Phytochemical Profile,Acute Toxicity, Antioxidant and Anti-inflammatory Activity of Hydroethanolic ExtractsFrom*Odontonema strictum*(Nees) Kuntze (Acanthaceae)Root and Stem

ABSTRACT

Background: The leaves of *Odontonema strictum* (Nees) Kuntze (Acanthaceae), a decorative plant native to Latin America, are used to treat hypertension in alternative medicine. However, the other parts of the plant have not yet been investigated for their antihypertensive properties. Aims: Our study aimed to investigate phytochemical groups with antihypertensive potential, antioxidant and anti-inflammatory properties, and the safety of using stems and roots hydroethanolic extracts.

Methodology: Phytochemical screening by HPTLC and determining flavonoids andtotal polyphenols were carried out. The antioxidant activity was evaluated usingABTS, FRAP, DPPH, and LPO methods. The inhibitory activity of Phospholipase A2 and 15-lipoxygenase was determined. Finally, acute oral toxicity was carried out on female mice NMRI.

Results: The hydroethanolic extracts of *Odontonema strictum* root (HE_ROS) and stem (HE_SOS) contain tannins, sterols, triterpenes, flavonoids, and saponosides. HE_SOS gave the highest levels of flavonoids (23.69±1.10 mg QE/g) and total phenolics (64.15±4.02 mg TAE/g. The two hydroethanolic extracts had antioxidant activity, with HE_ROS being the best. However, these activities were lower than Trolox ($p<0.05$). The extracts have a moderate inhibitory effect on 15-lipoxygenase and Phospholipase A2. Finally, HE_ROS and HE_SOS are practically non-toxic when administered once orally.

Conclusion: This study provided scientific data and could justify the use of *Odontonema strictum* in the treatment of hypertension.

Keywords:*Odontonema strictum*, Root, Stem, Antioxidant, Anti-inflammatory, Safety of use

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1. INTRODUCTION

The pathophysiology of cardiovascular disease is multifactorial. It involves inflammation, oxidative stress, vascular dysfunction, and sympathetic system activation[1, 2]. To cope with these disorders, patients, in addition to modern medicines, turn to alternative medicine. For example, the use of herbal medicines is steadily increasing due to greater adherence and acceptance in developing and developed countries[3, 4]. Furthermore, the growing interest in alternative medicine is thought to be explained by the failure of conventional medicine or the search for new bioactive compounds for disease management[5].Thus, "ethnopharmacological evidence could be considered valuable hypotheses to support preclinical or clinical research in phytomedicines in the current context of developing new or alternative therapeutic agents from natural sources.

Moreover, the only data available on the potential applications of plant species in phytotherapy are sometimes those provided by ethnopharmacology, given their limited use by indigenous populations"[6, 7].In addition, "among the plants used to treat chronic conditions such as hypertension is *Odontonema strictum* (Nees) Kuntze (Acanthaceae), a decorative plant from Latin America. It is known to be used in alternative medicine to treat arterial hypertension"[8, 9]."Using pharmacological tests, the authors reported that aqueous, methanolic, and ethyl acetate extracts of the plant's leaves have antihypertensive/hypotensive properties *in vivo* and vasorelaxant properties *ex vivo* on the coronary arteries of mice, rats, and pigs"[9, 10]. "The extracts have also shown antioxidant properties"[11, 12]. "Phytochemically, flavonoids O-heterosides and C-heterosides have been isolated from the plant's leaves"[8]and "polyphenols such as verbascoside and isoverbascoside"[13]. "*Odontonemastrictum* leaves, and flowers contain carbohydrates, saponins, flavonoids, sterol-terpenes, tannins, anthocyanins, β-Sitosterol, and Stigmasterol"[10, 13-15]. Moreover, all the findings on the plant concern the leaves. Therefore, this study investigated phytochemical groups with antihypertensive potential, antioxidant and anti-inflammatory properties, and the safety of using stems and roots hydroethanolic extracts.

