

## Phytochemical Screening and Study of the Acute Oral Toxicity of the Aqueous Extract of The Leaves of *Diospyros hoyleana* F.white (Ebenaceae)

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### Abstract

Despite the advent of generic drugs, many treatments still remain financially inaccessible to economically disadvantaged populations who constantly continue to turn to bioactive molecules from nature to find essential remedies that are gentle, more effective and with few side effects. Medicinal plants are still the primary reservoir of new drugs. The material used during this study consisted of plant material, animal material and technical material. 500 g of the sprayed plant drug was macerated in 2.5 L of distilled water for one duration of 48 hours. Numerous secondary metabolites of the aqueous extract of the leaves of *Diospyros hoyleana* F. White have been demonstrated. The acute oral toxicity experiment was conducted according to OECD protocol guideline 423. The mass of crude extract obtained after drying in an oven at 60 °C. was 64 g with a yield of 12.8%. Tests for Flavonoids, Saponins, Phenols, Tannins and Anthraquinones were found to be positive with the aqueous extract of the leaves of *Diospyros hoyleana* F. White while those for Alkaloids, Sterols, Terpenes and Coumarins were found to be Negative. After the acute oral toxicity study, no abnormalities of the physiological parameters observed in rats were observed. In addition, no deaths have been recorded. The LD<sub>50</sub> was therefore greater than 5000 mg/kg. The body masses of the animals were generally increasing during the study regardless of the chosen batch. The rats were sacrificed and the masses of the internal organs were weighed. Four blood biochemical parameters of the groups of rats studied were assayed.

**Keywords:** Medicinal plants, phytochemical screening, acute oral toxicity, *Diospyros hoyleana* F. White

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### INTRODUCTION

Cameroon is an undisputed potential of biodiversity because of the diversity and the richness of its flora. In addition to their role in the balance of ecosystems, plants provide humans with essential natural resources for their survival and development. Plants contribute to food security and primary health care for nearly 80% of the population in developing countries [1]. Furthermore, despite the advent of generic drugs, many treatments still remain financially inaccessible to economically disadvantaged populations who constantly continue to turn to bioactive molecules from nature, in search of effective essential remedies with few side effects. Medicinal plants are still the primary reservoir of new drugs. They are considered a source of essential raw material for the discovery of

new molecules necessary for the development of future drugs through their active compounds [2].

Secondary metabolites are a group of molecules which intervene in the adaptation of plants to their environment. They are present in all higher plants, and have a limited distribution in the plant organism. More than 200,000 structures have been defined. They are of extraordinary structural variety but are produced in small quantities [3]. These molecules mark in an original way, a family, a genus or a species of plant and sometimes make it possible to establish a chemical taxonomy. They represent 2 to 3% of the organic matter of plants and in some cases up to 10% and even more. Polyphenols or phenolic compounds form a large class of chemicals found in plants' surface tissues. They are

polyhydroxylated phytochemicals and comprising at least one aromatic ring with 6 carbons. Tannins are polyphenolic substances of plant origin that have the property of transforming fresh skin into a rot-resistant material [4]. Flavonoids represent a class of secondary metabolites widely distributed in the plant kingdom. They are almost universal plant pigments which are partly responsible for the coloring of flowers, fruits and sometimes leaves [5]. Flavonoids, mainly known for their antioxidant action, help fight free radicals from oxygen and nitrogen. The oxidative stress caused by these free radicals seems to weaken the good health of the body [6]. They have antibacterial, antiviral, anti-cancer, anti-tumor, anti-inflammatory, anti-allergic, anti-hepatotoxic, anti-spasmodic activities [7]. Flavonoids may have a positive impact on diabetes by inhibiting the enzyme aldose reductase. Alkaloids are natural and organic substances which originate mainly from plants and which contain at least one nitrogen atom in their chemical structure, with varying degrees of alkalinity [8]. They find several pharmaceutical applications in humans. They are anti-tumor (vincalcoblastine, vincristine, taxol, camptothecine), analgesics (morphine, codeine), spasmolytics (tubocurarine and papaverine); vasodilators (vincamine and ajmalicin), emetics (emetine), cough suppressants (codeine), antiarrhythmics (quinidine and ajmaline) and antimalarials (quinine). They are also agents for the treatment of Alzheimer's disease (galanthamine). Saponins are heterosidic substances with hemolytic, antimicrobial, insecticidal, anti-inflammatory and analgesic properties. However, the majority of plants used in traditional medicine can have toxic effects.

