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PHYTOCHEMICAL CHARACTERIZATION AND EVALUATION OF THE ACUTE ORAL TOXICITY OF AQUEOUS EXTRACTS FROM THE BARK, STEM, AND ROOT OF *ZANTHOXYLUM ZANTHOXYLOIDES* (LAM.) A MEDICINAL PLANT IN HIGH DEMAND IN NORTHERN CÔTE D'IVOIRE

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ABSTRACT

This study focuses on the phytochemical screening and acute toxicity assessment of aqueous extracts from the bark, stem, and root of *Zanthoxylum zanthoxyloides*, a plant harvested in the town of Dikodougou, in the Poro region of Côte d'Ivoire. The plant samples were dried, ground, and extracted by aqueous maceration. The phytochemical compounds investigated included alkaloids, flavonoids, terpenoids, polyphenols, and saponosides. Acute oral toxicity was evaluated in Wistar rats following a single administration of 2,000 mg/kg body weight, with clinical and weight monitoring for 14 days. Extraction yields were 15.04% for root bark and 13.06% for stem bark. Phytochemical analysis revealed the presence of the five categories of metabolites under investigation, with no statistically significant difference between the two extracts. Terpenoids had the highest concentrations, with 127.96 ± 15.77 for the root extract and 173.80 ± 17.88 for the stem extract, according to the units indicated in the figures. After administration of the extracts at 2,000 mg/kg, no mortality or major clinical signs of toxicity were observed during the 14-day follow-up period. The median lethal dose (LD50) is therefore estimated to be greater than

2,000 mg/kg body weight. However, differences in weight gain were observed among the treated groups. These results characterize the phytochemical composition of the extracts studied and their acute oral toxicity profile under the experimental conditions used.

KEYWORDS: *Zanthoxylum zanthoxyloides*; phytochemical screening; aqueous extracts; secondary metabolites; acute toxicity.

INTRODUCTION

Plants are an important source of natural compounds with various biological activities. Studying their chemical composition makes it possible, in particular, to identify secondary metabolites that may contribute to the observed biological effects and to guide research into their pharmacological properties. These compounds include alkaloids, polyphenols, flavonoids, saponosides, and terpenoids, the presence of which may vary depending on the plant organs studied. *Zanthoxylum zanthoxyloides* is a plant species whose chemical properties have been the subject of several studies. In particular,[1] reported the presence of alkaloids, phenolic compounds, saponins, sterols, and triterpenes in the stems and roots of this plant harvested in Mali. Meanwhile,[2-4] reported high levels of terpenoids and alkaloids in extracts of *Zanthoxylum zanthoxyloides*. Furthermore,[5] and[6] reported anti-inflammatory effects associated with these classes of compounds. These observations highlight the importance of characterizing the phytochemical constituents of this species. However, the study of its chemical composition must be accompanied by an assessment of the extracts' safety. Acute toxicity testing is a crucial step in determining the adverse effects that may occur following a single administration. To this end, Guideline No. 423 of the Organization for Economic Cooperation and Development[7] proposes a method for assessing acute oral toxicity based, in particular, on the observation of animals following administration of the test substance. In this context, the present study aims to conduct a phytochemical screening and to evaluate the acute oral toxicity of aqueous extracts from the bark, stem, and root of *Zanthoxylum zanthoxyloides*, harvested in Dikodougou, in the Poro region of Côte d'Ivoire. The study involves determining extraction yields, quantifying the main categories of secondary metabolites of interest, and observing the effects of a single oral dose of 2,000 mg/kg body weight administered to Wistar rats over a 14-day period.

MATERIALS AND METHODS

Plant Material

The plant material consists of the bark of the stem and root of *Zanthoxylum zanthoxyloides*,

harvested in the Poro region (Korhogo), specifically in the municipality of Dikodougou (Ivory Coast). Both parts were harvested in August 2024, air-dried for two weeks, and then ground into powder. The plant was identified at the National Center for Floristics under herbarium number CSRS006424.