2. MATERIAL AND METHODS

2.1. Material

2.1.1. Plant Material

*Odontonemastrictum*roots and stems wereharvested in June 2022 from the "Institut de Recherche en Sciences de la Santé / Centre National de la Recherche Scientifique et Technologique (IRSS/CNRST)"botanicalgarden(GPS coordinates N 12°22.161', W 001°29.088'), Ouagadougou (Burkina Faso). The reference specimen (HNBU 8702), after identification of the plant, was prepared and deposited at the"DépartementEnvironnement et Forêts (DEF)/CNRST," Ouagadougou, Burkina Faso. The collected samples were protected from sunlight and dust, dried in the open air, and ground using a mechanical grinder (Gladiator Est. 1931 Type BN 1 Mach. 404611083) to a powder. The roots and stem powder obtained were used to prepare the study extracts.

2.1.2. Experimental Animals

Female NMRI (Naval Medicinal Research Institute) mice with average weights of 27 ± 2 gfrom the animal house of the "Institut de Recherche en Sciences de la Santé / Centre National de la Recherche Scientifique et Technologique (IRSS/CNRST), Burkina Faso."They were reared in

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plastic cages containing wood shavings at a temperature of 21-23 °C with a relative humidity of 55-60% and subjected to light (12/24 h) according to the rearing conditions of these species. Standard laboratory pellets enriched with proteins (29%) and water were provided for satiation to mice [16].

2.2.Methods

2.2.1. Preparation of HydroethanolicExtract

Extraction was performed using the maceration exhaustion technique described by Nitiéma et al.[17] brieflyfor 24 hours at room temperature. 200 g of each plant powder was macerated in 1000mL of 80% ethanol (Ethanol 96%/Water; 80/20; v/v). After 24 hours, the mixture was filtered through a fine mesh nylon cloth and centrifuged at 2000 rpm for 5 min. “Each residual pomace wassuccessively extracted twice under the same conditions with 80% ethanol. The filtrates obtained were concentrated in a rotavapor (electronic microprocessor controller CPS ventilated type) under reduced pressure at a constant temperature of 60 °C and then frozen and lyophilized.The hydroethanolic extracts of *Odontonema strictum* root (HE_ROS) and *Odontonema strictum* stem (HE_SOS) were then recovered and stored in a hermetically sealed plastic bottle protected from light and humidity for the various tests” [16].

2.2.2. Phytochemical screening by High-Performance Thin-Layer Chromatography

“High-performance thin-layer chromatography (HPTLC) detectedsaponosides, flavonoids, sterol-triterpenes, and tannins in the two extracts. HPTLC was carried out on chromatoplates (60 F_{254}, 10 x 5 cm, glass support 10 x 20 cm, Merck) [18]. Approximately 20 µL of each extract was streaked with a semi-automatic sample dispenser (CAMAG, Linomat 5, Switzerland) along the baseline 0.8 cm from the bottom edge of the plate. After deposition and drying, the plates were placed in a tank containing eluent previously saturated (20 × 10 cm, saturation time: 30 min). The solvent system used depended on the metabolite to be identified: Ethyl acetate/formic acid/H_2O (18/2/4/2/1 v/v/v/v) for tannins; Ethyl acetate/formic acid/H_2O, (8/2/1 v/v/v/) for flavonoids; Hexane/ethyl acetate/methanol (10/5/5 v/v/v) for saponosides; Ethyl acetate/hexane (8/2 v/v) for Sterol-triterpenes. After migration over 80mm in length, the plates were dried, and Neu reagent for flavonoids, Sulphuric anisaldehyde reagent for saponosides, Liebermann-Burchard reagent for Sterol-triterpenes and 5% of $FeCl_3$ for tannins revealed the chromatographic profiles. The phytochemical profiles of the two were then observed under visible light (tannins) and at UV wavelengths of 366 nm” [16].

2.2.3. Determination of Total Phenolic Compounds

“The total phenolic content of HE_ROS and HE_SOSwas determined using the Folin-Ciocalteu Reagent (FCR) [19]. Gallic acid, the reference compound, was used to construct the standard curve. Briefly, 25 µl of sample at a 1 mg/mL concentration was mixed with 125 µL of FCR. One hundred (100) µL of 7.5% w/v sodium carbonate solution was added to the mixture. Absorbance at 760 nm was measured using a microplate reader (Spectro UV, Epoch Biotek, USA) after one hour's incubation. Results were expressed as mg gallic acid equivalent (GAE)/g dry extract” [16].