## MATERIAL AND METHODS

### Equipment

Plant material: the plant material used was the leaves of *Diospyros hoyleana* F. White collected in Dibombari, Littoral Region, of Cameroon. The plant was identified at the Cameroon's National Herbarium and compared to sample No. 1745 / HNC.

Animal material: Wistar albino rats were used, obtained at the animal breeding facility of the Herbarium the Plants Museum in Douala.

## METHODOLOGY

Leaf Drying and Spraying: Once harvested, the leaves of *D. hoyleana* were dried at room temperature away from direct sunlight, then spread out on plastic sheeting and turned daily for even drying. This operation lasted 2 weeks after which the leaves were pulverized as described elsewhere [9].

Maceration: an aqueous extract was obtained by macerating 500 g of the pulverized vegetable drug in 2.5l of distilled water for a period of 48 hours. This operation was repeated once, in order to maximize the extraction. At the end of this operation, the filtrate

obtained was brought to a water bath at a temperature of 60°C.

Calculation of the extraction yield: The extraction yield (EY) was calculated relative to the mass of dry plant matter according to the formula:

$$EY = \frac{\text{Mass of the extract}}{\text{Initial mass of powder}} \times 100$$

### Phytochemical screening

The phytochemical screening of the aqueous extract of the leaves of *D. hoyleana* was carried out at the Analytical Chemistry Laboratory, Faculty of Medicine and Pharmaceutical Sciences, University of Douala.

Phenol identification test: 2 ml of extract were placed in a test tube and 1 ml of 5% ferric chloride was added. The appearance of a thick blackish-blue color indicates the presence of phenols.

Shinoda test: for the identification of flavonoids, magnesium shavings, methanol, and hydrochloric acid were used as reagents. The dry extract was dissolved into 3 ml of methanol, and a few shavings of magnesium were added, followed by 5 drops of hydrochloric acid. The appearance of a purple or red-orange color indicates the presence of flavonoids.

Liebermann Burchard test: this test was used to identify sterols and triterpenes. The reagents used were chloroform, acetic anhydride, concentrated sulfuric acid. A small amount of dry extract was dissolved in a few drops of chloroform. A few drops of acetic anhydride were added, followed by sulfuric acid. A purple color that turns green indicates the presence of a sterol. An initial brick-red coloration that turns purple indicates the presence of a triterpene.

Dragendorff test: The purpose of the test is to identify alkaloids. The reagents used are bismuth nitrate, distilled water, acetic acid, potassium iodide. The Dragendorff's reagent was prepared by mixing a concentrated solution of potassium iodide (8g of KI with 200 ml of distilled water) with an equal volume of a bismuth (III) nitrate solution (0.85g of Bi(NO<sub>3</sub>)<sub>3</sub> in 100 ml of distilled water and 10 ml of acetic acid) and adding 100 ml of distilled water to 10 ml of this mixture, as well as 100 ml of acetic acid.

A small quantity of the extract was dissolved in methanol. The presence of alkaloids was materialized by the appearance of a precipitate when adding the Dragendorff's reagent or spraying a TLC plate. The color of the precipitate varied between yellow, orange, red and brown, depending on the nature of the alkaloid.

**Saponin identification test:** a small amount of dry extract was dissolved in 1 ml of distilled water. The aqueous solution obtained was subjected to energetic stirring for 3 seconds. The presence of saponins results in the formation of a thick foam which persists for about 30 minutes.

**Bornstrager test:** this test was used to identify of anthraquinones, by adding 1ml of 25% ammonia to an equal volume of methanolic extract. The appearance of a red coloration indicates the presence of anthraquinones.

