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Original Article

Phytochemical analysis, antioxidant, and antihyperlipidemic activities of *Teucrium takoumitense*

Otman El-Guourrami, Ph.D [a,*], Hamza Elbouny, Ph.D [b], Abdessamad Ait Benlabchir, Ph.D [c], Soufiane Drioua, Ph.D [a], Brahim Ouahzizi, Ph.D [b], Chakib Alem [b], Anass Doukkali [a] and Hanane Benzeid [a]

[a] *Laboratory of Analytical Chemistry, Faculty of Medicine and Pharmacy, Mohammed V University, Rabat, Morocco*
[b] *Laboratory of Biochemistry, Department of Biology, Faculty of Sciences and Techniques, University Moulay Ismail, Errachidia, Morocco*
[c] *Laboratory of Natural Substances, Pharmacology, Environment, Modeling, Health and Quality of Life, Department of Biology, Faculty of Sciences, Sidi Mohamed Ben Abdellah University, Fez, Morocco*

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المخلص

أهداف البحث: الغرض الرئيسي للدراسة الحالية هو تحديد التركيب الكيميائي وتقييم السلامة والنشاط المضاد للأكسدة والنشاط المضاد للدهون الزائدة للمستخلص المائي من الأعشاب الطبية التوكريوم تاكوميتينس.

طرق البحث: تم تقييم تحليل الفيتوكيميائي (المحتوى الكلي للفينول والفلافونويد وأحماض الهيدروكسي الحمضية)، والنشاط المضاد للأكسدة (قوة التخفيض الفريك للأكسدة، حمض الأزينوبيس الاثيل البنزوثيازولين السلفونيك، الديفينيل البيكريل الهيدرازيل، واختبارات القدرة الكلية المضادة للأكسدة)، السمية الحادة، والنشاط المضاد للدهون الزائدة وفقا لنماذج المقالات والدراسات. في حين تم تحديد التوصيف الفيتوكيميائي بواسطة كروماتوغرافيا الغاز المرتبطة بطيف الكتلة بعد التميثل.

النتائج: كشف المستخلص المائي من التوكريوم تاكوميتينس عن محتوى عالي من البوليفينولات الكلية وأحماض الهيدروكسي الحمضية وكمية قليلة من الفلافونويدات الكلية. بالإضافة إلى ذلك، أظهر المستخلص نشاط مضاد للأكسدة ملحوظ. كان البنزين، (هيكسيل أوكسي)-(19,32%) و 2,6 أ-ميثانو-6أ-اتش-اندينو[4,5-ب]أوكسيرين، أوكتاهيدرو – (1أ.ألفا.، 2.بيتا.، 3أ.ألفا.، 6أ.بيتا.، 6ب.ألفا.)-(32,42%)، دي-فوكوز (5,47%)، 5-هيدروكسيميثيلفورفورال (5,47%) والجواياكول (3,19%) هي المركبات الرئيسية في المستخلص. تم تقدير الجرعة الوسطى المميتة بين 500 و 2000 ملغم / كغ. علاوة على ذلك، أظهر المستخلص المائي من التوكريوم تاكوميتينس في 500 و 250 ملغم / كغ نشاطا مضادا للدهون الزائدة في الجسم مثيرا للاهتمام.

الاستنتاجات: الجدير بالذكر أن المستخلص من التوكريوم تاكوميتينس له قدرة دوائية هامة وتركيب كيميائي متنوع.

الكلمات المفتاحية: السمية الحادة؛ النشاط المضاد للدهون الزائدة؛ النشاط المضاد للأكسدة؛ التركيب الكيميائي؛ التوكريوم تاكوميتينس

Abstract

Objective: The main purpose of the present work was to determine the chemical composition, safety, and antioxidant and antihyperlipidemic activities of an aqueous extract of *Teucrium takoumitense*.