2.2.4. Determination of Flavonoid Compound

“The total flavonoid content of HE_ROS and HE_SOSwas assessed using an aluminumtrichloride reagent [19]. A standard calibration curve was plotted with quercetin as the reference. Briefly, 100 µL ofeach extract(1 mg/mL) was mixed with 100 µL of a 2% w/v aluminum trichloride solution. Absorbance at 415 nm was measured using a mass

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spectrophotometer(Epoch Biotek, USA) after 10 min's incubation. Results were expressed as mg quercetin equivalent (QE)/g dry extract of *Odontonema strictum" [16]*.

2.2.5. Assessment of Antioxidant Properties

2.2.5.1.Ferric Reducing Antioxidant Power (FRAP) Assay

"The FRAP assay was performed on HE_ROS, HE_SOS, and Trolox, as described previously[20]. The mixture of 1.25 mL potassium hexacyanoferrate aqueous solution (1%) and 0.5 mL samples with 1.25 mL phosphate buff er was incubated at 50°Cfor 30 min. Next, 1.25 mL of trichloroacetic acid (10%) was added and centrifuged at 3000×g for 10 min. $FeCl_3$ solution (0.125 mL, 0.1%) and distilled water (0.625 mL) were added to the supernatant (0.625 mL), and absorbance was measured at 700 nm using a spectrophotometer (Agilent, Santa Clara, CA) equipped with ChemStation UV-visible software. The reference substance Trolox was used to plot the calibration curve. The FRAP activity of the samples (HE_ROS and HE_SOS) was expressed as mmol Trolox equivalent/gram dry extract" [16].

2.2.5.2.DPPH• Essay

Hydroethanolic extracts of *Odontonema strictum* root and stem and Trolox free radical scavenging activity were performed using 2,2-diphenyl-1-picrylhydrazyl (DPPH)[21]. Briefly, after 30 min of incubation in the dark at room temperature,the absorbance of 10 µL of samples and Trolox added to 200 µLof DPPH (0.04 mg/mL) was measured at 490 nm using a Bio-Rad spectrophotometer (model 680, Japan). The result was expressed as antioxidant capacity equivalent to Trolox. As a function of sample concentration, a DPPH•-inhibition percentage curve was plotted. The inhibition (%) of the DPPH radical was calculated using the following formula:
Inhibition (%) = [(Ac - Ae)/Ac]x100; Ae and Ac represent, respectively,the absorbances of the extract/ascorbic acid and the control (DPPH solution without sample). The concentration required to inhibit 50% of DPPH (IC_{50}) was determined on the curve.
Anti-radical power (ARP) was determined by the formula: ARP= 1/ $IC_{50;}$ARP: Anti Radical Power; IC_{50}: 50% inhibitory concentration expressed in µg/mL

2.2.5.3.ABTS$•^{+}$Assay

The ABTS free radical scavenging activity of HE_ROS, HE_SOS, and Trolox was assessed using the previously described procedure[19]. A mass of 19.2 mg of ABTS was dissolved in 5 mL of distilled water.A 3.312 mg potassium persulphate mass was added to the ABTS solution at 3.84 mg/mL. The solution was left for 16 h in the dark at room temperature before useafter adding the potassium persulphate. On the day of the experiment, 4.5 mL of the mixture was diluted in 220 mL of absolute ethanol. The range of 8 dilutions to be tested was prepared from the parent concentration of the samples (1 mg/mL). On a 96-well microplate, 200 µL of ABTS solution mixed with 20 µL of the extract or reference was added to each well. After incubation at 25 ºC for 30 min, absorbances at 415 nm were read against a blank using an Agilent 8453 spectrophotometerwith ChemStation UV-visible software. Measurements were performed in triplicate. The percentage inhibition of absorbance at 415 nm was calculated according to the formula:
% Inhibition = [(A0-A)/ A0] x 100
A0 is the absorbance of the control; A is the absorbance of the sample.
The absorbance inhibition curve as a function of HE_ROS, HE_SOS, or Trolox concentration was constructed to determine the 50% inhibitory concentration (IC_{50}). Anti-radical power (ARP)

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was defined using the formula: ARP = $(1/CI_{50})$; ARP: Anti-radical power; IC_{50}: 50% inhibitory concentration expressed in µg/mL.

