secondary metabolite groups are extensively described in the literature for their diverse biological activities and are likely to contribute to the pharmacological effects of this species. Furthermore, the obtained results indicate that the oral LD50 of both aqueous and hydroethanolic extracts is greater than 5000 mg/kg body weight. According to the Globally Harmonized System (GHS), this value corresponds to a very low acute oral toxicity, resulting in no mortality or significant clinical signs of toxicity in Wistar rats. The absence of acute toxicity signs observed in this study suggests that *Daniellia oliveri* leaf extracts possess a favorable toxicological profile, supporting their traditional medicinal use.

KEYWORDS: *Daniellia oliveri*, phytochemical screening, acute toxicity, safety, medicinal plant.

1. INTRODUCTION

Interest in medicinal plants is steadily increasing worldwide due to their contribution to the prevention and management of various ailments. The recourse to plant-based treatments, as well as the search for new biologically active substances, constitutes one of the major scientific endeavors.

Daniellia oliveri (Fabaceae) is a tree widely distributed across sub-Saharan Africa, reaching heights of 15 to 30 meters. This species is extensively used in traditional medicine across several African countries. Within the rich Ivorian flora, Daniellia oliveri is among the medicinal species widely employed in traditional medicine (Mian et al., 2025). Various parts of this plant are used for treating different conditions. In particular, the leaves are utilized for managing headaches, fever, inflammation, glaucoma, dental pain, gastrointestinal disorders, burns, and epilepsy (Kaboré, 2009; Diaby, 2014; Soro et al., 2022; Mian et al., 2025).

Despite these numerous therapeutic applications and the pharmacological activities attributed to it, available scientific data remain insufficient to fully substantiate the traditional uses of Daniellia oliveri. Therefore, further investigations are required to characterize its

phytochemical composition and evaluate its toxicological profile, thereby contributing to the scientific validation of its traditional medicinal use.

2. MATERIALS AND METHODS

2.1. Biological Material

2.1.1. Plant Material

The leaves of Daniellia oliveri used in this study were collected in the Poro region, in the vicinity of the city of Korhogo, Côte d'Ivoire. Botanical authentication of the species was performed at the National Floristic Center (CNF) of Félix Houphouët-Boigny University in Abidjan.

2.1.2. Animal Material

The study was conducted on adult Wistar rats, with a mean body weight ranging between 120 and 130 g. The animals were obtained from the animal house of the Faculty of Pharmaceutical and Biological Sciences at Félix Houphouët-Boigny University (Abidjan, Côte d'Ivoire).

2.2. METHODS

2.2.1. Processing of Leaves

The harvested leaves were promptly transported to the Biotechnology and Agroresource Transformation Laboratory at Peleforo GON COULIBALY University (Korhogo, Côte d'Ivoire). After sorting, removal of debris, and separation of stems, the leaves were washed with distilled water. The leaves were then drained at room temperature (20 °C) and shade-dried on benchtop surfaces (25–28 °C) for 15 days. The dried plant material was ground using a micro-mill (CULATTI, France) fitted with a 10 µm sieve, and subsequently stored at 4 °C.

2.2.2. Preparation of Aqueous and Hydroethanolic Extracts

The aqueous extract of Daniellia oliveri was obtained according to the method described by Zirihi et al. (2003). A 100 g quantity of plant powder was macerated in 1 L of distilled water and stirred for 24 hours using a magnetic stirrer. After homogenization, the mixture was filtered sequentially through absorbent cotton and Whatman filter paper. The filtrate was evaporated in an oven at 40 °C to yield the crude aqueous extract.

The 70% hydroethanolic extract (1 L ethanol/water, 70:30, v/v) was prepared following the method of Guédé-Guina (1990). A 100 g sample of Daniellia oliveri leaf powder was subjected to extraction with the hydroethanolic mixture under magnetic stirring for 24 hours. Following filtration through absorbent cotton and then under vacuum under the same conditions as the aqueous extract, the filtrate was concentrated using a rotary evaporator and dried at 40 °C in an oven. The resulting dry product constituted the 70% hydroethanolic extract.

