

Evaluation of the nutritional profile, bioactive substances, antioxidant characteristics, and safety evaluation of *Euphorbia hirta* and *Euphorbia hyssopifolia*

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ABSTRACT

Background: *Euphorbia hirta* and *Euphorbia hyssopifolia* are traditionally used for various medicinal purposes, but evidence supporting their safety remains limited. Despite their widespread use in traditional medicine, systematic evaluation of their potential toxic effects is necessary before their medicinal applications can be fully supported. Therefore, this study investigated the safety of extracts of *E. hirta* and *E. hyssopifolia* by assessing biochemical and hematological parameters, together with macroscopic and microscopic changes in major organs of treated rats.

Methods: This research analyzes the nutritional composition, the bioactive compounds, and the antioxidant power, as well as the acute toxicity of two medicinal plants of the genus *Euphorbia*, *Euphorbia hirta* and *Euphorbia hyssopifolia*.

Result: The proximate analyses reveal that *E. hirta* has a content of dry matter (14.51%), proteins (1.46%), lipids (1.78%) and ash (3.68%) higher than that of *E. hyssopifolia*. *E. hirta* also has higher levels of calcium, potassium, phosphorus, copper and iron, while *E. hyssopifolia* is richer in zinc. Both plants have a low energy content (46.85 - 52.99 kcal.100⁻¹ g), however, they offer an interesting source of micronutrients. Qualitative phytochemical screening confirmed the presence of polyphenols, catechins and gallic tannins, as well as leucocyanidin, while alkaloids and quinones were absent. In quantitative terms, *E. hirta* had a higher content of phenolic compounds and flavonoids (1.57 mg GAE.g⁻¹ and 67.49 µg EQ.g⁻¹ respectively) compared to *E. hyssopifolia*. The evaluation of the antioxidant activity by the DPPH test indicates that *E. hirta* has a slightly higher antioxidant capacity (IC₅₀ = 0.30 mg.mL⁻¹) than that of *E. hyssopifolia* (IC₅₀ = 0.41 mg.mL⁻¹). The acute toxicity assessment showed no negative effects, no mortality or no modification of the biochemical and hematological parameters at a dose of 1000 mg.kg⁻¹. Histopathological examinations revealed no damage to the target organs (liver, spleen, kidneys), thus confirming the short-term safety of the aqueous extracts.

Conclusion: In summary, these two species of *Euphorbia* present interesting prospects for the development of phytotherapeutic agents.

INTRODUCTION

Plants have always served as both food and medicinal, with herbalism being the oldest known method for treating diseases [1]. They provide countless opportunities for the development of novel medications, whether in the form of an extract, a pure component, or a derivative. The biological activities of the majority of the natural ingredients utilized in folk medicines are well supported by scientific research. However, there is limited data or evidence regarding the potential toxicity that consumers may experience from medicinal herbs [2]. The welfare of animals and the prompt availability of safe and efficacious pharmaceuticals are paramount concerns for the general populace, patients, and consumers alike. One might expect that the botanical species utilized in traditional medicinal practices would exhibit minimal toxicity, considering their long-standing application in human health care. Nevertheless, contemporary studies have revealed that a substantial number of the medicinal herbs employed in traditional therapies possess adverse side effects [3].

Consequently, it is essential to underscore that the longstanding application of any botanical species for therapeutic purposes does not inherently assure the safety of such plants. This situation prompts apprehensions regarding the possible toxicological repercussions stemming from both acute and chronic utilization of these medicinal botanicals [4]. To enhance confidence in the safety profile of medicinal flora or the formulations derived therefrom for human application, particularly in the development of pharmaceutical products, it is imperative to gather evidence from toxicity assessments [5]. The escalation of morbidity and mortality linked to the utilization of plants or so-called traditional remedies has garnered global scrutiny in recent years [6].

Comprising approximately 2100 species, the genus *Euphorbia*, which is classified within the Euphorbiaceae family, represents a notably diverse assemblage predominantly located in regions such as the Mediterranean Basin, the Middle East, Southern Africa, and the southern United States [7-9].

Euphorbia hyssopifolia is an herbaceous plant that is usually found in tropical and subtropical regions of Africa and America [10]. It often grows along roads and in fields up to 25.89 cm high, with opposite leaves, cyathias with glands and triangular capsules containing blackish seeds with transverse furrows [11,12]. It is rich in flavonoids, tannins, alkaloids, steroids, diterpenoids, and triterpenoids such as lupeol, betulin, lanosterol, and cycloartenol, as well as phenolic compounds including pyrocatechol and p-hydroxybenzoic acid. These bioactive compounds are directly associated with the medicinal qualities of *E. hyssopifolia*, which include notable antioxidant, anticancer, diuretic, and purgative effects, anti-inflammatory effects on the respiratory system, bronchial relaxation for colds and asthma [10], as well as relief from indigestion and back pain when taken as a tea or tonic [13].