2.2.5.4.Lipid Peroxidation Inhibition (LPO) Assay

The lipid peroxidation activity of Wistar rat liver was determined using 2-thiobarbituric acid, described previously [19]. $FeCl_2$-H_2O_2 was used to induce peroxidation of liver homogenate. A 0.2 mL volume of HE_ROSandHE_SOSat a concentration of 1.5 mg/mL was mixed with 1 mL of 1% rat liver homogenate, then 50 µL H_2O_2 (0.5 mM) and 50 µL $FeCl_2$ (0.5 mM) were added. The mixture was incubated for 60 min at 37°C, and then 1 mL of 2-thiobarbituric acid (0.67%) and 1 mL of trichloroacetic acid (15%) were added. For 15 min,the mixture was heated in boiling water. The experiment was performed in triplicate, and the absorbance at 532 nm was read. Trolox was used as the reference substance. The percentage inhibition was calculated using the following formula:Percentage inhibition (%) = [1-(A1 - A2)/A0]x100

A1 is the absorbance of the control (without sample); A2 is the absorbance with the sample; A0 is the absorbance without liver homogenate.

2.2.6. Anti-Inflammatory Activity

2.2.6.1.Lipoxygenase Inhibition Assay

Lipoxygenase inhibition was determined using linoleic acid (1.25 mM) as substrate [19]. Inhibitors (HE_ROS orHE_SOS/ Zileuton: reference substance) were prepared to a final 100 µg/mL concentration. A volume of 146.25 µL of 15-lipoxygenase solution (820.51 U/mL) was added to 3.75 µL of each inhibitor. Next, 150 µL of linoleic acid was added. A spectrophotometer (Epoch Biotek Instruments, USA) was used to measure absorbances at 234 nm against enzyme-free blanks. The tests were carried out in triplicate, and the percentage of lipoxygenase inhibition was calculated using the formula:

Inhibition (%) = [(AE - AI)/AE]x100

AE: Absorbance enzyme test - Absorbance blank; AI: Absorbance inhibition test - Absorbance blank.

2.2.6.2. Phospholipase A2 (sPLA2) Inhibition Assay

The sPLA2 activity of bee venom was determined according to the instructions of the manufacturer Abcam (Japan) described in catalog no. ab133089[17, 19]. To perform the sPLA2 inhibition assay,a 96-well microplate was used. A final 100 µg/mL concentration of HE_ROS, HE_SOS,and reference compounds (Betamethasone and Acetylsalicylic acid) was used. Absorbances were read spectrophotometrically (Agilent 8453) at 415 nm against a blank that had not received the enzyme. The experiment was performed in triplicate.The percentage inhibition of sPLA2 at 100 µg/mL was calculated using the following formula: Inhibition (%) = [(AE - AI)/AE]x100.

AE: Absorbance of enzyme assay - Absorbance of blank; AI: Absorbance of inhibition assay - Absorbance of blank.

2.2.7. Acute Oral Toxicity

The acute oral toxicity test for HE_ROS andHE_SOSwas performed on female mice following OECD guideline 423[21, 22]. Two (02) batches, each consisting of three mice, were placed separately in polypropylene cages: a test batch of three miceand a control batch of three mice. The extracts were administered by gavage using a single dose of an esophageal tube to the test

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mice after fasting for three hours. A dose of 2g/kg body weight (bw) of HE_ROS andHE_SOSwas chosen as the starting dose. The control batch received the solvent for dissolving the extract (distilled water, 10 mL/kg). The mice were observed individually for two hours after administration, at the end of which food was restored. They were then observed twice daily for 14 days to monitor for mortality and toxidrome (signs of toxicity), such as changes in Hair standing up, Lack of appetite, convulsions, diarrhea, Sleepiness, and coma. The quantities of food and water consumed and the weight of each mouse were measured every 2 days during the 14 days of experimentation. The mice were sacrificed, and an autopsy was performed on the organs (liver, lungs, kidneys, heart,and spleen),then weighedon day 15. The relative weight of each organ was calculated [(Organ weight (g) / Fasting mouse weight on the day of sacrifice (g)) ×100]. After the mice were killed, the test was repeated under the same conditions as the first test (test batch and control batch).

2.3. Statistical Analysis

Values are given as arithmetic means ± SD. The graphics were constructed using GraphPad Prism 8.4.3 Software, San Diego, CA. Statistical comparisons were performed usingStudent's T-test and one- and two-way ANOVA. A post hoc test was performed using Bonferroni's test analysis to compare all the groups. A *p*-value < 0.05 was considered statistically significant.