Methods: Phytochemical analysis (total phenolic, total flavonoid, and total hydroxycinnamic acid contents), antioxidant activity (ferric-reducing antioxidant power, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid, 2,2-diphenyl-1-picrylhydrazil, and total antioxidant capacity tests), acute toxicity, and antihyperlipidemic activity were evaluated according to established models. In addition, the phytochemical profile was determined by methylation followed by gas chromatography-mass spectrometry (GC/MS).

Results: The aqueous extract of *T. takoumitense* had a high content of total polyphenols (87.01 ± 0.31 mg gallic acid equivalent (GAE)/g extract) and hydroxycinnamic acid (2.28 ± 0.1 g/100 g Powdered Material) and a low content of

* Corresponding address: Laboratory of Analytical Chemistry, Faculty of Medicine and Pharmacy, Mohammed V University, Rabat, Morocco.
E-mail: otman_elguourrami@um5.ac.ma (O. El-Guourrami)
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total flavonoids (2.99 ± 0.16 mg GAE/g extract). In addition, the extract demonstrated remarkable antioxidant activity (DPPH IC_{50} = 76.67 ± 0.56 µg/mL, ABTS IC_{50} = 89.65 ± 0.27 µg/mL, FRAP EC_{50} = 296.32 ± 0.86 µg/mL, TAC value = 43 ± 0.27 mg EAA/g extract). The main compounds were identified as benzene, (hexyloxy)- (19.32%), 2,6a-methano-6aH-indeno[4,5-b]oxirene, octahedro-(1a.alpha., 2.beta., 3a.alpha., 6a.beta., 6b.alpha.)- (32.42%), D-fucose (5.47%), 5-hydroxymethylfurfural (5.47%) and guaiacol (3.19%). The LD_{50} was estimated to be between 500 and 2000 mg/kg. Furthermore, at 500 and 250 mg/kg, the aqueous extract of *T. takoumitense* exhibited good antihyperlipidemic activity *in vivo*.

Conclusion: *T. takoumitense* extract has significant pharmacological potential and a varied chemical composition.

Keywords: Acute toxicity; Antihyperlipidemic activity; Antioxidant activity; Chemical composition; *Teucrium takoumitense*

Introduction

Medicinal and aromatic herbs are widely used by the world's population due to their abundance of secondary metabolites, which serve as the foundation for many medical therapies.[1,2] Secondary metabolites exhibit several potential biological properties; this forms the scientific basis for the use of herbs in folk medicine. These metabolites have been described as antivirals and antibiotics and they allow good absorption of UV radiation; furthermore, it has been demonstrated that some herbs can play a key role in animal reproduction by exhibiting estrogenic properties.[3] These metabolites have been used across human history as spices, condiments, pigments, and pharmaceuticals. The three most significant categories of secondary metabolites are terpenes, polyphenols, and alkaloids.[4] According to statistics, over 80% of people in developing nations use natural plant-based medicines.[5]

Traditional medicine may have a greater acceptance rate since it is more widely available, less expensive, and has a long history of experimental use.[6,7] Effective medications typically have negative side effects. However, because herbal medicines are typically seen as being both safe and efficient, people continue to use these natural resources.[8] Morocco is a Mediterranean country with a rich history of medicine and a profound understanding of common herbal remedies.[9,10] Due to its unique phytogeographical characteristics, Morocco is considered as one of the most important reservoirs of biodiversity for many species. More than 4200 plants species have been identified in Morocco, including 800 endemic species and 600 classified as medicinal plants.[11] A significant number of the Moroccan population are thought to continue to use the majority of these species.[12–14]

Teucrium is the largest genus of the Lamiaceae family in Morocco. Numerous academics have investigated this genus from the end of the 18th century to the present day. In Morocco, the genus *Teucrium* is known to consist of 60 taxa divided into 52 species and split into eight divisions.[15] Of these species, *Teucrium takoumitense* Förther & Podlech (*T. takoumitense*) is an endemic plant that grows in a very limited area in the village of Tazougart in the Errachidia region. To the best of our knowledge, there are no documented reports on the phytochemical or biological properties of this species. Traditionally, the administration of aqueous extracts of *Teucrium* plants is most frequently used method to treat several disorders, including cardiovascular diseases and disorders related to oxidative stress.[16,17] Thus, the main objective of the present work was to evaluate the antioxidant and antihyperlipidemic activities, quantify the key families of secondary metabolites, and determine the chemical composition of the aqueous extract of *T. takoumitense* (AETT).