Euphorbia hirta, is a medicinal plant widespread all over the world [4] with specific morphological characteristics, in particular a thin stem, purple or reddish in color, up to about 40 cm in height and elliptical-oblong leaves. The plant produces tricellular capsules containing three brown angular seeds, each enclosed in a yellow and hairy envelope [14-17]. The extract from *E. hirta* is traditionally ingested in the form of an aqueous decoction for various medicinal purposes. The plant, known by its common English name, has always been used to treat respiratory problems such as asthma [18]. In addition, existing research regularly indicates its potential effectiveness against dengue fever [19,20]. Despite this, research has shown that *E. hirta* hosts a diverse range of biologically active compounds [21]. *E. hirta* is highly appreciated for its important antimalarial, antifungal, antipyretic, antispasmodic, sedative, antiasthmatic, anthelmintic and antibacterial properties [17]. The extraction of leaves from *E. hirta* resulted in an elevation of urinary excretion and electrolyte levels in rodent subjects [22]. Phytochemical studies have demonstrated that *E. hirta* L. is rich in bioactive constituents, including flavonoids, tannins, alkaloids, saponins, glycosides, steroids, and triterpenoids such as β -amyirin, taraxerol, and euphorbol. These substances are known to support the diuretic, galactogenic, antianaphylactic, antimicrobial, antioxidant, anticancer, antiplatelet aggregation and anti-inflammatory, aflatoxin inhibitory, antifertility, anthelmintic, antiparasmodic, antiamebic, antimalarial, larvicidal and repellent and antifeedant activities against *Plutella xylostella* [22,23].

Despite their potential hazards, medicinal plants harbor the capacity to produce more effective and less harmful therapeutic agents if subjected to comprehensive research. It is imperative to evaluate the quality, efficacy, and safety of plant-derived medications. The results of this

preliminary study will subsequently be incorporated into an essential database for the evaluation of this plant's safety profile. This research aims to perform both qualitative and quantitative analyses to determine the nutritional composition and bioactive constituents of *E. hyssopifolia* and *E. hirta*, in addition to exploring their antioxidant and antibacterial properties and assessing their safety.

METHODS

Plant material

The collection of the two plants took place in October 2022, with specimens gathered in Rabat (33°57'55.1" N 6°53'37.1" W). For this study, we utilized the entire plant to prepare our extracts.

Proximate analysis

Dry matter content

To find the percentage of dry matter (DM), the sample must be placed in an oven set at 70 °C until there is a difference of at least 3 mg between two separate, consecutive weigh-ins spaced two hours apart [24].

Ash content

Total ash from samples was measured using the gravimetric method at 550°C in accordance with ISO 936 [25], the findings were shown as a percentage (g of total ash per 100 g of dry matter).

Protein content

The average total protein was estimated using the Association of Official Analytical Chemists technique (AOAC 928.08) [26]. Grams of protein per 100 grams of dry weight were used to express the results. A Jones factor of 6.25 was used to convert nitrogen content to protein content.

Lipid content

The mean lipid content was determined using the Soxhlet technique and reported as g.100 g⁻¹ dry matter [26].

Total carbohydrate content

Equation (1) was used to calculate the quantity of carbohydrates in both plants by deducting the quantities of fat, protein, and ash from the dry matter value [27].

$$\text{Carbohydrates (g.100 g}^{-1}\text{)} = \text{DM} - (\text{P} + \text{A} + \text{F}) \quad (1)$$

Where:

DM: is the percentage of total dry matter;

P: is the percentage of protein;

A: is the percentage of ash;

F: is the percentage of total lipids.

Mineral composition

Using a One gram test sample, the mineral components in the calcined residue were identified after 16 hours at 480 °C. After being diluted with 25% concentrated nitric acid, the ash was filtered. The purpose of this extract was to determine the minerals. Inductively Coupled Argon Plasma Atomic Emission Spectrometry (Rf power 1500-watt, plasma gas flow rate (Ar) 8 L/min, auxiliary gas flow rate (Ar) 0.2 L.min⁻¹, axial view size, total analysis time ≈ 45 min, and integration (read) time = 15 s per element).

Since phosphorus is present in organic molecules, a digestion or calcination process is necessary to oxidize the organic material and release the phosphorus. The total phosphorus concentration was then ascertained colorimetrically using AOAC Method 965.17 [28].

Pesticide analysis

Acetonitrile is used to extract the pesticides from the samples. Following the addition of buffer citrate salts, sodium chloride, and magnesium sulfate, the liquid is vortexed and centrifuged. The filtrate is frozen for a minimum of three hours when dealing with fatty compounds. To remove the interfering chemicals and any remaining water, the organic phase is purified using a solid extraction phase (dispersive SPE) made up of magnesium sulfate and primary secondary amine (PSA). A tiny quantity of formic acid is added to the extracts to acidify them. Liquid chromatography combined with mass spectrometry is then used to assess the purified extract [29].

Extraction and Phytochemical screening of plants

Extraction

Maceration was used to create aqueous extracts of *E. hirta* and *E. hyssopifolia*. However, 200 mL of distilled water was combined with 20 g of powder from each plant. For 48 hours, the mixture was left to macerate at room temperature without any light. After this period, a paper filter was used to filter the mixture. The extracted materials were kept in storage at 4 °C.

The extraction yield was computed using the following formula in proportion to the dry plant matter weight:

$$EY = (\text{Obtained extract mass} / \text{Powder initial mass}) \times 100 \quad (2)$$

Phytochemical Screening

Phytochemical screening was done to find out which important groups of secondary metabolites existed in our extracts and might be responsible for the effects we saw. Alkaloids, reducing chemicals, flavonoids, polyphenols, tannins, anthocyanins, and quinones were among the chemical groups that were identified using conventional characterization reagents [30].

Quantification of Bioactive Contents

These analytical techniques, which were modified from Zargoosh et al. [31] to identify phenolic components, are based on the reduction of the Folin-Ciocalteu reagent during polyphenol oxidation. The researchers modified the methods described in the Chang et al. [32] study in order to determine the concentration of flavonoid compounds in their samples. On the other hand, the Hagerman [33] technique employs vanillic acid as a reagent for quantifying tannin.