3.RESULTS

3.1. Phytochemical Screening

The phytochemical analysis of HE_ROS andHE_SOShighlighted the presence of saponins, flavonoids,steroids, triterpenes, and tannins (Table 1).

Table1:Phytochemical profile of hydroethanolic extractsof *c* revealed by HPTLC

Extracts	Compounds			
	Saponosides	flavonoids	steroids and triterpenes	tannins
HE_ROS	+	+	+	+
HE_SOS	+	+	+	+

HE_SOS: Hydroethanolic extract of *O. strictum*stem; HE_ROS: Hydroethanolic extract of *O. strictum* root

3.2. Total phenolic and flavonoid contents in *Odontonema strictum* root and stem

The contents of total phenolics and flavonoidsin HE_ROS andHE_SOSare recorded in Table 2.HE_ROS and HE_SOS showed similar values for total phenolic compounds. However, the flavonoid content of HE_SOS was higher than that of HE_ROS (p<0.01).

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Table 2: Total phenolics and flavonoid contents in *Odontonema strictum* root and stemextracts

Extracts	**Total phenolics**(mg TAE/g)	**Flavonoids** (mg QE/g)
HE_SOS	64.15±4.02	23.69±1.10
HE_ROS	57.85±3.69	12.92±1.96**

*QE: quercetin equivalent; TAE: tannic acid equivalent; **p<0.01 vs HE_SOS; n = 3*

3.3. Antioxidant activity of extracts

The antioxidant activity of HE_ROS andHE_SOSis shown in Table3.The IC_{50} of the ABTS and DPPH tests were lower with Trolox than with the 02 extracts ($p < 0.001$).The percentage inhibitions of the LPO test were lower with Trolox than with the 02 extracts ($p < 0.001$).HE_ROS and HE_SOS at 100 µg/mL were more effective than Trolox at inhibiting lipid peroxidation (LPO)($p < 0.001$).However, there was no significant difference between the inhibition percentages of 02 extracts by the DPPH and FRAP tests.

Table 3:*In vitro* antioxidant activity of hydroethanolic extractsof *O. strictum* root and stem

Extracts	ABTS		DPPH		FRAP	LPO
	IC_{50} (µg/mL)	ARP	IC_{50} (µg/mL)	ARP	mmol EAA/g	Inhibition (%) (at 100 µg/mL)
HE_SOS	$22.58±2.83^{***}$	0.04	$32.59±4.35^{***}$	0.03	565.39±12.89	$62.24±5.38^{*}$
HE_ROS	$9.97±2.75^{***ϕϕ}$	0.10	$29.18±2.94^{***}$	0.03	$719.34±6.49^{ϕϕϕ}$	$73.61±6.42^{**}$
Trolox	$2.04±0.12^{ϕϕϕ}$	0.49	$5.06 ± 0.15^{ϕϕϕ}$	0.19	-----	$48.11±3.88^{ϕϕ}$

*IC_{50}: inhibition concentration 50%; ARP: anti-free radical power; n = 3; *p < 0.05 vs Trolox; ^{ϕϕ}p< 0.01 vs HE_SOS, EAA: Ascorbic acid equivalent.*

3.4. Anti-inflammatory Activity *InVitro*of *Odontonema strictum*Root and Stem Extracts

The evaluation of the *in vitro* anti-inflammatory activity of *Odontonema strictum* root and stem extracts by inhibiting 15-lipoxygenase and Phospholipase A_2 is recorded in Table 4.HE_ROS and HE_SOS have weak inhibitory effects on 15-lipoxygenase and Phospholipase A_2 compared with Betamethasone and Zileuton($p<0.001$).