Animal Materials

The acute toxicity assessment was conducted on twenty (9) male and female Wistar rats, weighing between 100 and 250 g. These rats were obtained from the animal facility of the National Center for Floristics (CNF) at Félix HOUPHOUËT BOGNY University (UFHB). These rats were housed at laboratory temperature (24°C) and fed a standard diet. Each rat was individually identified by a number marked on its tail with a permanent marker.

Methods

Preparation of Aqueous Extracts

The plant powder obtained by grinding the bark of the stem and root of *Zanthoxylum zanthoxyloides* after harvest and subsequent air-drying in the shade was used to prepare the aqueous extract, according to the method described by.[8] Thus, one hundred grams (100 g) of plant powder were macerated in one liter of distilled water by homogenization in a blender for five (5) minutes. The resulting homogenate was filtered through a sieve. Next, the filter residue was returned to the blender and macerated again for five (5) minutes in 500 ml of distilled water. The resulting homogenate was filtered a second time through a sieve. The residues from this filtration were returned to the blender and macerated again in 500 ml of distilled water under the same conditions. The homogenate obtained from these residues was also filtered through a sieve. Subsequently, the total filtrate obtained underwent a series of three (3) successive filtrations, first through clean cloth, then through cotton wool. The final filtrate obtained from these operations was dried in an oven at 50 °C for four (4) days. The dry evaporate was recovered in powder form for the two different parts of the plant, resulting in the dry extracts of the bark of the *Zanthoxylum zanthoxyloides* root (THOZA-ER) and the stem of *Zanthoxylum zanthoxyloides* (THOZA-ET), respectively.

Calculation of Yield

Yield is the ratio of the amount of extract obtained to the amount of material. The result is expressed as a percentage of the dry plant powder and calculated using the formula above:

$R\ (\%) = M1 \times 100 / M0$

R: Extract yield expressed as a percentage (%)

M1: Mass of the dry extract (in g)

M0: Mass of the plant powder (in g)

Quantitative Analysis of Phytochemicals

Analysis of Alkaloids

Principle

100 mg of the extract was dissolved in 4 mL of ethanol to obtain a concentrated stock solution with a concentration of 25 mg/mL. Next, the pH of this solution was measured and maintained between 2 and 2.5 by adding a few drops of hydrochloric acid (HCl). Next, 2 mL of Dragendorff's reagent was added to the extract, and the mixture was centrifuged at 7,000 rpm for 3 minutes. A few drops of Dragendorff's reagent were added to the centrifugate to complete the precipitation, followed by a second centrifugation. The supernatant was completely decanted, and the precipitate was recovered and washed with ethanol. The filtrate was discarded, and the residue was treated with 2 mL of disodium sulfate solution. The black-brown precipitate that formed was then centrifuged. Two drops of disodium sulfate are added to the medium to complete the precipitation, followed by a second centrifugation. The residue is removed and dissolved in 2 mL of concentrated nitric acid, heating as needed. The resulting solution is then made up to 10 mL by adding distilled water. One milliliter of this diluted solution is then mixed with 5 mL of thiourea, and the absorbance is measured at 435 nm against a blank consisting of nitric acid, distilled water, and thiourea prepared using the same procedure.[9]

Preparation of the Calibration Curve

17 g of atropine powder were dissolved in 17 ml of nitric acid to prepare a stock solution with a concentration of 1 mg/ml. Dilutions were made from this stock solution and made up to 10 ml with distilled water. Subsequently, 1 mL of each dilution was added to 5 mL of 3% thiourea. The absorbance (X) was measured at a wavelength of 435 nm.

Polyphenol Assay

Preparation of Reagents

To prepare a 20% $Na_2 CO_3$ solution, 10 grams of $Na_2 CO_3$ were dissolved in 50 mL of distilled water and stirred until completely dissolved. The mixture was allowed to stand for at least 1 hour, resulting in a 20% $Na_2 CO_3$ solution. To prepare the Folin-Ciocalteau solution (1:10), 5 mL of Folin's reagent was taken and diluted in 50 mL of distilled water.