Antioxidant activity and toxicity of the aqueous extract

Antioxidant Activity

The antioxidant activity of extracts from *E. hirta* and *E. hyssopifolia* was measured by looking at DPPH radical scavenging activity, described in our previous study [34]. From a series of tubes, 500 µL of ethanol solutions containing different concentrations of extracts were taken, and 2500 µL of DPPH solution (0.2 mM) was added to all tubes. The contents were shaken well and allowed to react at room temperature for 30 min. The absorbance of the samples was recorded

at 517 nm against the control. A negative control was made by mixing 500 µL of methanol and 2500 µL of DPPH solution, and the methanol was used as a blank in parallel with the test sample. The percentage inhibition was calculated as follows in Equation:

$$\% \text{ Activity} = ((\text{Abs Cn} - \text{Abs Ech})) / (\text{Abs Cn}) \times 100$$

Where: Abs Ech: absorbance of the sample and Abs Cn: absorbance of negative control.

Evaluation of acute toxicity

The Organization for Economic Cooperation and Development dose- adjustment approach, which can be found in line OECD 423 [35], was used to conduct toxicity studies. The experiment was carried out on eighteen female Wistar albino rats in the lab. The number of deaths throughout a 14-day period was calculated by observing the rats' behavior. The rats were divided into three groups, each with six females, after a 6- hour fast before the test [35]. Aqueous extracts of *E. hirta* and *E. hyssopifolia* were given orally to three groups at doses of 1000 mg.kg⁻¹, while the control group was given 10 mL.kg⁻¹ of distilled water. An hour following administration, behavioral observation was carried out.

The rats received equal amounts of daily food and water throughout the experiment. Furthermore, during the first several hours following gavage and then every day for 14 days, toxicity indicators were recorded, including coat changes, motility, tremors, changes in body weight (mass), food intake, grooming, breathing, stool appearance, mobility, respiration, convulsions, and the number of deaths. This work is carried out in accordance with the OECD Guidelines for Animal testing.

After two weeks of daily treatment, the rats were euthanized under anesthesia with chloral hydrate (50 mg.kg⁻¹), and blood samples were taken in tubes for hematological analyzes. Then, the rats were sacrificed to take their kidneys, spleen and livers in order to perform a macroscopic examination. The organs were then preserved in paraformaldehyde until the histological analysis carried out according to the usual technique of staining with hematoxylin and eosin.

Statistical Analysis

Using statistical software (SPSS, ver. 20), the data was subjected to a one-way ANOVA, followed by Tukey's test ($\alpha = 5$) for multiple comparisons and significance level determination. $p < 0.05$ was regarded as statistically significant.

RESULTS

Proximate analysis

Table 1 shows the approximate composition of *E. hirta* and *E. hyssopifolia*. The dry matter content is $14.51 \pm 0.28\%$ and $11.92 \pm 0.04\%$ respectively for *E. hirta* and *E. hyssopifolia*, presenting a notable difference. The protein content of *E. hirta* ($1.46 \pm 0.20\%$) was significantly higher compared to the protein content of *E. hyssopifolia* ($1.07 \pm 0.09\%$). The ash content of *E. hirta* ($3.68 \pm 0.15\%$) was higher compared to *E. hyssopifolia* ($2.11 \pm 0.06\%$). The fat content of *E. hirta* ($1.78 \pm 0.02\%$) exceeded that of *E. hyssopifolia* ($1.38 \pm 0.02\%$). The carbohydrate content of *E. hirta* ($7.58 \pm 0.34\%$) was observed to be higher than that of *E. hyssopifolia* ($7.36 \pm 0.09\%$).

Minerals content

The mineral composition of the two plants was evaluated and documented in Table 1. *E. hirta* has a higher concentration of Ca (0.06 ± 0.01 mg.100 g⁻¹), K (0.02 ± 0.01 mg.100 g⁻¹), P (0.07 ± 0.01 mg.100 g⁻¹), Cu (0.02 ± 0.00 mg.100 g⁻¹) and Fe (0.05 ± 0.00 mg.100 g⁻¹) compared to *E. hyssopifolia*; 0.05 ± 0.00 mg.100 g⁻¹, 0.01 ± 0.00 mg.100 g⁻¹, 0.06 ± 0.00 mg.100 g⁻¹, 0.00 ± 0.00 mg.100 g⁻¹ and 0.04 ± 0.00 mg.100 g⁻¹ respectively. However, the level of Zn (0.03 ± 0.04 mg.100 g⁻¹) in *E. hyssopifolia* are significantly higher than those of *E. hirta* (Table 1).

Calorific content and nutritional value

The caloric value of *E. hirta* (52.2 ± 1 Kcal.100 g⁻¹) exceeds that of *E. hyssopifolia* (46.17 ± 0.45 Kcal.100 g⁻¹). A 100 g serving of *E. hirta* contributes moderately to the necessary daily energy consumption, i.e. approximately 221.49 Kj. In addition, it provides 1.54% of the RDI for carbohydrates, 0.81% of the RDI for fats and 0.30% of the RDI for proteins. A 100 g serving of *E. hyssopifolia* also contributes moderately to the required daily calorie intake, which is approximately 195.85 Kj. In addition, it provides 1.50% of the RDI for carbohydrates, 0.63% of the RDI for fats and 0.22% of the RDI for proteins.

