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# Phytochemical screening and acute toxicity of aqueous and hydroethanolic extracts of the stem bark and leaves of *Albizia zygia* harvested in Korhogo

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## Abstract

The global increase in the number of reported diabetes cases each year is alarming, particularly in developing countries. Due to limited financial resources, many patients rely on herbal medicine. *Albizia zygia* is a plant commonly used in West Africa for traditional treatments of various diseases, including diabetes. The objective of this study was to identify the families of chemical compounds in the aqueous and hydroethanolic extracts of the bark and leaves of *Albizia zygia* and to assess acute toxicity. The stem bark and leaves of *Albizia zygia* were collected from the botanical garden of Peleforo GON COULIBALY University in Korhogo and extracted using water and a hydroethanolic mixture (70:30, v/v). Phytochemical screening was performed using thin-layer chromatography, and acute toxicity was assessed using the OECD 423 method. Phytochemical analysis revealed the presence of polyphenols, alkaloids, tannins, sterols, and flavonoids in the various *Albizia zygia* extracts. Acute toxicity testing showed that this plant is non-toxic at a dose of 2000 mg/kg body weight. The results obtained may support its use in traditional medicine.

**Keywords:** *Albizia zygia*; Phytochemical screening; Polyphenols; OECD 423; Acute toxicity

## 1. Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by persistent hyperglycemia resulting either from insulin deficiency or peripheral resistance to its action (Aranaz et *al*., 2023). The prevalence of type 2 diabetes is steadily increasing, making it a major public health problem in both developed and developing countries (American Diabetes Association). In Côte d'Ivoire, the prevalence rose from 5.7% in 1997 to 6.2% in 2017 (PNLMM/PMNT), and the WHO estimates that in Africa, the number of people with diabetes could reach 47.1 million adults aged 20 to 79 by 2047 (Yao et *al.*, 2023). This rapid increase places considerable pressure on health systems responsible for managing non-communicable diseases. Medical management of this disease requires significant financial resources. Consequently, patients turn to traditional medicine, which is perceived as more accessible (WHO, 2011). *Albizia zygia* is a medicinal plant used in the treatment of diabetes in Korhogo. This study aimed to characterize the chemical composition of this plant and determine its acute toxicity in order to better document its pharmacological profile.

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## 2. Materials and Methods

### 2.1. Plant Material

Leaves and stem bark of *Albizia zygia* were collected in March 2023 from the botanical garden of Peleforo GON COULIBALY University. The bark was removed using a knife.

### 2.2. Methods

#### *2.2.1. Preparation of AMETe, AMETm, JFe, and JFm powders*

The various parts of *Albizia zygia* were dried at room temperature for two weeks (leaves) and four weeks (stem bark). Subsequently, these parts were ground into a fine powder using a Binatone Blender-BLG-450P MK2-1. 5L blender. The resulting powder was then stored in containers for our various analyses.

AMETe: aqueous extract of *Albizia zygia* stem bark; AMETm: hydroethanolic extract of *Albizia zygia* stem bark; JFe: aqueous extract of *Albizia zygia* leaves; JFm: hydroethanolic extract of *Albizia zygia* leaves

#### *2.2.2. Extraction of AMETe, AMETm, JFe, and JFm*

Preparation of AMETe, AMETm, JFe, and JFm

After drying and grinding the plant material, extractions were performed. Thus, 250 g of the powder was dissolved in 2.5 L of distilled water for AMETe and JFe, and in 1.75 L of ethanol and 0.75 L of distilled water (70:30, v/v) for AMETm and JFm (Yohanna B. et *al.*, 2022; N'guessan et *al*., 2025a; Able et *al.*, 2025). The mixture was homogenized by magnetic stirring for three hours at room temperature, followed by maceration at the same temperature for 24 hours prior to further processing. The supernatant was then filtered through cotton placed in a funnel after an additional 5 minutes of stirring and sieving. The resulting filtrate was centrifuged at 10,000 rpm (Jouan centrifuge, TH 12) for 15 minutes at room temperature. The resulting solution was then placed in an oven at a temperature of 50°C. These four steps, performed individually yielded the crude extracts AMETe, AMETm, JFe, and JFm.