Principe

10 mg of extract were weighed and dissolved in 10 mL of methanol to obtain a concentrated stock solution with a concentration of 1 mg/mL. Next, 1 mL of this solution was withdrawn and added to 1 mL of FCR. The mixture was homogenized using a vortex mixer for 6 minutes. After mixing, 1 mL of sodium carbonate was added, and distilled water was added to bring the total volume to 10 mL. The mixture was kept in the dark for one hour. The absorbance was measured at 735 nm, using as a blank an identical preparation in which the extract was replaced with distilled water.

Preparation of the Gallic Acid Calibration Series

5 mg of gallic acid was dissolved in 5 mL of distilled water; the mixture was then shaken to produce a stock solution with a concentration of 1 mg/mL. Subsequently, a series of dilutions of the gallic acid stock solution was prepared using distilled water. Specifically, 1 mL of each dilution was transferred to test tubes (labeled C0 through C10), and 1 mL of FCR (1:10) was added to each tube; the mixture was then allowed to stand for 6 minutes. After this time, 1 mL of 20% sodium carbonate was added to each tube. Finally, the entire mixture was incubated in the dark for 1 hour. Absorbance was measured at 735 nm.Dosage des terpènoïdes.

Preparation of the 50 mg/mL stock solution of extract

250 mg of dry extract was weighed out and dissolved in 5 mL of distilled water to obtain a concentrated stock solution (CSM = 50 mg/mL).

Procedure

1 mL of the previously prepared stock solution was taken and transferred to a test tube; then, 2 mL of chloroform was added. After mixing thoroughly, the mixture was allowed to stand for 3 minutes. Next, 200 µL of concentrated sulfuric acid was added, and the solution was incubated in the dark for 2 hours. After incubation, the reddish-brown precipitate formed was carefully separated from the supernatant by decantation without disturbance. Subsequently, 3 mL of absolute methanol was added, and the mixture was shaken thoroughly until the precipitate was completely dissolved. Absorbance was measured at 538 nm using a blank prepared in the same manner, substituting methanol for the plant extract.

Flavonoid Assay

Preparation of the Stock Solution 100 mg of dry extract was weighed out and dissolved in 4 mL of distilled water to obtain a concentrated stock solution (CSM).

Principle

400 µl of the stock solution were taken and transferred to a test tube, to which 120 µl of 5% sodium nitrite ($NaNO_2$) was added. After 5 minutes, 120 µl of 10% $AlCl_3$ was added, and the mixture was then shaken vigorously. Six minutes later, 800 µl of 1 M NaOH was added. The absorbance was immediately measured at 510 nm against the blank. A stock solution of quercetin in methanol was prepared and then diluted to various concentrations ranging from 0 to 1000 µg/mL to plot the calibration curve.[10]

Determination of Saponins

Preparation of the Reagent

8 g of vanillin were dissolved in 100 mL of 96° ethanol, resulting in an 8% vanillin concentration.

Principle

25 mg of extract were weighed and dissolved in 1 mL of distilled water to obtain a concentrated stock solution (CSM = 25 mg/mL of extract). Then, 100 µL of this solution were withdrawn and transferred to a test tube. Next, 500 µL of 8% vanillin was added to this mixture, which was placed in an ice bath; then, 5 mL of 72% sulfuric acid was added. The mixture was stirred and then held at room temperature (21 ± 2°C) for 3 min. After this time, the mixture was incubated for another 10 minutes at 60°C, and finally, the mixture was allowed to cool. The absorbance was measured at 544 nm against a reagent blank prepared as follows (ethanol + 8% vanillin + 72% sulfuric acid).

Evaluation de la toxicité

The Organization for Economic Cooperation and Development[7] Test Guideline No. 423 for acute oral toxicity was used to evaluate the acute toxicity of our aqueous extracts. This approach does not seek to establish a precise LD50, but rather to estimate the dose range at which a substance could pose a lethal risk.