In accordance with daily nutritional guidelines predicated on a caloric intake of 2000 Calories and the recommended dietary allowances stipulated in European regulations [36], a more favorable daily consumption of 100 g of *E. hirta* contributes approximately 2.93% of the RDI for proteins, 2.55% of the RDI for lipids, and 2.92% of the RDI for carbohydrates. Conversely, 100 g of *E. hyssopifolia* yields approximately 2.15% of the RDI for proteins, 1.97% of the RDI for lipids, and 2.83% of the RDI for carbohydrates.

Pesticide residue analysis

The results indicate an absence of detection of the targeted pesticides, which is encouraging.

Extraction yields

The aqueous extracts of *E. hirta* and *E. hyssopifolia* were prepared from 20 g of dry plant material, and the yield was quantified as a percentage, with results illustrated in Figure 1. The yields of *E. hirta* and *E. hyssopifolia* are $5.0 \pm 0.2\%$ and $4.3 \pm 0.3\%$, respectively.

Phytochemical Analysis

Phytochemical analysis of extracts derived from *E. hirta* and *E. hyssopifolia* indicated the presence of phenolic compounds, flavonoids, and both Catechic and gallic tannins (Table 1).

Quantitative phytochemical analysis

The quantification of total phenol, flavonoid, and tannin content within aqueous extracts of *E. hirta* and *E. hyssopifolia* was undertaken, as depicted in Figure 1. Total phenol content was expressed as gallic acid equivalents (mg GAE.g⁻¹ DM). Analysis revealed a significantly greater total phenol content in *E. hirta* (1.57 ± 0.11 mg GAE.g⁻¹) compared to *E. hyssopifolia* (1.21 ± 0.04 mg GAE.g⁻¹). Similarly, the total flavonoid content was found to be higher in *E. hirta* (67.49 ± 0.27 µg EQ.g⁻¹) than in *E. hyssopifolia* (53.83 ± 1.95 µg EQ.g⁻¹). Conversely, *E. hirta* exhibited the lowest tannin content (0.32 ± 0.02 mg CE/g), while *E. hyssopifolia* displayed the highest (0.51 ± 0.03 mg CE.g⁻¹). Statistical analysis indicated significant interspecies differences with respect to phenolic compounds, flavonoids, and tannins.

Antioxidant activity

This study determined the half-maximal inhibitory concentrations (IC₅₀) of plant extracts necessary to scavenge 50% of DPPH radicals. The antioxidant activity of *E. hirta* and *E. hyssopifolia* extracts is presented in Figure 1. Results indicated that the *E. hirta* extract exhibited a greater antioxidant capacity (0.30 ± 0.01 mg.mL⁻¹) than the *E. hyssopifolia* extract (0.41 ± 0.01 mg.mL⁻¹). The antioxidant activity of both plant extracts was observed to be dose-dependent (Figure 1). L-ascorbic acid, used as a standard, demonstrated significantly higher DPPH radical scavenging activity (0.11 ± 0.00 mg.mL⁻¹) compared to both plant extracts.

Acute toxicity

The findings regarding the toxicity of aqueous extracts from *E. hirta* and *E. hyssopifolia* are detailed in Figure 2. Oral administration of varying doses of the extracts did not induce mortality or observable behavioral alterations in rats throughout the experimental period. These trials indicate that the LD₅₀ exceeds 1000 mg.kg⁻¹. Furthermore, no toxicological signs, such as reduced sensitivity to stimuli (both pain and noise), decreased mobility, softened stool, altered

behavior, body weight loss, or mortality, were observed for a duration of 14 days post-administration. These effects were also absent within the initial four-hour post-administration observation period.

Aqueous extracts of *E. hirta* and *E. hyssopifolia* are classified under Category 4 of the OECD Globally Harmonized System of Classification and Labelling of Chemicals (GHS), indicating non-toxicity via the oral route [35].

The results of repeatedly administering aqueous extracts of *E. hirta* and *E. hyssopifolia* orally every day were greatly valued. Weight gain and behavioral parameter examination, however, revealed no alterations in the previously indicated parameters. Behavior observation during the study period revealed that no behavioral alterations occurred during the experimental period, irrespective of the dose given. The rats' body weight increased progressively over time, as the image illustrates. In contrast to the controls, the body weight of the rats that received the extract did not significantly alter over time.

In Figure 2, the resulting organ weights are displayed. There was no discernible difference in the weight, color, or texture of the target organs (liver, kidney and spleen) compared to the control group, suggesting that the administration of the aqueous extracts had no effect on their normal growth.

Table 2 indicates that *E. hirta* and *E. hyssopifolia* had no effect on the biochemical constants. This is statistically validated by the observation of non-significant differences compared to the control group ($p>0.05$).

Table 2 summarizes the results of the hematological analysis. No significant differences ($p>0.05$) in the hematological status between the groups of rats that received the extracts and the control group were found.

The macroscopic evaluations of the organs of rats to which extracts of *E. hirta* and *E. hyssopifolia* were administered revealed no modification of the coloration when they were juxtaposed with the organs of the control group of rats. In addition, microscopic evaluations indicated that none of the organs of the rats treated with the extract showed changes in cell architecture or negative repercussions. No pathology was observed during the histological analyzes of the main organs (liver, spleen, kidneys) of the control group.