#### *2.2.3. Phytochemical Screening*

Phytochemical analyses aimed to detect the various categories of specialized metabolites present in plant organs through qualitative evaluation. Numerous families of chemical compounds have been examined. These include alkaloids, coumarins, flavonoids, tannins, saponins, sterols, and polyterpenes.

Detection of saponins using the foam test

Saponins are detected by dissolving 2 g of powder in 100 mL of distilled water. The resulting solution is then boiled for 30 minutes. After cooling, the solution is filtered, and the filtrate is adjusted to 100 mL with distilled water. The stock solution is divided into 10 test tubes (1 mL, 2 mL, 3 mL, 4 mL, 5 mL, 6 mL, 7 mL, 8 mL, 9 mL, and 10 mL). Each tube must be brought to 10 mL with distilled water. The solution is vigorously shaken for 15 seconds, then allowed to stand for 15 minutes. Finally, the height of the foam in each tube is measured. The presence of saponins is evaluated as follows (Trease and Evans, 1987):

- -No foam = negative test
- -Foam < 1 cm = weakly positive test
- -Foam 1–2 cm = positive test
- -Foam more than 2 cm = strongly positive test

The foam index (I) is determined using the following formula: I = 1000/N where N is the number of the tube. In this equation, N represents the tube number in which the foam reaches a height of 1 cm. A foam index greater than 100 suggests the presence of saponins.

- Screening for saponosides (steroidal and triterpenic) by TLC

A qualitative method was performed using thin-layer chromatography (TLC) on aqueous and hydroethanolic extracts of the AMET trunk bark and JF leaves of *Albizia zygia*. The support used was aluminum, Merck, TLC Silica Gel 60 F 254, and the optimal solvent was a mixture of $CH_2Cl_2$/AcOEt/acetic acid (7:2:1, v/v/v). A few drops of each extract were applied with a capillary tube to spots spaced 1 cm apart along the baseline, which was drawn 1 cm from the bottom of

the chromatographic plate. Once dried, the plates were placed in a container containing a solvent mixture corresponding to each extract. After the plant compounds had eluted, the plate was dried. Visualization was performed using a 1% $SbCl_3$ solution in ethanol (Bekro et *al.*, 2007).

- Test for Alkaloids in Test Tubes

Alkaloids were identified using Burchard's reagent (iodo-iodide reagent) and Dragendorff's reagent (potassium iodo-bismuthate reagent). For this experiment, 6 mL of the solution was completely evaporated. The resulting residue was dissolved in 6 mL of 60% ethanol. The addition of 2 drops of Dragendorff's reagent to this alcoholic solution resulted in the formation of an orange precipitate or coloration. Similarly, the addition of 2 drops of Burchardat's reagent produced a reddish-brown precipitate, thus indicating a positive reaction (Konan, 2010).

Detection of alkaloids by TLC using Dragendorff's reagent

The stationary phase of the chromatographic plate is silica gel 60F 254, with a rigid aluminum backing. The solvent (or mobile phase) used is a mixture of ethyl acetate and methanol in a 90:10 (v/v) ratio. Using a capillary tube, a few drops of each extract are applied at points spaced 1 cm apart on the baseline, located one centimeter from the bottom of the chromatographic plate. Once dry, the plates are placed in a chamber containing a mixture of migration solvents, appropriate for each extract. After the phytochemical compounds have migrated, the plate is left to dry. Then, by spraying Dragendorff's reagent, visible light are attributed to alkaloids (Lagnika, 2005).

- Testing for coumarins in a test tube

To test for coumarins, take 2 mL of the 5% infusion and place it in a test tube. Then, add 3 mL of 10% NaOH. After mixing the resulting solution, a yellow coloration appears, indicating the presence of coumarins (Diallo, 2000).

Detection of coumarins by thin-layer chromatography (TLC)

The detection of coumarins was performed using Godin's reagent. The chromatographic plates are first sprayed with this reagent, followed by a 10% (v/v) ethanolic solution of $H_2SO_4$ (5.5 mL of sulfuric acid with a density of 1.84 mixed with 95 mL of ethanol). They are then heated to 100°C until blue spots become visible (Lagnika, 2005).