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Table 4:15-Lipoxygenase and Phospholipase A_2 inhibitory activity of *Odontonemastrictum* root and stem extracts

Extracts	15-Lipoxygenase IC_{50} (µg/mL)	Phospholipase A_2 Inhibition (%)
HE_SOS	18.31±1.09***	7.39±1.04$^{\phi\phi\phi\ \#\#\#}$
HE_ROS	15.73±2.23***	6.77±0.56$^{\phi\phi\phi\ \#\#\#}$
Zileuton	2.92±0.32	----
Betamethasone	----	35.39±3.31
Acetylsalicylic acid	----	58.18±3.53

HE_SOS: Hydroethanolic extract of *O. strictum*stem; HE_ROS: Hydroethanolic extract of *O. strictum* root; ***p<0.001 vs Zileuton; $^{\phi\phi\phi}$p<0.001 vs Betamethasone;$^{\#\#\#}$p<0.001 vs Acetylsalicylic acid; n = 3

3.5. Acute Oral Toxicity

3.5.1. Mortality and Toxidrome

The dose of 2000 mg/kg body weight (bw) showed no remarkable behavioral changes ormortality in female mice at the first and second stages of administration of HE_ROS andHE_SOS(Table 5).

Table 5:Mortality of female mice administered a single dose of *O. strictum* root (HE_ROS) and stem(HE_ROS) extracts

Extracts administered / Behaviour of mice	Mortality	
	1st test	2nd test
Control (Distilled water)	0/3	0/3
HE_ROS(2000 mg/kg)	0/3	0/3
HE_SOS(2000 mg/kg)	0/3	0/3
Convulsions	-	-
Sleepiness	-	-
Hair standing up	-	-
Lack of appetite	-	-
Diarrhea	-	-
Hyperventilation	-	-

HE_SOS: Hydroethanolic extract of *O. strictum* stem; HE_ROS: Hydroethanolic extract of *O. strictum* root; n = 6

3.5.2. Changes in Body Weight, Food, and Water Consumption of Mice

Figure 1 shows the mean weight gain, feed consumption, and water consumption for two weeks in female mice given a vehicle (distilled water), and a single dose (2000 mg/kg) of HE_ROS and HE_SOS. No statistically significant differences were observed between the batches in the three parameters assessed(p > 0.05).

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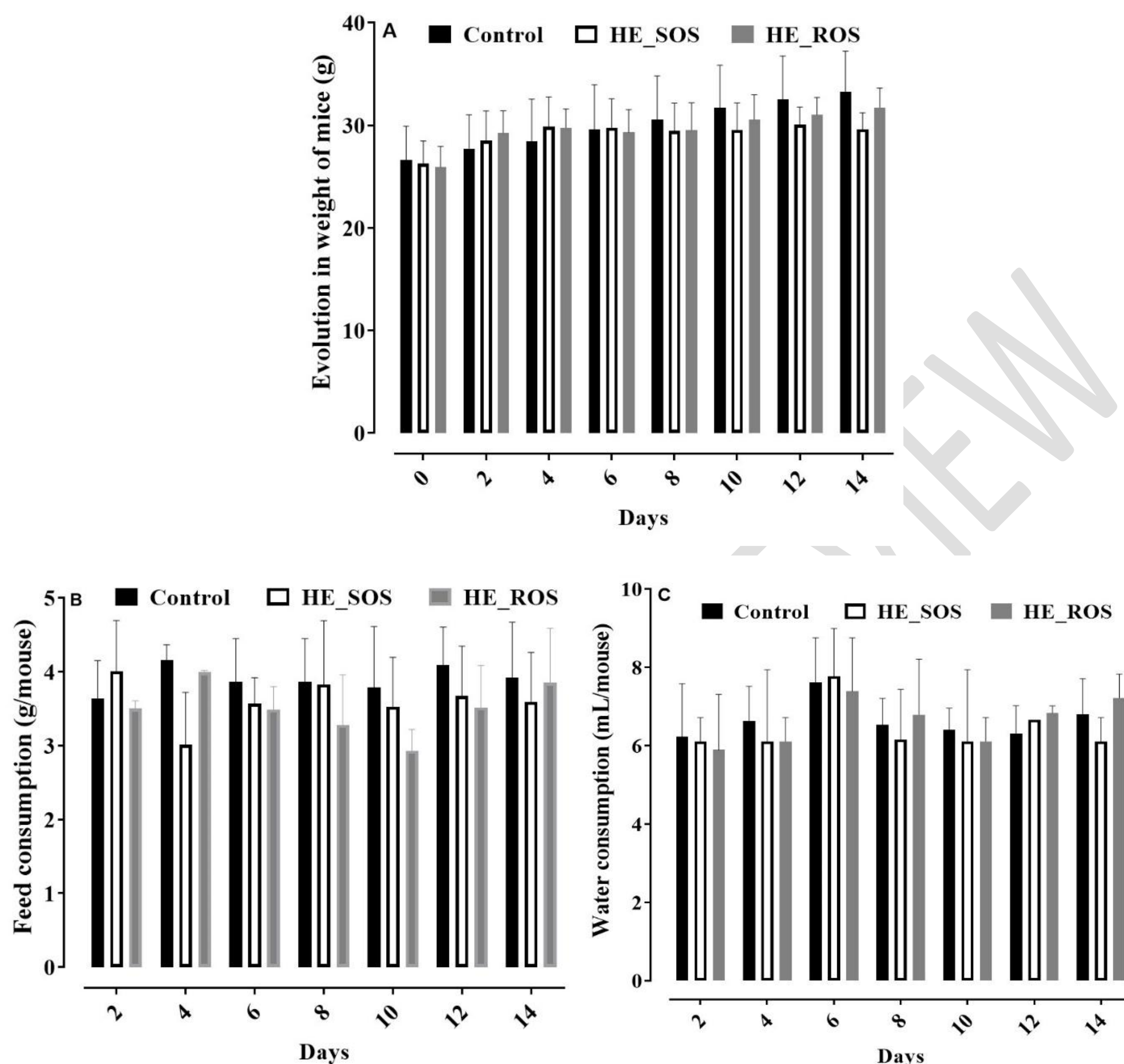