Figures

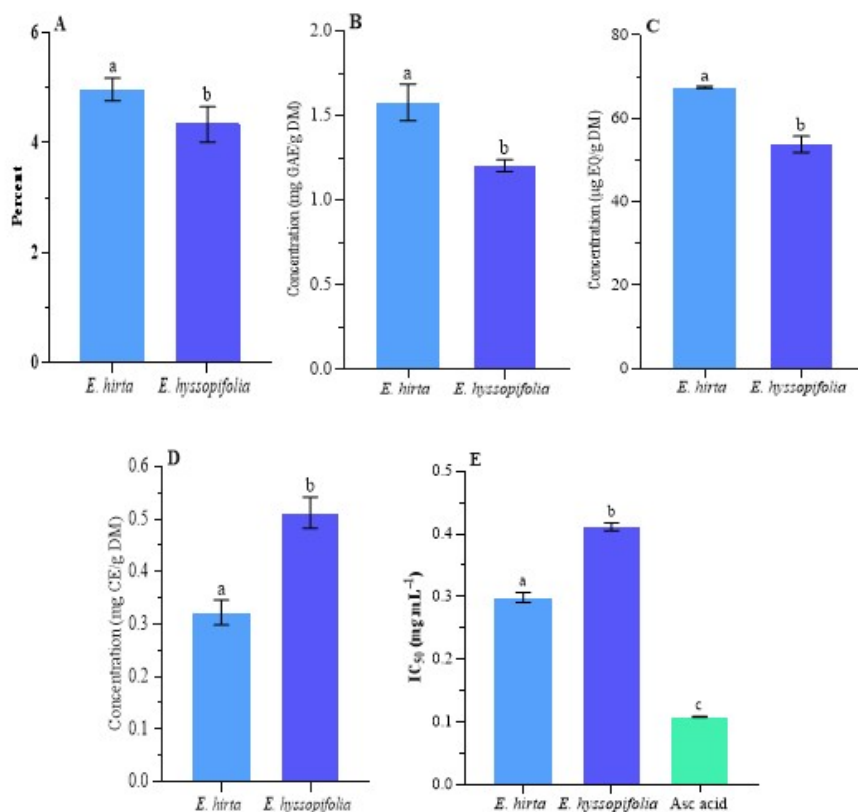


Figure 1: Histogram of the extraction yields (A), total content of phenolic compounds (B), flavonoids (C), tannins (D), and Antioxidant activity (E) of the *E. hirta* and *E. hyssopifolia*. The significant difference ($p < 0.05$) between the different extracts is illustrated by the letters a, and b.

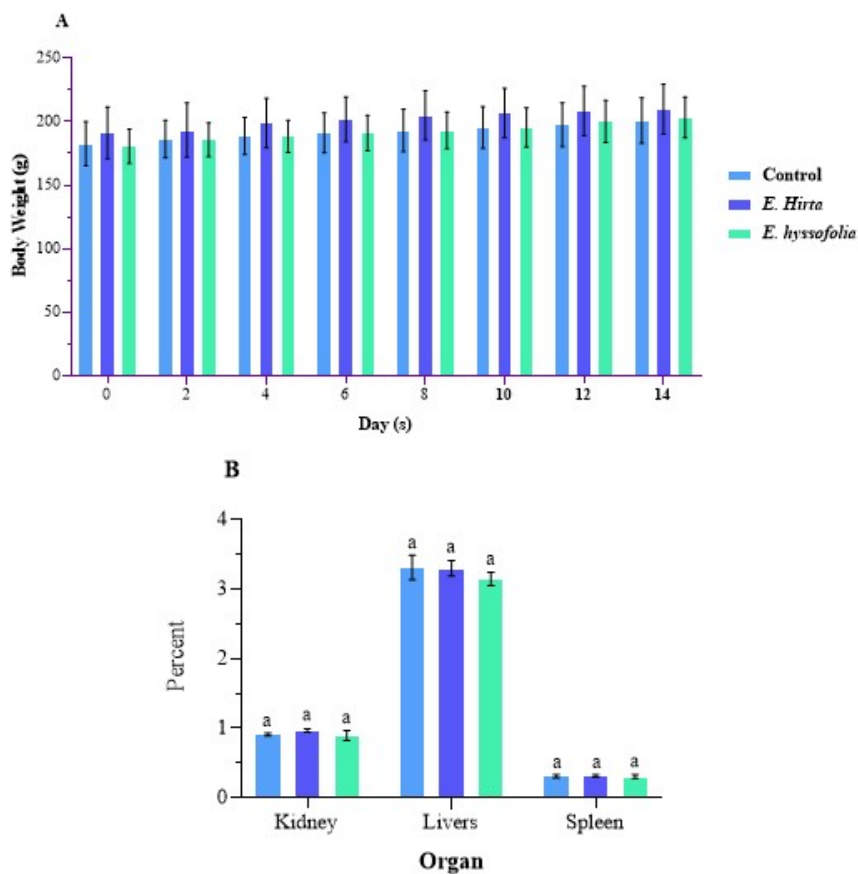


Figure 2: Effects on body weight (A) and on relative organ weights (B) in rats. The significant difference ($p < 0.05$) between the different extracts is illustrated by the letters a, and b.