- Screening for sterols and polyterpenes by TLC

The Liebermann-Bürchard reagent highlighted the sterols, which appear yellow-green under UV light at 366 nm. This chemical agent is used to detect oleanane- and ursane-type triterpenes, which appear red, as well as lupane-type triterpenes, which appear as yellow-orange spots. Using vanillin sulfate, terpenes are visualized in the visible spectrum as well as under UV light at 366 nm, appearing in shades of violet, pink, and orange. Additionally, terpenes were detected as violet under UV/366 nm using Godin's reagent (Bekro et *al.*, 2007).

- Test-tube detection of Polyphenols

To detect polyphenols, 2 mL of each extract solution is added to a few drops of a 2% (w/v) aqueous solution of ferric chloride ($FeCl_3$). The appearance of a blue-black or greenish-black coloration indicates the presence of polyphenols (Ladyguina et al., 1983; Békro et *al.*, 2007).

Detection of polyphenols by TLC

The extracts obtained were dissolved in diethyl ether and examined on a silica gel plate. The mobile phase used for separation consisted of acetic acid and chloroform in a 1:9 (v/v) ratio. Once the plate was developed, the spots were visualized under UV light at wavelengths of 254 nm and 365 nm (Harborne, 1998). The formation of a blue-black or green-black spot indicates the presence of polyphenols.

- Screening for flavonoids by thin-layer chromatography

The four extracts were applied to a TLC plate. The mobile phase used for separation was 50% AcOEt/MeOH/$NH_4OH$ (9:1:1). Detection occurs at 365 nm after spraying with Neu's reagent (2-aminoethyl-diphenylborate) at 1% in pure MeOH (Konan, 2010).

Detection of Flavonoids Using the Cyanidin Test

To detect flavonoids, the method known as the "cyanidin reaction" was employed. In practice, 2 mL of the aqueous and hydroethanolic extracts were evaporated, and the residue was dissolved in 5 mL of hydrochloric acid diluted twice. The

addition of 2 to 3 pieces of magnesium generated heat, followed by an orange-pink or purplish coloration. Upon adding 3 drops of isoamyl alcohol, this color intensified, confirming the presence of flavonoids.

- -An orange-pink coloration indicates the presence of flavones.
- -A pink-purple color indicates the presence of flavanones.
- -A red color indicates the presence of flavanols and flavanonols.

In the absence of magnesium shavings, after heating for 1 to 5 minutes in a water bath, a cherry red or purplish color appears in the presence of leucoanthocyanins. Catechols produce a reddish-brown coloration (Mibindzou, 2004).

- Testing for tannins in a test tube

To detect tannins, dissolve 2 mg of extract in 2 mL of ethanol. Then add 2 to 3 drops of 2% $FeCl_3$. A positive result is indicated by the appearance of a blue-black or blue-green color (Trease and Evans, 1987).

Detection of tannins by TLC

To identify tannins, the reagent was sprayed onto the chromatographic plate. In the visible spectrum, the tannins appear as gray spots (Lagnika, 2005).

*2.2.4. Acute Toxicity*

Assessment of the acute toxicity of the extracts in mice

This study was conducted due to the possibility of contamination or the presence of foreign substances in the various batches. During the tests, we followed OECD Protocol 423 (OECD, 2001). In each phase, three mice were used, weighing between 19 and 26 grams. For the first dose, we selected values of 5, 50, 300, and 2000 mg/kg body weight (Figure 1).