**Tannin identification test:** the purpose of the test was to highlight tannins in the extract. The reagent used was 5% ferric chloride (FeCl<sub>3</sub>). In a test tube, 2 ml of extract was introduced, then 1 ml of FeCl<sub>3</sub> solution. The appearance of a bluish to black color indicated the presence of tannins.

#### Acute toxicity assessment

The acute oral toxicity experiment was conducted according to OECD protocol guideline 423 [10]. 10-week-old female Wistar strain rats were fasted overnight before the experiment from 8 p.m. to 8 a.m. Four batches of three randomly assigned rats were dosed with 50, 300, 2000 and 5000 mg/kg body weight of the aqueous extract from the leaves of *D. hoyleana*. The control batch received distilled water. The rats were observed for 2 hours after administration of the extract, then fed. Furthermore, they were observed after 4 hours, 8 hours and then 14 days, during which the symptoms of intoxication (change in coat, motility, tremors, grooming, breathing, sensitivity to noise, as well as deaths) were noted. The dead rats in each batch were counted for the determination of the LD<sub>50</sub>. The extract was administered to the animals orally.

## RESULTS

**Extraction Yield:** The aqueous extract was prepared from 500g of macerated powder from the leaves of *D. hoyleana*. The mass of crude extract obtained after drying in an oven at 60°C was 64 g, representing a yield of 12.8%.

**Phytochemical screening:** the phytochemical study of the aqueous extract of *D. hoyleana*'s leaves has revealed the presence of various groups of molecules. Various metabolites (9) were therefore highlighted. Five tests returned positive results, namely that for flavonoids, saponins, phenols, tannins and anthraquinones, while the remaining 4 turned out negative: alkaloids, sterols, terpenes and coumarins (Table I).

**Table-I: Secondary metabolites tested**

Secondary metabolites	Test result
Alkaloids	-
Sterols	-
Flavonoids	+
Saponins	+
Phenols	+
Tannins	+
Terpenes	-
Coumarins	-
Anthraquinones	+

(+) = positive test; (-) = negative test.

#### Acute toxicity of aqueous extracts of *Diospyros hoyleana*

The acute toxicity study looked at physiological changes in Wistar-strain albino rats. The aqueous extract of *D. hoyleana*'s leaves was administered to the rats and observed during the first 2h, then at 4h, 8h and 14 days after administration. At the end of the fourteen days of observation, no anomalies were found in the parameters studied. In addition, no deaths were being recorded. The LD<sub>50</sub> is therefore greater than 5,000 mg/kg (Table II).

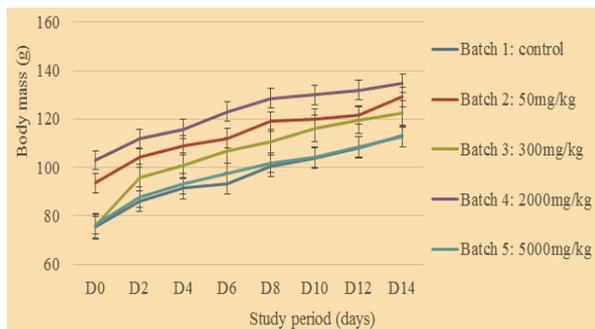
**Table-II: Observation of physiological parameters of rats**

Groups Criteria	Batch 1 control	Batch 2 50mg / kg	Batch 3 300mg/kg	Batch 4 2000mg/kg	Batch 5 5000mg/kg
Grooming	N	N	N	N	N
Drowsiness	Abs	Abs	Abs	Abs	Abs
Salivation	Abs	Abs	Abs	Abs	Abs
Eye colour	N	N	N	N	N
Behaviour	N	N	N	N	N
Coat	N	N	N	N	N
Tremor	N	N	N	N	N
Motility	N	N	N	N	N
Sound reaction	N	N	N	N	N
Appearance of salts	N	N	N	N	N
Number of deaths	0	0	0	0	0

N = normal; Abs = absent; 0 = zero.