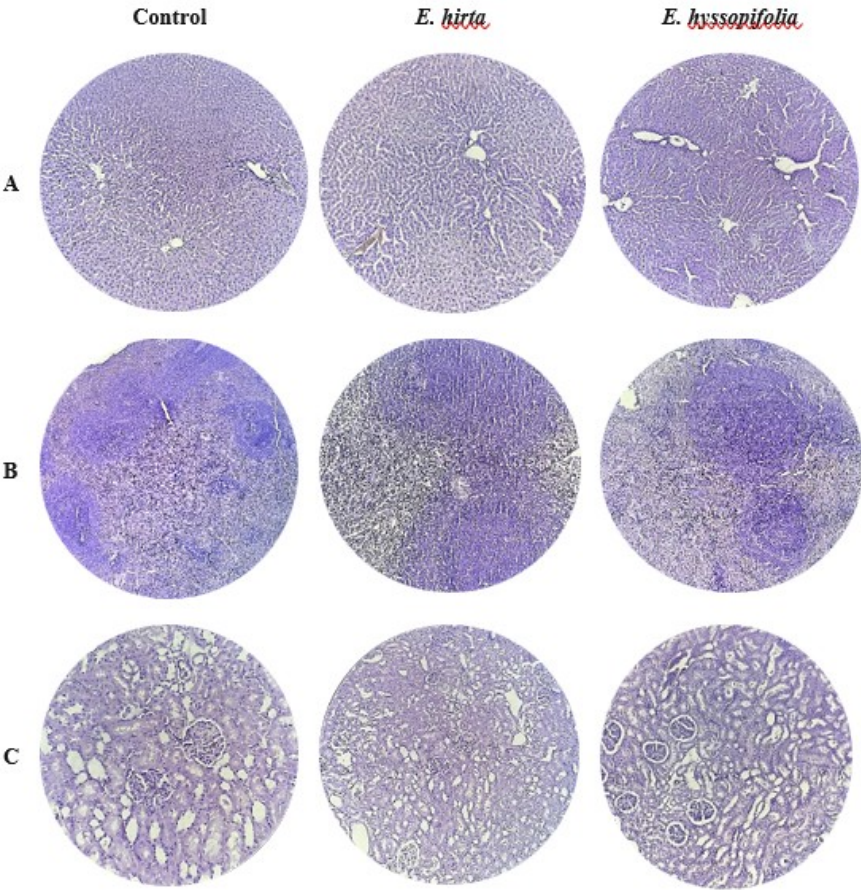


Figure 3: Representative histological sections of the liver, spleen, and kidney of rats following administration of *E. hirta* and *E. hyssopifolia* extracts. (A) Liver, (B) spleen, and (C) kidney sections from the control, *E. hirta*-treated, and *E. hyssopifolia*-treated groups.

Tables

	<i>E. hirta</i>	<i>E. hyssopifolia</i>
Proximate composition (%)		
Dry matter	14.51 ± 0.28 ^a	11.92 ± 0.04 ^b
Protein	1.46 ± 0.20 ^a	1.07 ± 0.09 ^a
Crude fat	1.78 ± 0.02 ^a	1.38 ± 0.02 ^a
Carbohydrate	7.58 ± 0.34 ^a	7.36 ± 0.09 ^a
Ash content	3.68 ± 0.15 ^a	2.11 ± 0.06 ^b
Energy	52.2 ± 1.0 ^a	46.2 ± 0.5 ^b
Minerals (mg.100 g⁻¹)		
Calcium	0.06 ± 0.00 ^a	0.05 ± 0.00 ^a
Sodium	0.02 ± 0.00 ^a	0.01 ± 0.00 ^a
Potassium	0.02 ± 0.00 ^a	0.01 ± 0.01 ^a
Magnesium	0.03 ± 0.01 ^a	0.02 ± 0.01 ^a
Iron	0.05 ± 0.00 ^a	0.04 ± 0.00 ^b
Manganese	0.21 ± 0.06 ^a	0.14 ± 0.00 ^a
Zinc	0.02 ± 0.00 ^a	0.01 ± 0.00 ^a
Copper	0.02 ± 0.00 ^a	0.01 ± 0.00 ^b
Phosphorus	0.07 ± 0.00 ^a	0.06 ± 0.00 ^b
Phytochemical analysis		
Polyphenols	+++	+++
Catechic tannins	++	+
Gallic tannins	+++	+++
Leucocyanidins	++	+
Cyanidins	–	–
Anthocyanins	–	+
Quinones	–	–
Alkaloids	–	–

Table 1: Proximate composition, Minerals content and Qualitative profile of the phytochemical compounds of *E. hirta* and *E. hyssopifolia*. The significant difference ($p \leq 0.05$) between the different extracts is illustrated by the letters a, and b. +++: Very Abundant; ++: Moderately Abundant; +: Present; –: Absent.

	Control	<i>E. hirta</i>	<i>E. hyssopifolia</i>
Biochemical constants			
ALT (U/L)	60.70 ± 1.41 ^a	60.10 ± 1.56 ^a	59.50 ± 5.52 ^a
AST (U/L)	91.55 ± 2.11 ^a	90.20 ± 1.41 ^a	91.10 ± 1.56 ^a
Chol (g/L)	0.52 ± 0.04 ^a	0.52 ± 0.02 ^a	0.51 ± 0.04 ^a
Blood Glucose (g/L)	1.76 ± 0.07 ^a	1.74 ± 0.04 ^a	1.67 ± 0.03 ^a
Hematological constants			
WBC (10 ³ .ml ⁻¹)	2.45 ± 0.35 ^a	2.56 ± 0.64 ^a	2.41 ± 0.56 ^a
RBC (10 ⁶ .ml ⁻¹)	8.44 ± 0.60 ^a	8.61 ± 0.42 ^a	8.79 ± 0.27 ^a
HGB (g.dl ⁻¹)	12.95 ± 0.35 ^a	13.05 ± 0.50 ^a	13.65 ± 0.64 ^a
HCT (%)	46.15 ± 1.49 ^a	46.80 ± 1.13 ^a	47.15 ± 0.92 ^a
MCV (fL)	53.55 ± 0.35 ^a	53.90 ± 0.57 ^a	53.65 ± 0.64 ^a
MCH (pg)	14.75 ± 0.21 ^a	15.75 ± 0.92 ^a	15.55 ± 1.20 ^a
MCHC (g.dl ⁻¹)	27.55 ± 0.64	27.70 ± 0.71 ^a	28.45 ± 1.20 ^a
PLT (10 ³ .ml ⁻¹)	918.00 ± 2.83 ^a	936.50 ± 24.75 ^a	984.00 ± 21.21 ^a
MPV (fL)	7.20 ± 0.28 ^a	7.55 ± 0.35 ^a	7.45 ± 0.07 ^a
NEUT (%)	16.00 ± 1.41 ^a	17.50 ± 2.12 ^a	16.00 ± 2.83 ^a
LYMPH (%)	82.00 ± 8.49 ^a	86.50 ± 7.78 ^a	79.50 ± 7.78 ^a
MONO (%)	4.58 ± 0.49 ^a	4.36 ± 0.09 ^a	5.735 ± 0.29 ^a
EOS (%)	1.23 ± 0.25 ^a	1.55 ± 0.30 ^a	1.63 ± 0.83 ^a
BASO (%)	1.20 ± 0.24 ^a	1.02 ± 0.22 ^a	1.14 ± 0.17 ^a