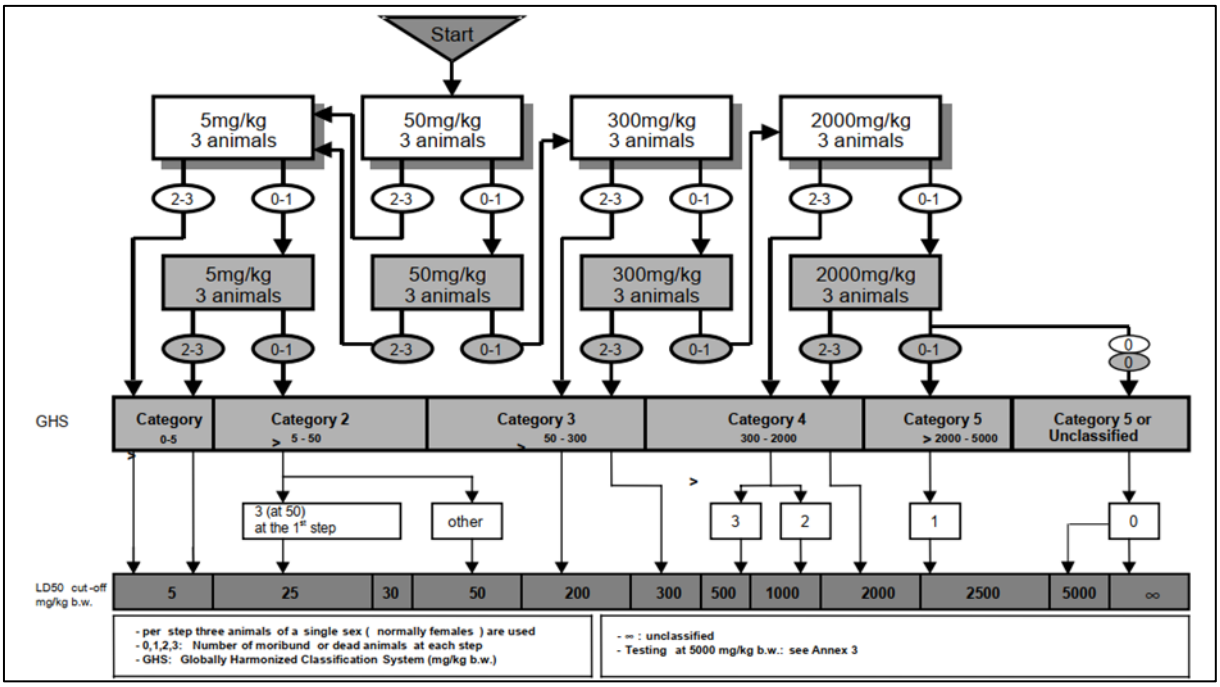


**Figure 1** Test protocol with an initial dose of 5 mg/kg body weight (OECD 423)

The toxicity level selected was the one at which mortality was expected among some of the treated animals. According to the aforementioned guideline, it was necessary to select female mice due to their increased sensitivity. Each group of animals was orally administered and observed at 30-minutes intervals. When the treated group showed no deaths or other signs of toxicity (such as aggression, mobility, alertness, stool consistency, vomiting, deaths, etc.), the next group was then treated in turn. The animals were monitored on the first day and then regularly over a 14-day period.

*2.2.5. Method of Administration*

After fasting the mice for 24 hours, they were fed via a rigid feeding tube. The method is based on a simple concept. The mice were securely restrained. Once the tube was inserted into the animal's throat, the extract was also administered carefully (Silué et *al.*, 2018; N'guessan et *al.*, 2025c). The protocol for treating the mice in the experiments proceeded as follows: the animals were divided into 5 groups of 3 mice, which received the following treatment:

- -Group I (control): the animals received distilled water.
- -Group II (treated): the animals received 5 mg/kg body weight of extract.
- -Group III (treated): the animals received 50 mg/kg body weight of extract.
- -Group IV (treated): the animals received 300 mg/kg body weight of extract.
- -Group V (treated): the animals received 2000 mg/kg body weight of extract.

After treatment, the mice were monitored every hour during the first day and daily for a period of two weeks (14 days).

The animals' behaviors and clinical signs were recorded throughout the study. Changes in the animals' weight were assessed every three days. The study lasted 14 days.

## 3. Results and Discussion

### 3.1. Extraction Yields

The results show that the highest yields were obtained by extraction using the binary solvent mixture of distilled water and ethanol. Furthermore, the JFm extract provided the highest yield (Table 1).