Grouping of Animals

The rats were divided into three groups of three animals each: a control group that received no treatment, Group 1 treated with the aqueous extract of THOZA-ET, and Group 2 treated with the aqueous extract of THOZA-ER.

Procedure

The rats in each group were weighed and deprived of food, but not water, overnight. The following day, a second weighing was performed before administration of a single dose of the extracts. Rats in Group 1 received a dose of 2,000 mg/kg body weight of the THOZA-ET aqueous extract orally via a gastric tube, while those in Group 2 were treated in the same manner with a dose of 2,000 mg/kg body weight of the THOZA-ER aqueous extract. After administration, the rats were observed every 30 minutes for one hour. After one hour of observation, the rats were fed and then observed daily for 14 days and weighed every two days. Mortality and any behavioral changes, such as changes in eye color, salivation, diarrhea, lethargy, and morbidity, were recorded during the observation period.

RESULTS AND DISCUSSION

Results

Extraction Yield

The extraction yield defined as the ratio of the weight of the extract to the weight of the plant powder is shown in Table I. The average mass obtained from an initial mass of 100 g of plant powder for aqueous extraction was m = 15.04 g with a yield R = 15.04% for root bark and m = 13.06 g with a yield R = 13.06% for the stem bark of *Zanthoxylum zanthoxyloides*.

Secondary Metabolite Composition of the Extracts

Phytochemical analysis of the aqueous extracts from the stem bark and root of *Zanthoxylum zanthoxyloides* revealed the presence of various chemical compounds, including alkaloids, polyphenols, terpenoids, flavonoids, and saponosides. Furthermore, statistical analysis indicated that there are no significant differences between the chemical compositions of the extracts derived from the stem bark and the root. However, among the various extracts, terpenoids are the most abundant, with 173.80 mg/mL in the stem bark extract and 127.96 mg/mL in the root extract, followed by polyphenols, at 1.91 mg/mL in both extracts. The phytochemical composition of the various aqueous extracts is illustrated in Figures 1, 2, 3, 4, and 5.

Table I: Extraction Yields for THOZA-ER and THOZA-ET.

Plant material	Mass (g)	extract (g)	Yield (%)
THOZA-ER	100g	15,04g	15,04%
THOZA-ET	100g	13,06g	13,06%

THORA-ER: Aqueous extract of the root bark of *Zanthoxylum zanthoxyloides*.

THOZA-ET: Aqueous extract of the stem bark of *Zanthoxylum zanthoxyloides*

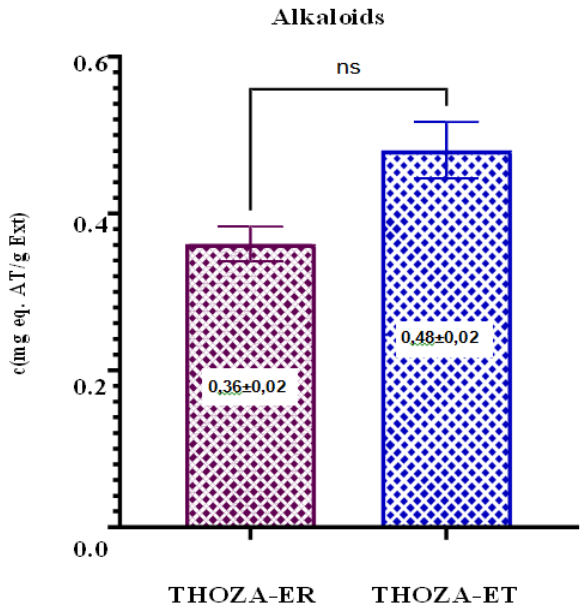


Fig. 1: Alkaloid Content of THOZA-ER and THOZA-ET.