Effects of the aqueous extract *Diospyros hoyleana*'s leaves on the evolution of body masses of batches of rats during the study of acute oral toxicity

The body masses of the animals were generally increasing during the acute oral toxicity study regardless of the batch chosen. Those of the control batch increased from 75.37g on D0 to 112.6g on D14, an increase of 37.23g. Those of batch 2 increased from 93.55g on D0 to 129.1g on D14, an increase of 35.55g. Rats in the third batch went from 75.82g on D0 to 122.3g on D14, an increase of 46.48g. Batch 4 went from 102.98g on D0 to 134.7g on D14, an increase of 31.72g. Finally, rats in batch 5 went from 76.51g on D0 to 112.6g on D14, an increase of 36.09g. Comparing the body masses of the same batch on D0 and D14 using the ANOVA test revealed very significant differences regardless of the batch chosen (Figure 1).

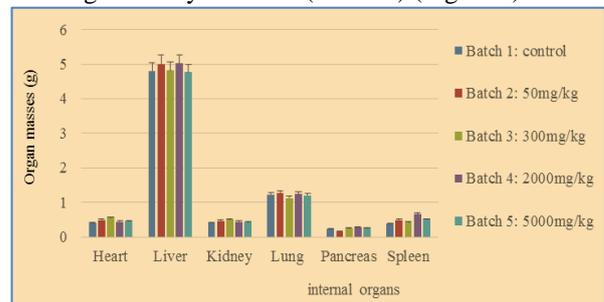


**Fig-1: Evolution of the body masses of batches of rats**

**Effects of the aqueous extract of the leaves of *Diospyros hoyleana* on the masses of the internal organs of batches of rats**

After 14 days observing the rats following the acute oral toxicity study, they were sacrificed and the average internal organ masses were weighed. The highest average mass of hearts (0.55 g) was obtained in batch 3 while the lowest (0.4 g) was obtained in batch 1. The highest average mass of livers (5.02 g) was obtained in batch 4 while the lowest (4.76 g) was obtained in batch 5. The highest average mass of the kidneys (0.5g) was obtained in batch 3 while the lowest one (0.41 g) was obtained in batch 1. The highest average lung mass (1.28 g) was obtained in batch 2 while the lowest one (1, 13 g) was obtained in batch 3. The highest average pancreas mass (0.29 g) was

obtained in batch 4 while the lowest one (0.16 g) was obtained in batch 2. The highest average mass of spleens (0.66 g) was obtained in batch 4 while the lowest one (0.38 g) was obtained in batch 1. Compared to the average mass of hearts of Control batch, the mean heart masses of rats in batches 2 and 3 were found to be significantly different ( $P < 0.01$ ), while those of lots 4 and 5 were not significantly different ( $P > 0.05$ ). Likewise, the mean masses of the livers of the rats from batches 2 and 4 were found to be significantly different ( $p < 0.001$ ) from that of the control batch, while those from batches 3 and 5 were not found to be significantly different ( $P > 0, 05$ ). The mean masses of the kidneys of the rats from batches 2 and 3 were found to be significantly different ( $P < 0.01$ ) from that of the control batch, while those from batches 4 and 5 were not found to be significantly different ( $P > 0.05$ ) (Figure 2).



**Fig-2: Internal organs' mass within batches of rats**

**Effects of the aqueous extract of the leaves of *Diospyros hoyleana* F. White on the blood biochemical parameters of the batches of rats studied**

Four blood biochemical parameters of the groups of rats studied were assayed to understand the state of functioning of the kidneys and liver at the end of the study period. The highest urea rate (3.46 mmol/l) was obtained in batch 4 while the lowest rate (2.53 mmol/l) was obtained in batch 2. The highest creatinine level (28.33 mmol/l) was recorded in batch 5 (5000 mg/kg) while the lowest level was obtained in batch 3 (300 mg/kg). The highest level of ALAT (22.33 IU/l) was recorded in lots 2 (50 mg/kg) and 4 (2000 mg/kg) while the lowest level (18.67 IU/l) was obtained in batch 3 (300 mg/kg). The highest ASAT level (67.33 IU/l) was recorded in batch 4 (2000 mg/kg) while the lowest level (58.33 IU/l) was obtained in batch 3 (300 mg/kg) (Table III).