**Table 1** Yields of aqueous and hydroethanolic extracts of *Albizia zygia*

| **Extracts** | **AMETe** | **AMETm** | **JFe** | **JFm** |
|---|---|---|---|---|
| Yields | 13.33±0.26 | 13.01±0.20 | 17.06±0.06 | 23.00±2.27 |

### 3.2. Phytochemical Screening

Phytochemical screening revealed the presence of the main chemical groups in the various extracts studied. The results of the phytochemical tests conducted on the various extracts are summarized in Table 2. The results indicate that certain families of chemical compounds may be present or absent depending on the type of extract studied.

The study of the extracts indicates the presence of sterols, alkaloids, terpenes, polyphenols, flavonoids, and tannins in all the extracts studied. However, saponins were not detected in the extracts.

**Table 2** Phytochemical composition of the various extracts

| **Compound family** | **Alkaloids** | **Polyphenols** | **Flavonoids** | **Tannins** | **Saponins** | **Sterols** | **Terpenes** | **Coumarins** |
|---|---|---|---|---|---|---|---|---|
| AMETe | + | + | + | + | - | + | + | + |
| AMETm | + | + | + | + | - | + | + | + |
| JFe | + | + | + | + | - | + | + | + |
| JFm | + | + | + | + | - | + | + | + |

**+: present; -: absent**

### 3.3. Acute Toxicity of the Extracts

The following results were obtained from observations conducted over a 24-hour period and a 14-day interval (for the delayed effects of the extracts). Neither the control mice nor those that received the various extracts showed any signs of immediate toxicity or mortality.

Regarding the control mice ($H_2O$), no signs of immediate toxicity were observed. The mice were in good physical condition, with normal skin and fur, as well as eyes and mucous membranes in normal condition. Furthermore, no abnormal behavior was noted, such as diarrhea, salivation, lethargy, aggression, or drowsiness. They had a normal heart rate and maintained good mobility. There were no recorded deaths.

As for the mice treated with the various extracts, the following observations were noted:

The mice exhibited physical condition comparable to that of the control group, with no signs of toxicity or side effects observed for the AMETe, AMETm, JFe, and JFm extracts.

No adverse effects were observed in the groups that received the extracts, all of which exhibited a health status comparable to that of the control mice throughout the duration of the study. The mortality rate was 0% during the 14-day experiment. (Table 3).

**Table 3** Observations over a 24 hours period and 14 days in the control and treated groups of mice

| **Observations** | **Control Group** | | **Experimental Group** | |
|---|---|---|---|---|
| | **6 hours** | **12 hours** | **6 hours** | **12 hours** |
| Skin and fur | Normal | Normal | Normal | Normal |
| Eyes | Normal | Normal | Normal | Normal |
| Mucous membranes | Normal | Normal | Normal | Normal |
| Diarrhea | None | None | None | None |
| Salivation | None | None | None | None |
| Lethargy | None | None | None | None |
| Heart rate | Normal | Normal | Normal | Normal |
| Aggression | None | None | None | None |
| Drowsiness | None | None | None | None |
| Feeding | Yes | Yes | Yes | Yes |
| Mobility | Yes | Yes | Yes | Yes |
| Mortality | None | None | None | None |

The extracts were administered at doses of 5, 50, 300, and 2000 mg/kg body weight. After administration of the AMETe extract, the average weight of the mice, which was 26.66 g, increased to 29.33 g on the 14th day (Figure 2). For the AMETm extract, the average weight showed an increase from 27.33 g to 29.66 g on the 14th day (Figure 3). Administration of different doses of the JFe extract to the treated groups of mice, whose average weight was 26.33 g, resulted in an increase to 28.66 g on the 14th day (Figure 4). For the JFm extract, the average weight of the mice, which was 27.33 g, increased to 30.66 g on the 14th day (Figure 5).