Table 2: Effects of extracts on biochemical and hematological constants. Values are presented as mean ± standard deviation (SD). Biochemical parameters included alanine aminotransferase (ALT), aspartate aminotransferase (AST), cholesterol (Chol), and blood glucose, while hematological parameters included white blood cell count (WBC), red blood cell count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), mean platelet volume (MPV), neutrophils (NEUT), lymphocytes (LYMPH), monocytes (MONO), eosinophils (EOS), and basophils (BASO). Values bearing the same superscript letter are not significantly different (*p*>0.05).

DISCUSSION

Although the plant serves as a moderate source of protein, it is insufficient when juxtaposed with the recommended nutritional intake of protein, set at 56 g for individuals weighing 70 kg and 46 g for adults weighing 50 kg. Proteins of plant origin are generally considered to be of inferior quality, but when integrated with other protein sources such as proteins of animal origin, they can result in a sufficient nutritional profile [37].

A diet providing 1 to 2% of its caloric intake in the form of lipids is considered adequate for human consumption, since excessive fat consumption has been linked to the development of obesity and cardiovascular diseases [37]. Furthermore, lipids are integral to nutritional intake, functioning as a significant energy reservoir, aiding in the absorption of fat-soluble vitamins, and supporting various cellular processes. Carbohydrates are integral in supplying the energy required for physiological processes, functioning as vital nutrients within a well-rounded diet [37]. They are responsible for feeding various cells of the body, including those of the brain, muscles and blood [38].

Our results are in agreement with the results reported by Igwenyi et al. [39] for *E. hyssopifolia*. The fertility of soil, the content of moisture, and the temperature conducive to growth are the primary environmental determinants that affect the nutritional profile of flora cultivated in various geographic locales.

The significance of minerals in human dietary intake is extensively acknowledged, as they contribute substantially to the enhancement of both physical and cognitive well-being. Indeed, an extensive array of crucial micro and macro elements, commonly designated as trace elements, is indispensable for a variety of physiological functions [37]. Minerals are integral to

the optimal performance of tissues and serve as auxiliary signaling molecules in a multitude of biochemical pathways [40]. Iron constitutes a vital component of proteins and functions as a catalyst for particular enzymes, including cytochrome oxidase [41], and it is vital for the synthesis of hemoglobin [42]. It serves a critical function in the transference of energy throughout the plant organism and additionally modulates adiposity by facilitating the oxidation of biological molecules [41]. Sodium serves as a principal cation within both extracellular and intracellular fluids, playing a crucial role in sustaining the homeostasis of electrolytes within the organism [43]. Zinc is integral to the biochemical processes of protein synthesis, the replication of DNA, cellular differentiation, immune responses, and reproductive mechanisms [44]. Calcium constitutes a critical element of osseous and dental structures, playing a vital role in hemostasis, myocyte contraction, and acting as a necessary cofactor in various enzymatic processes [45].

Azaat et al. [46] reported a 1.5% yield for n-hexane, 2.8% for ethyl acetate, 13.7% for aqueous, and 10.6% for methanolic extracts of *E. hyssopifolia*, while Koffi et al. [47] obtained 22.5% for aqueous and 12.2% for ethanolic extracts of *E. hirta*.

The concentration of phenolic compounds in natural products constitutes a crucial metric for the quantitative assessment of extracts and their associated biological activity, owing to the significant roles these compounds play in fundamental physiological processes [48]. These findings are consistent with those reported by Azaat et al. [46], who observed a total phenol content of 0.45 mg.g⁻¹ and a flavonoid content of 0.11 mg.g⁻¹. A separate investigation demonstrated that the n-hexane extract of *E. dracunculoides* possessed a total phenol content of 8.21 mg.g⁻¹ and a flavonoid content of 4.18 mg.g⁻¹ [49].

Phenolic compounds exhibit a broad range of biochemical activities, including antioxidant, antimutagenic, and anticancer properties, and the capacity to modulate gene expression. Flavonoids are a class of active ingredients that exhibit a variety of biological actions, including the suppression of mitochondrial adhesion formation and resistance to microbial, ulcerative, arthritic, angiogenic, and carcinogenic illnesses [50].