2.3. Phytochemical Study

The phytochemical study of the aqueous and hydroethanolic leaf extracts of Daniellia oliveri was conducted using standard qualitative tests to identify major chemical groups according to methods described by Trease and Evans (2002). The target metabolites included alkaloids, polyphenols, flavonoids, tannins, polyterpenes and sterols, leucoanthocyanins, and saponins.

The presence of sterols and polyterpenes was determined using the Liebermann-Burchard reaction. Polyphenols were detected using the ferric chloride test ($FeCl_3$), while flavonoids were identified via the cyanidin reaction. Tannins were revealed using Stiasny's test. Alkaloids were characterized using Dragendorff's reagent. The presence of saponins was assessed by observing persistent foam formation after shaking aqueous solutions. Finally, leucoanthocyanins were detected by the development of a red to reddish-purple coloration following the addition of concentrated hydrochloric acid (HCl).

2.4. Acute Toxicity Study

The evaluation of the acute toxicity of the aqueous and hydroethanolic leaf extracts of Daniellia oliveri was carried out in compliance with Guideline 423 of the Organisation for Economic Co-operation and Development (OECD, 2001). A 0.9% sodium chloride (NaCl) solution was used to prepare the different extract doses. The administered concentrations were established based on the mean animal weight and expressed in mg/kg body weight (b.w.). The mean weight of the rats ranged from 120 ± 0.03 to 130 ± 0.05 g.

Animals were fasted for 24 hours prior to the administration of the extract doses and saline solution. For administration corresponding to a dose of 100 mg/kg b.w., a volume of 1 mL of solution was administered to each experimental group. The control group (Group 1) received 0.9% NaCl solution. Groups 2, 3, and 4 received doses of 300, 2000, and 5000 mg/kg of D. oliveri aqueous extract, respectively. Groups 5, 6, and 7 received doses of 300, 2000, and 5000 mg/kg b.w. of the 70% hydroethanolic extract, respectively. Following administration, the animals were continuously observed during the first 24 hours and subsequently monitored daily for 14 days. Particular attention was paid during the first 24 hours to observe clinical signs of toxicity and potential mortality in each experimental group.

3. RESULTS

3.1. Phytochemical Study

Phytochemical screening of the aqueous and hydroethanolic leaf extracts of D. oliveri revealed the presence of various secondary metabolites, including alkaloids, polyphenols, flavonoids, polyterpenes, and sterols. In contrast, tannins and saponins were not detected in either extract (Table 1).

Table 1: Target secondary metabolites in the two types of D. oliveri extracts.

Target Secondary Metabolites	Test	AqE	HEe
Alkaloids	Bouchardat / Dragendorff	+	+/--
Flavonoids	Cyanidin	+	++
Polyphenols	2% Ferric trichloride	+	++
Leucoanthocyanins	Acidification	+	--
Catechic tannins	Stiasny	+	--
Gallic tannins	Stiasny	--	--
Polyterpenes and sterols	Liebermann-Burchard	+	++
Saponins	Foam formation	+	--

Strong presence: ++; Presence: +; Trace: +/--; Absence: --; AqE = aqueous extract; HEe = hydroethanolic extract.

3.2. Acute Toxicity

Administration of the aqueous and hydroethanolic leaf extracts of D. oliveri at doses of 300, 2000, and 5000 mg/ kg b.w. induced no clinical signs of toxicity or behavioral alterations in adult female rats. No mortality was recorded throughout the 14-day observation period (Table 2).

Table 2: Clinical signs, behavior, and mortality observed following administration of aqueous and hydroethanolic leaf extracts of D. oliveri in female rats.