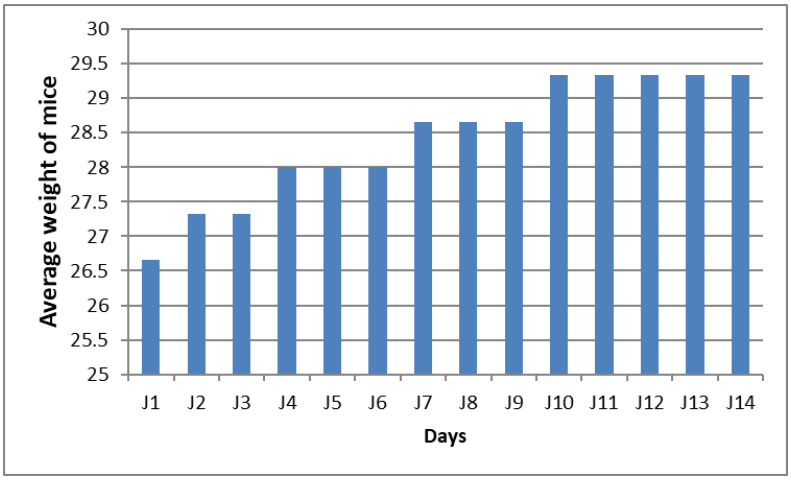


**Figure 2** Average weight of mice by day (AMETe)

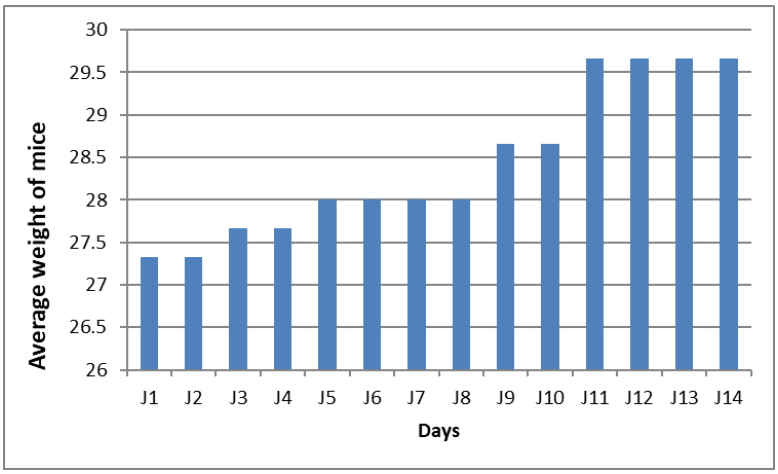


**Figure 3** Average weight of mice by day (AMETm)

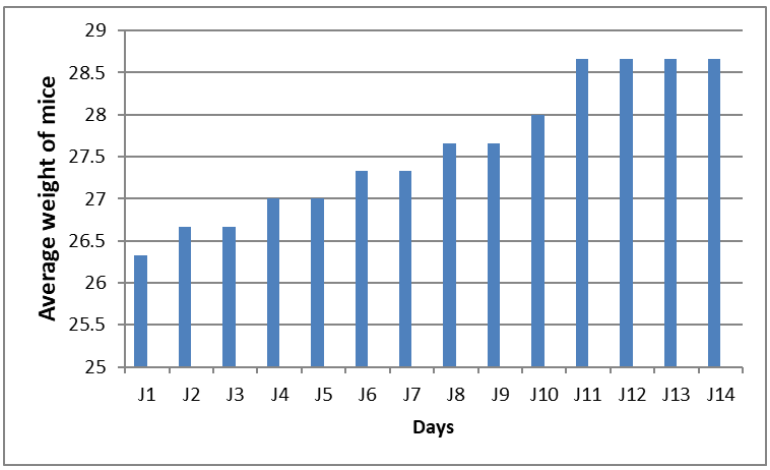


**Figure 4** Average weight of mice by day (JFe)

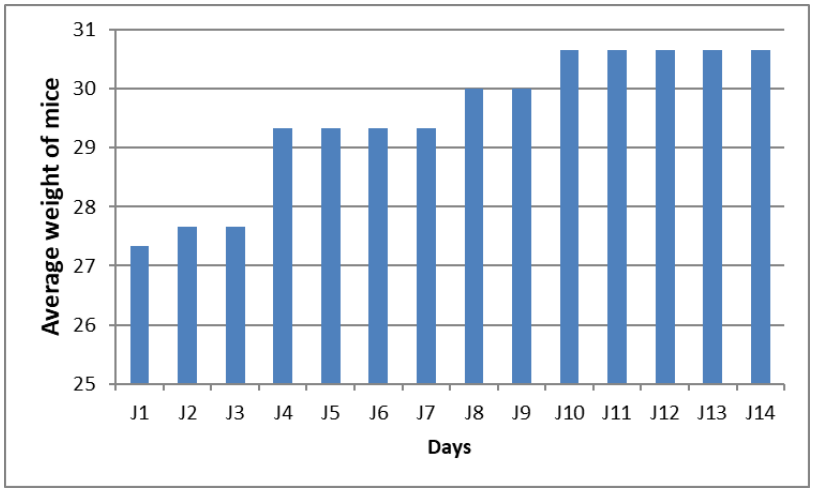


**Figure 5** Average weight of mice by day (JFm)

## 4. Discussion

In this study conducted on *Albizia zygia*, extraction using a mixture of water and ethanol proved to be more effective than aqueous extraction. Furthermore, extracts derived from the leaves yielded higher yields than those obtained from the bark of the trunk. This variation in yields could be attributed to the nature of the solvent used for extraction (N'guessan et *al*., 2025a), as well as to the composition of the sample.

A phytochemical study is very useful for analyzing certain biologically active compounds found in medicinal plants (Akinsola et *al.,* 2022). Phytochemical analysis of the bark and leaves of *Albizia zygia* revealed the presence of sterols, alkaloids, terpenes, polyphenols, flavonoids, and tannins in all the extracts analyzed. These results were observed in both aqueous and hydroethanolic extracts of the bark and leaves of *Albizia zygia*. This finding is similar to that reported by Okoro (2023) in a study conducted on the leaves of *Albizia zygia*. Indeed, the author demonstrated the presence of alkaloids, flavonoids, tannins, saponins, glycosides, steroids, anthraquinones, anthocyanins, and terpenoids in both types of extracts—aqueous and ethanolic—from the leaves.

Alkaloids are naturally occurring nitrogen-containing chemical compounds that exhibit physiological effects in addition to sedative and analgesic actions.

They are useful for alleviating symptoms of depression and anxiety. Due to their stimulating properties, which cause excitement linked to effects at the cellular and nervous system levels, alkaloids can be dangerous if ingested in excessive amounts.