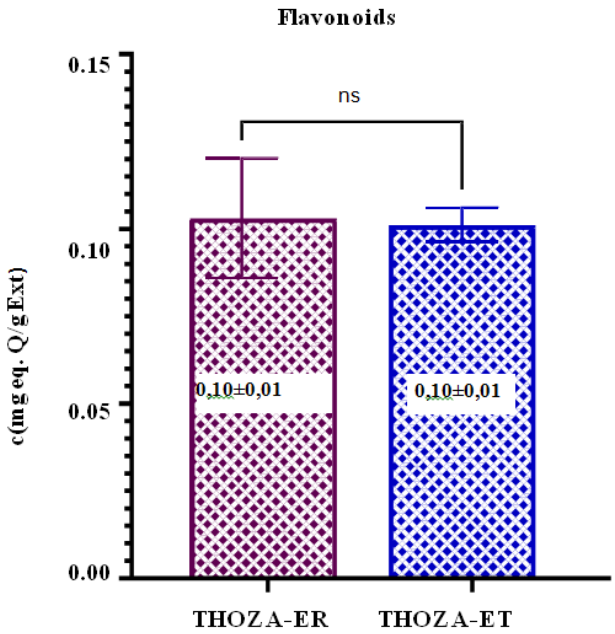


Fig. 2: Flavonoid Content of THOZA-ET and THOZA-ER.

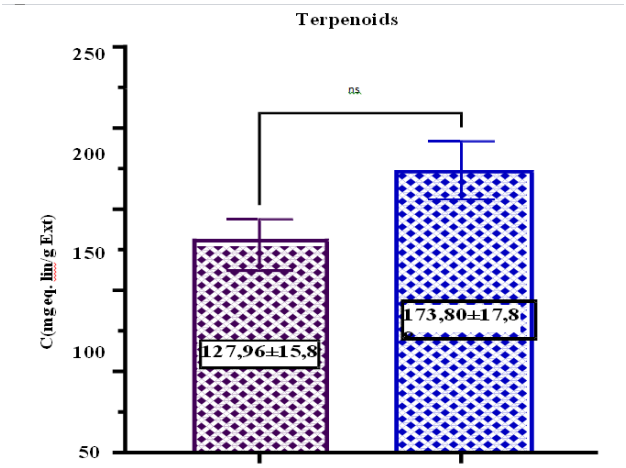


Fig. 3: Terpenoid Content of THOZA-ET and THOZA-ER.

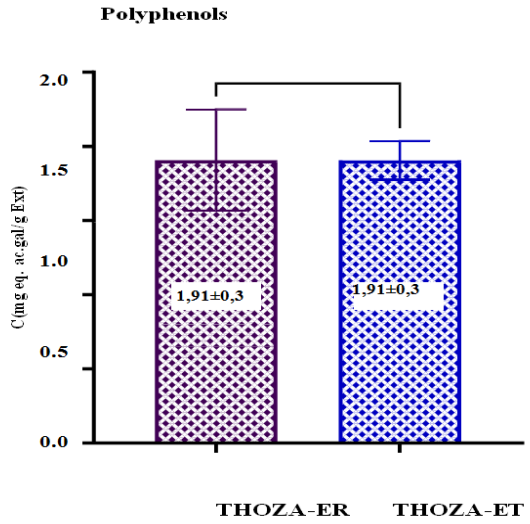


Fig 4: Polyphenol Content of THOZA-ET and THOZA-ER.

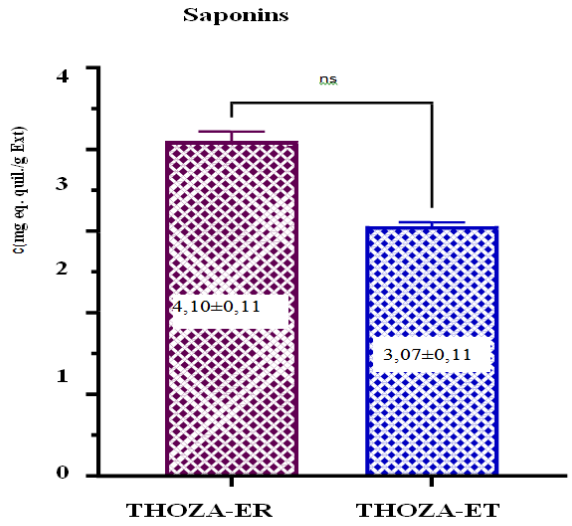


Fig 5: Saponin Content of THOZA-ET and THOZA-ER.