**Table-III: Biochemical parameters of batches of rats**

Groups	urea (mmol/l)	Creatinine (mmol/l)	ALAT (UI/l)	ASAT (UI/l)
batch 1: control	2,56 ± 0,30	25,20 ± 7,70	20,00 ± 4,35	63,33 ± 7,638
batch 2: 50 mg/kg	2,53 ± 0,56	25,33 ± 10,07	22,33 ± 7,50	61,67 ± 14,19
batch 3: 300 mg/kg	2,73 ± 0,32	23,33 ± 10,12	18,67 ± 3,51	58,33 ± 18,93
batch 4: 2000mg/kg	3,46 ± 0,45*	26,33 ± 3,21	22,33 ± 3,78	67,33 ± 8,021
batch 5: 5000mg/kg	2,86 ± 0,05	28,33 ± 12,01	22,00 ± 6,24	59,33 ± 10,50

## DISCUSSION

The presence of secondary metabolites in plant extracts is responsible for their various pharmacological properties [9]. Saponins are heterosidic substances with a surface-active property. Alkaloids have anti-ulcer, anti-hypertensive, anti-inflammatory, anti-cancer analgesic, sedative and neurological properties. The flavonoids have anti-inflammatory, antidiabetic, antiviral, antimicrobial, antimalarial properties, and also intervene in the fight against venereal diseases. Tannins are antioxidants, antibacterial, and antifungals [9]. The phytochemical study of the aqueous extract of *D. hoyleana*'s leaves revealed the presence of various groups of molecules. The tests for the presence of various metabolites revealed the presence of five, namely: the flavonoids, saponins, phenols, tannins and anthraquinones. No alkaloids, sterols, terpenes or coumarins were found in the extract. These results are similar to those of Tankeu *et al.* [9] who worked on extracts from the bark of *Musanga cecropioides* and the fruits of *Picalima nitida* obtained by maceration with several types of solvent. They obtained positive tests for flavonoids, saponins, phenols and tannins among those tested in this study. Etame *et al.* [12] found similar results.

At the end of fourteen days of observation, corresponding to the period of study of the acute oral toxicity of the aqueous extract of *D. hoyleana*'s leaves on Wistar albino rats, no abnormalities were found in the physiological parameters observed and no deaths were recorded, leaving the LD<sub>50</sub> above the dose 5000 mg/kg. Therefore, the aqueous extract of *D. hoyleana*'s leaves can be classified as non-toxic according to the Hodge and Stenner toxicity scale [13]. Tankeu *et al.* [9] found results going in the same direction whereas Bounihi [14] in his work found an LD<sub>50</sub> of 2500 mg/kg.

The increase in body masses of the batches of rats during this study would be due to the non-toxicity of the aqueous extract of *D. hoyleana*'s leaves. This result supports that of the observation of physiological parameters which also showed that the aqueous extract of *D. hoyleana*'s leaves would be of low toxicity and could be used as a traditional medicine. Dibong *et al.* [15] found similar results. They showed that the weight growth of males and females increased overall regardless of the lot chosen.

## CONCLUSION

The general objective of this work was to identify secondary metabolites and study the acute oral toxicity of the aqueous extract of *Diospyros hoyleana*'s leaves. At the end of the study, the mass of the extract obtained after drying in an oven at 60°C was 64 g with an extraction yield of 12.8%. Tests for flavonoids, saponins, phenols, tannins and anthraquinones were found to be positive with the extract, while those for

alkaloids, sterols, terpenes and coumarins were negative. At the end of fourteen days of observation, corresponding to the period of study of the acute oral toxicity of the aqueous extract on Wistar albino rats, no abnormalities were found in the physiological parameters observed. In addition, no deaths were recorded, proving an LD<sub>50</sub> greater than 5000 mg/kg. Such a species deserves to be protected for a rational and sustainable management because it is part of the heritage of the African pharmacopoeia in general and Cameroonian in particular. An in-depth study of the pharmacological properties of the extract of this species would favor the formulation of improved traditional medicines.

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