Tannins are secondary compounds found in plants, consisting of a group of polyphenols. Tannins exhibit a variety of physical and chemical characteristics depending on the type of plant, the part of the plant used, and the season (Alagbe, 2019).

Furthermore, *Albizia zygia* extract caused a 60% reduction in blood glucose levels after four hours in antihyperglycemic studies and an 85% reduction in streptozotocin-induced diabetes models (Faloye et *al*., 2025).

Regarding toxicity, the four extracts caused no mortality or clinical signs of toxicity at an $LD_{50}$ of 2000 mg/kg body weight; furthermore, Faloye et *al.* (2025) demonstrated the non-toxicity of this plant. These data support the use of this plant in the treatment of diabetes in the city of Korhogo.

## 5. Conclusion

This study highlights the presence of alkaloids, tannins, polyphenols, and sterols in *Albizia zygia*. These compounds confer biological properties to the bark and leaves of this plant. The plant exhibits low acute toxicity ($LD_{50}$ > 2000 mg/kg bw). These results demonstrate the promising potential of this plant for the treatment of diabetes. In the next stage of this study, the antidiabetic activity of the extracts and the isolated pure compounds will be assessed.

## Compliance with ethical standards

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*Disclosure of conflict of interest*

No conflict of interest to be disclosed.

*Author contributions Conceptualisation and literature acquisition:*

[Author 1]. Original draft preparation: [Author 1, Author 2]. Critical revision: [Author 3]. Supervision and final approval: [Author 4, Author 5]. All authors approved the submitted version and agree to be accountable for all aspects of the work.

*Data availability statement*

No new datasets were generated or analysed during the preparation of this review. All data cited are available in the published literature referenced herein.

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