Acute Toxicity of Aqueous Extracts from the Bark, Stem, and Root of *Zanthoxylum zanthoxyloides*

General Observations on the Rats

The animals were generally in very good health prior to administration of the aqueous extracts of THOZA-ER and THOZA-ET. Immediately after administration of the plant extracts, the rats in the groups treated with a dose of 2,000 mg/kg body weight licked their muzzles, huddled together, and a few minutes later remained calm. All these physical signs ceased half an hour after gavage, and all animals resumed their normal behavior. No clinical signs of toxicity such as circling, respiratory distress, lethargy, morbidity, salivation, or mortality were observed in animals that received aqueous extracts of THOZA-ER and THOZA-ET at a dose of 2,000 mg/kg body weight. Administration of the aqueous extracts of THOZA-ER and THOZA-ET did not cause any changes in the nature of the feces, urine, or, even less so, changes in the rats' general appearance, such as coat, skin, or the condition of the eyes, ears, and mouth. No diarrhea was observed throughout the experiment. There was no hematuria or uncoordinated movements in the rats during the study period. A summary of the clinical signs is provided in (Table II).

LD50 of aqueous extracts from the stem and root of *Zanthoxylum zanthoxyloides*

Following a single administration of aqueous extracts of THOZA-ER and THOZA-ET at a dose of 2,000 mg/kg body weight, no deaths were observed in the animals during the 14-day follow-up period. This indicates that the LD50 is greater than 2,000 mg/kg body weight.

Effects of the Extracts on Weight Gain in Rats

Figure 6 shows the trend in weight gain (%) in rats from the control, THOZA-ER, and THOZA-ET groups during the follow-up period. According to the curve, the control group reached its highest weight gain around day 12, after which the value decreased by the last data point shown. The THOZA-ET group showed an increase in weight gain during the follow-up period, although the values remained below the peak observed in the control group; the curve did not show a peak exceeding 11% on day 12. The THOZA-ER group also exhibited lower weight gain than the other two groups starting on day 6.

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Table II: Clinical Signs Observed Over a 14-Day Period.

Signs	Control batch in	Treatment group treated with THOZA-ER at 2,000 mg/kg	Treatment group treated with THOZA-ET at 2,000 mg/kg
Respiratory distress	AB	AB	AB
Walking in circles	AB	AB	AB
Morbidity	AB	AB	AB
Diarrhea	AB	AB	AB
Lethargy	AB	AB	AB
Salivation	AB	AB	AB
Mortality	AB	AB	AB

AB stands for "no sign"