HE_SOS: Hydroethanolic extract of O. strictum stem (2000 mg/kg); HE_ROS: Hydroethanolic extract of O. strictum root (2000 mg/kg)

Figure 1: Evolutionin weight (A), feed (B), and water (C) consumption of female mice from *Odontonema strictum* rootand stemextracts tests and control batches during 14 days of follow-up; n = 6

3.5.3. Macroscopic Observation and Relative Organ Weights of Mice

Fresh macroscopic examination of vital organs such as the lungs, liver, heart, kidneys, and spleen of control mice and mice treated with HE_SOS (2000 mg/kg) and HE_ROS (2000 mg/kg) showed no lesions or changes in the color or appearance of the various organs.Figure 2 shows the relative organ weights of the control and extract-treated batches of mice. No statistically significant variation was observed between the relative organ weights of the 03 batches of mice.

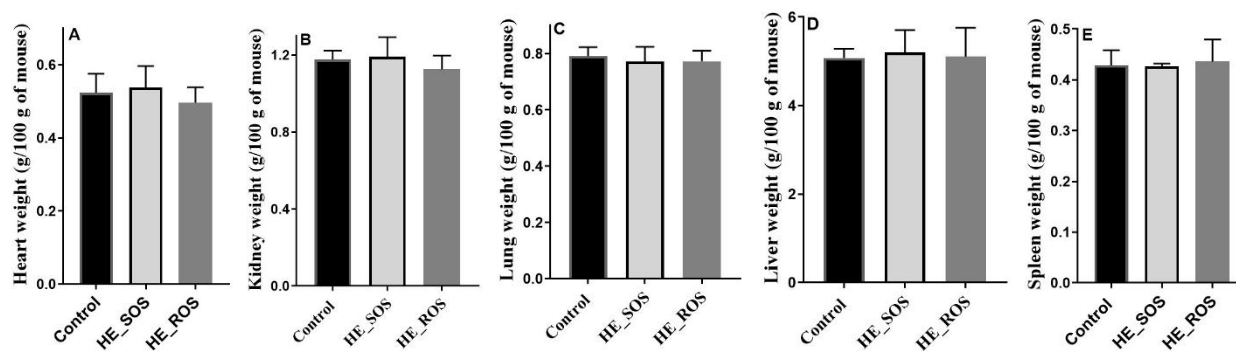

Figure 2: Relative weights of vital organs of control and hydroethanolic extractsof *O. strictum* root (HE_ROS, 2000 mg/kg) and Stem(HE_SOS, 2000 mg/kg)treated mice; n=6