Parameters	Aqueous Extract			Hydroethanolic Extract (70%)		
	300 mg/kg	2000 mg/kg	5000 mg/kg	300 mg/kg	2000 mg/kg	5000 mg/kg
Mobility	Good	Good	Good	Good	Good	Good
Limb paralysis	No	No	No	No	No	No
Respiration	Normal	Normal	Normal	Normal	Normal	Normal
Stool appearance	Normal	Normal	Normal	Normal	Normal	Normal
Aggressiveness	No	No	No	No	No	No
Convulsion	No	No	No	No	No	No
Feeding	Good	Good	Good	Good	Good	Good
Mortality	0	0	0	0	0	0

4. DISCUSSION

The utilization of medicinal plants has grown considerably over recent decades, holding a prominent place in the healthcare systems of many countries. Concurrently, the discovery and characterization of novel plant-derived bioactive molecules remain major scientific research objectives aimed at developing new therapeutic approaches.

Phytochemical analysis allowed for the identification of the main secondary metabolites present in both types of Daniellia oliveri leaf extracts. The major bioactive compounds identified are alkaloids, polyphenols, flavonoids, polyterpenes, and sterols. The results obtained align with those of N'Guessan et al. (2009), who likewise highlighted the presence of identical secondary metabolite groups in several Ivorian medicinal plants used in Krobou country, notably in the aqueous extract of Paullinia pinnata and methanolic extracts of Trema orientalis leaves and Petersianthus macrocarpus bark. Conversely, the findings of the present study differ from those reported by Soro et al. (2016), who did not detect saponins in the aqueous leaf extract of Daniellia oliveri during their investigation on its anti-inflammatory activity. Variations in the phytochemical profile of Daniellia oliveri observed across different studies can be attributed to several factors, including biological factors related to the specific plant organ analyzed and its developmental stage, environmental factors associated with ecological conditions and harvest period, and methodological factors such as the extraction process, solvent nature, and detection method sensitivity. The abundance of bioactive compounds in the aqueous and hydroethanolic extracts attests to their biological potential. The presence of these secondary metabolites could contribute to the pharmacological activities reported in the literature and suggests potential utility in the prevention or management of pathologies associated with oxidative stress (Diomandé et al., 2018).

The oral acute toxicity study, conducted in accordance with OECD Guideline 423 (OECD, 2001), demonstrated that administration of the aqueous and hydroethanolic leaf extracts of Daniellia oliveri did not induce mortality, clinical signs of toxicity, or behavioral alterations in female rats, even at the limit dose of 5000 mg/kg b.w. The results of the present study agree with those reported by Mian et al. (2025), who evaluated the anti-inflammatory activity of an aqueous leaf extract fraction (FDo4) of Daniellia oliveri. The observations of this study are also consistent with those of Ibe et al. (2022), who demonstrated the protective effects of an ethanolic leaf extract of Ficus capensis in a phenylhydrazine-induced anemia model in Wistar rats. These authors reported no mortality, clinical signs of toxicity, or behavioral changes in female rats following oral

administration of aqueous and ethanolic extracts of these plants up to the limit dose of 5000 mg/kg b.w., in agreement with OECD Guideline 423.

The LD_{50} of both aqueous and hydroethanolic leaf extracts of Daniellia oliveri is estimated to be greater than 5000 mg/kg b.w. Although phytochemical screening revealed traces of alkaloids in the hydroethanolic extract, no mortality or clinical signs of toxicity were observed at the administered doses. These results suggest that the alkaloids present are either weakly toxic or present in insufficient concentrations to induce acute toxicity under the experimental conditions of this study (Behidj-Benyounes et al., 2013; Guergour, 2018). The low toxicity of the aqueous extract could be attributed to the extraction process, as distilled water likely does not extract certain potentially toxic compounds localized in mucilage cells (Bruneton, 1999). According to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS), as applied under OECD Guideline 423 (2001), the aqueous extract of Daniellia oliveri is classified under Category 5 acute toxicity, indicating low toxicity.

5. CONCLUSION

This study revealed the presence of various secondary metabolites in the aqueous and hydroethanolic leaf extracts of Daniellia oliveri, including flavonoids, polyphenols, alkaloids, polyterpenes, and sterols. The presence of these compounds may contribute to the pharmacologically significant biological activities attributed to this species. Acute toxicity evaluation categorized Daniellia oliveri among non-toxic substances under the experimental conditions tested. These findings suggest that this plant represents a valuable source of bioactive compounds for the traditional management of numerous pathologies.

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