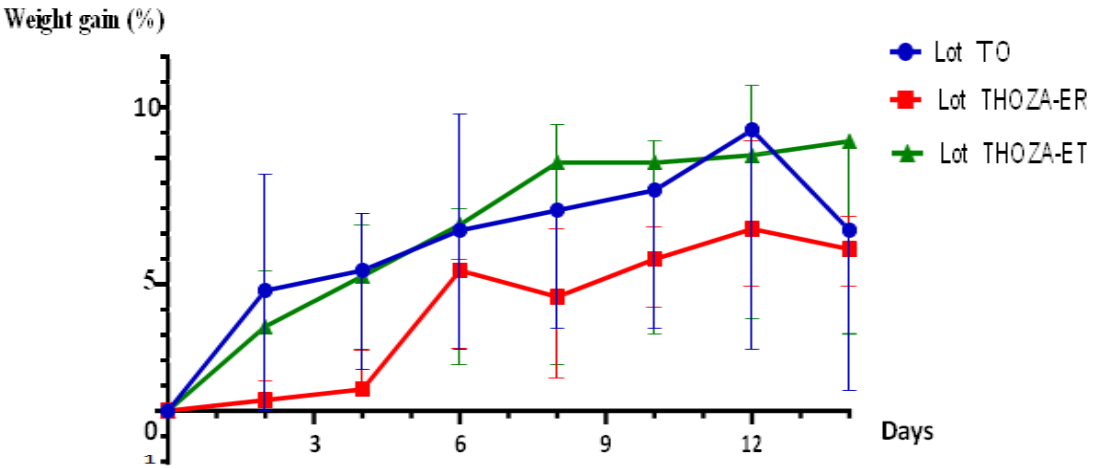


Fig 6: Effect of the extracts on weight gain in rats.

DISCUSSION

Aqueous extraction was used to evaluate the yield of the different parts of the plant. The aqueous extract of the root bark of *Zanthoxylum zanthoxyloides* had an average yield of 15.04%, slightly higher than that of the aqueous extract of the stem bark of *Zanthoxylum zanthoxyloides* (13.06%), and both yields are acceptable for aqueous maceration extractions. These values are similar to those reported by[8], who used a similar method. This difference between the two organs could be explained by a higher metabolite density in the roots than in the stems. Phytochemical analysis identified several categories of secondary metabolites with biological activity: alkaloids, polyphenols, flavonoids, saponosides, and terpenoids. These results are consistent with those reported by other Researchers have confirmed that powder derived from the stems and roots of *Zanthoxylum zanthoxyloïdes* harvested in Mali contains alkaloids, phenolic compounds (flavonoids, tannins, coumarins), saponins, sterols, and

triterpenes.[9] The root bark of *Zanthoxylum zanthoxyloides* harvested in Côte d'Ivoire contains monoterpenes and flavonoids. Terpenoids were found to be the most abundant, particularly in the stem extract (173.80 mg/mL compared to 127.96 mg/mL for the root), but this difference is not statistically significant. These results are consistent with the observations of[2-4], who reported high levels of terpenoids and alkaloids in *Zanthoxylum zanthoxyloides* extracts. These compounds are known for their anti-inflammatory effects[5,6] The absence of mortality or clinical signs of toxicity at a dose of 2,000 mg/kg over 14 days, in accordance with the Organization for Economic Cooperation and Development[7] suggests that aqueous extracts of *Z. zanthoxyloides* are non-toxic when administered orally. In accordance with OCDE criteria[7] and the[11] aqueous extracts of *Z. zanthoxyloides* belong to Category 5 and are considered non-toxic when administered orally. These results are consistent with those of[4,12] who determined an LD50 well above 2000 mg/kg bw. These results are also similar to those of[13], who reported that the FACA® capsule can be classified in Category 5 of substances with no acute toxic effects, with an LD50 greater than 2,000 mg/kg bw.[14] also stated that the FACA® syrup had an estimated LD50 of 5,000 mg/kg bw. Monitoring of body weight in the treated rats indicated a change in weight gain in the animals at the administered dose compared to the control group. Rats treated with the aqueous extract of THOZA-ET gained weight, whereas rats treated with the aqueous extract of THOZA-ER lost weight. The weight gain observed in the rats could be attributed to excessive food intake.[15] In contrast, the weight loss could be correlated with the animal's physiological state. The spontaneous reduction in growth could be due to decreased food intake, stress experienced by the animals, and interactions between the dose and its absorption.[16]

CONCLUSION

This study revealed the presence of alkaloids, polyphenols, flavonoids, terpenoids, and saponosides in aqueous extracts of the stem and root of *Zanthoxylum zanthoxyloides*. Terpenoids constitute the major compounds, while extraction yields reached 13.06% and 15.04% for the stem and root, respectively. No mortality or major clinical signs of acute toxicity were observed at a dose of 2,000 mg/kg during the 14-day follow-up period. These preliminary results warrant further toxicological investigations to better characterize the safety of the extracts studied.

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