4. DISCUSSION

"To our knowledge, this is the first work carried out on the plant's stems and roots. In the search for the biological properties of a plant drug, phytochemistry is an essential tool for responding to certain research hypotheses. Thus, we have searched the presence of tannins, saponosides, sterol-triterpenes, and flavonoids in hydroethanolic extracts from *O. strictum* root (HE_ROS) and Stem(HE_SOS). These compounds are known for their vasorelaxant properties"[10, 21]. *O. strictum*, an ornamental plant from Latin America, is traditionally used to manage arterial hypertension[9]. In addition, our findings corroborate previous studies. Indeed, authors have isolated flavonoid O-heterosides and flavonoid C-heterosides from *O. strictum* leaves [8]. In addition, "the leaves and flowers of *Odontonema strictum* contain polyphenols such as verbascoside and isoverbascoside), carbohydrates, flavonoid saponins, glycosides, tannins, steroids, and terpenoids as well as β-Sitosterol and Stigmasterol"[13, 15]."The pathophysiological mechanism of cardiovascular pathologies is multifactorial and includes inflammation, oxidative stress, the renin-angiotensin system, and vascular dysfunction"[23-25].To this end, "we have formulated research hypotheses that both extracts possess antioxidant and anti-inflammatory properties. In addition, natural antioxidants act beyond their capacity to mitigate oxidative stress by blocking intracellular signaling cascades. Indeed, the imbalance between oxidizing species and antioxidant compounds can increase the harmful species that induce functional modulation of lipids, proteins nucleic acids, and structural modifications. The result of these reaction cascades is chronic inflammatory and cardiovascular disease"[26, 27]. "Interestingly, our research highlighted these two properties of the extracts. These results are in line with the literature, which has documented the antioxidant properties of extracts from the leaves of this plant"[11-13]. These properties could provide a vascular protective effect by blocking reactive oxygen species, known to have vasoconstrictive effects and lead to endothelial dysfunction. Antioxidant compounds have already been shown to play an essential role in preventing cardiovascular disease[28, 29].Both extracts, therefore,can trap free radicals. Several concordant studies have shown a correlation between the presence of phenolic compounds and flavonoids and antioxidant activity based on DPPH, ABTS, and FRAP tests [16, 30]. Moreover, in the hypertension mechanism, inflammation of the vessel cannot be ignored, hence the search for anti-inflammatory properties in the extracts. Overall, HE_SOS and HE_ROS showed inhibition of phospholipase A_2 and 15-lipoxygenase. These anti-inflammatory activities were less effective than Betamethasone and Zileuton. The flavonoids and sterols/triterpenes in HE_SOS and HE_ROS are known for inhibiting pro-inflammatory enzymes[19]. However, a medicinal product can only be used if it is both effective and non-toxic. So, the safety of a pharmaceutical

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product is a fundamental criterion in therapeutics. As a result, usingplant extracts in alternative medicine requires vigilance in terms of safety. With this in mind, the acute oral toxicity of HE_SOS and HE_ROS was assessed. The results showed that HE_SOS (2000 mg/kg bw) and HE_ROS (2000 mg/kg bw) did not cause any mortality or visible behavioral changes in female NMRI mice. The LD_{50} of HE_SOS and HE_ROS is estimated at 5000 mg/kg bw according to the United Nations Globally Harmonised System with demonstrated adverse effects [22, 31]. These results show that extracts from the stems and roots of *Odontonema strictum* can be used safely. About the literature, this study constitutes, to our knowledge, the first toxicity studies on extracts from this plant. However, the mechanism of action of these extracts on cardiovascular pathologies requires preclinical investigations, particularly thebioguided evaluation of their muscle relaxant properties *ex-vivo*, their antihypertensive properties *in vivo,* and their oral toxicity at repeated doses.

5. CONCLUSION

This study is the first to identify phytochemical groups such as saponosides, tannins, flavonoids, sterol-triterpenes, and polyphenols in the stems and roots of *Odontonema strictum*. These two hydroethanolic extracts' antioxidant and anti-inflammatory properties have also been proven. These phytoconstituents and properties against free radicals are beneficial in treating cardiovascular pathologies. Extracts from the stems and roots of *Odontonema strictum*are potential research materials in the pre-clinical investigation of the antihypertensive properties of this plant, which is widely used in traditional medicine.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The experiment was carried out following the procedures of the Guide of Good Practices in Animal Experimentation under the Declaration of Helsinki.Furthermore, the experimental animal procedures have been performed by the Guide for the Care and Use of Laboratory Animals of the US National Institutes of Health and the EU Directive 2010/63/EU for animal experiments.

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