Plants represent a substantial reservoir of bioactive compounds with potential therapeutic applications, including the development of novel chemotherapeutic agents. Their utilization in both traditional and modern medical practices encompass the treatment of diverse ailments and the management of various pathogens. The pharmacological activity of substances derived from medicinal plants is the subject of ongoing investigation by researchers globally. Notably, herbal remedies are employed by approximately 80% of the world's population, attributable to their perceived efficacy, affordability, non-narcotic characteristics, and limited adverse effects [51].

Phytochemicals are classified as naturally occurring secondary metabolites found in plants. The identification and functional characterization of bioactive substances with medical significance have advanced significantly [52]. The phytochemical screening methodology encompasses both qualitative and quantitative analyses of the chemical classes present in plant species utilized in ethnomedicine.

Given the observed substantial variation in phytochemical content between the two plant species, a hypothesis regarding potential differences in their pharmacological effects was formulated. To empirically investigate this hypothesis, the antioxidant and antibacterial activities of both plants were subsequently assessed.

The scavenging of free radicals by 2,2-diphenyl-1-picrylhydrazyl (DPPH) constitutes a validated methodology for assessing the antioxidant capacity of plant extracts. This technique is characterized by its reduced analysis time, thereby facilitating its widespread application in quantifying the antioxidant potential of plant-derived materials. DPPH's efficacy as an antioxidant stems from its hydrogen-donating capability. The elimination of these free radicals is crucial for mitigating their deleterious effects in diverse pathologies, including cancer [53,54].

The observed antioxidant activity of the plant extracts is attributed to the presence of phenolic compounds. The antioxidant activity of aqueous, methanolic, ethyl acetate, and n-hexane extracts (0.31, 0.08, 0.19, and 2.08 mg.mL⁻¹, respectively) has been previously demonstrated by Azaat et al. [46]. Observed variations in the antioxidant potential among different plant species

can be attributed to significant disparities in their respective phenol and flavonoid concentrations. Furthermore, the choice of extraction solvent has been demonstrated to influence anti-radical scavenging activity [52].

For many centuries, natural substances, especially medicinal plants, have served as fundamental treatments for many ailments [55]. When evaluating the pharmacological effectiveness of natural substances, the evaluation of the toxicological characteristics of an extract, a fraction or a compound of a natural product generally constitutes an initial phase. Despite the advantageous pharmacological properties of *E. hirta* and *E. hyssopifolia*, knowledge about the chronic toxicology of these well-known plants remains insufficient. Therefore, the present study was conducted to evaluate and focus on their acute toxicity using an animal model.

These findings show that the extract's oral treatment has no influence on rats' regular growth. In comparison with other studies, Capule [56] found that the aqueous extract of *E. hirta* seemed non-toxic and that the mice remained intact throughout the experiment.

Weight variations serve as a measure of drug-related adverse reactions [57]; however, the absence of noticeable weight fluctuations in treated rats suggests that the extract does not have an impact on their growth, since appetite suppression can lead to weight loss due to metabolic disorders [58].

It is common practice to use an organ's relative weight to determine the extent of toxic damage to that organ. Given that the liver is the primary organ implicated in harmful metabolism, the hepatochemical coefficient is typically used to assess the level of toxicity of tested sample. The increase in the weight of the liver is a characteristic of the toxic impact. In the realm of toxicological research, the comparative mass of various organs is considered a relatively rational metric [59].

A common technique for assessing the severity of toxic organ damage is the proportional organ weight analysis. The major organ responsible for the metabolism of toxic substances is the liver, and the hepatic coefficient is often used to judge the toxicity of the samples tested. An index of toxicity is an increase in liver weight. The target organs did not differ significantly in weight, color or texture from the control group, which indicates that the treatment with *E. hirta* and *E. hyssopifolia* had no impact on their normal development. According to the toxicity studies, the relative weight of the organs is considered as a relatively sensitive indication [59].

According to Mukinda and Syce [60], the examination of blood parameters is crucial, since it can provide information on hematopoietic function, detect possible allergies and observe intravascular effects such as hemolysis. AST and ALT levels are reliable indicators of possible liver tissue damage [61]. Although AST is widely present in the liver and heart, ALT is found exclusively in the cytoplasm of hepatocytes. Thus, liver damage can cause elevated AST and ALT levels in the blood [62].

It is clear that hematological analyzes are used to prove the toxicity and judge the safety of the product. Since the hematopoietic system can serve as an essential indicator to define physiological and pathological conditions in humans and animals, it represents one of the most exposed targets to harmful chemicals [63].

The enlargement of the organs constitutes a first indicator of the potential toxicity associated with a chemical or biological agent. Nevertheless, this study did not document organ enlargement in all the groups examined.

This study demonstrates that *E. hirta* and *E. hyssopifolia* have a varied nutritional and phytochemical composition, with significant contents of polyphenols, flavonoids and essential minerals. Their antioxidant properties support their traditional use in the treatment of various ailments. Moreover, the absence of acute toxicity at 1000 mg.kg⁻¹ confirms their safety profile, reinforcing their potential as natural sources of bioactive molecules. However, additional research, in particular on chronic toxicity and precise mechanisms of action, is necessary to validate their application in pharmacology and human nutrition. These plants could thus constitute a promising alternative in the development of food supplements or natural

therapeutic agents.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Author Contributions

All authors took part in conceptualization of the study, execution of experiments, data analysis, writing manuscript and finally doing revisions.

AI Use Statement

The authors used an artificial intelligence (AI) tool solely for language editing and improvement of the manuscript. The AI tool was not used for data generation, data analysis, interpretation of results, scientific conclusions, or preparation of figures. The authors carefully reviewed and edited all AI- assisted content and take full responsibility for the accuracy, integrity, and originality of the manuscript and the data presented therein.

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