Material and methods

Chemicals and apparatus

All chemicals and reagents were of analytical grade. Sodium carbonate and gallic acid were obtained from Fluka Co (Spain). The other products were purchased from Sigma–Aldrich Chemical Co (USA). Spectrophotometric measurements were performed on a visible spectrophotometer VIS-723G (Beijing Beifen-Ruili Analytical Instrument, China).

Plant material and extraction process

The aerial parts of *T. takoumitense* were harvested in Tazouguerte village (Errachidia region, Morocco) in April 2022. The plant material was botanically identified in the Department of Botany at the Faculty of Sciences and Techniques of Errachidia (FSTE). Voucher specimens (Tt HerbFST # 95) were deposited in the Herbarium at FSTE. The aerial parts were dried in the shade for 7 days. Next, 5 g of the pulverized material was placed in 500 mL of distilled water and boiled for 30 min (decoction). The extract was then filtered and the solvent evaporated using a rotary evaporator (BUCHI RE-111 Rotavapor W/461 water bath) at 40 °C before freeze-drying. The untreated aqueous extract (AETT) was then stored at 4 °C until use.

Chemical composition

The profiles of volatile phytochemicals were identified using gas chromatography (GC) (Agilent 7890A Series) coupled to mass spectrometry (MS) equipped with a multimode injector and a 123-BD11 column, 15 m × 320 µm × 0.1 µm in size. Using helium as the carrier gas, a 4 µL volume of the soluble extract was injected into the column in split 1/4 mode at 2 mL/min. The temperatures of

the ion source and quadrupoles were 230 °C and 150 °C, respectively. The oven temperature program began at 30 °C and ended at 360 °C. Identification was performed using the NIST 2017 MS Library.

Phytochemical screening

Detection of flavonoids

The cyanidin reaction was used to identify flavonoids. A small amount of magnesium and a few drops of strong hydrochloric acid were added along with 2 mL of AETT. When flavonoids were present, a reddish-orange color developed.[18]

Detection of tannins

This test was carried out by adding a few drops of 10% (m/v) $FeCl_3$ aqueous solution to 3 mL of AETT, the presence of tannins was then confirmed. A positive test was revealed by the appearance of a blue-black or blue-green coloration.[19]

Detection of anthocyanins

One milliliter of concentrated sulfuric acid (H_2SO_4) and 1 mL of ammonium hydroxide (NH_4OH) are added to 1 mL of AETT. The presence of anthocyanins was indicated by a red color in an acidic medium and a purplish blue color in a basic medium.[20]

Detection of coumarins

A volume of 0.5 mL of NH_4OH (25%) was added to 2 mL of AETT. Analysis was carried out under a UV lamp at 366 nm and an intense fluorescent light indicated the presence of coumarins.[21]

Detection of free quinones

The detection of free quinones was confirmed by adding a few drops of 10% NaOH (m/v) to 3 mL of AETT; a change in coloration to yellow, red, or purple indicated the presence of free quinones.[22]

Detection of terpenoids

Terpenoids were identified by mixing 3 mL of AETT with 0.3 mL of chloroform; then, 1.2 mL of concentrated H_2SO_4 was added. The formation of a brownish, red, or purple ring at the contact zone indicated the presence of terpenoids.[18]

Detection of alkaloids

Alkaloids were identified based on precipitation reactions using Bouchardat, Mayer, and Dragendorff reagents. One milliliter of each reagent (Mayer, Dragendorff, and Bouchardat) was added to 3 mL of AETT; then, the solution was allowed to stand for 10 min. A positive test was revealed by the appearance of an orange precipitate with Dragendorff's reagent, a yellowish-white precipitate with Mayer's reagent, and a brown precipitate with Bouchardat's reagent.[23]

Detection of saponins

The foaming test was used to indicate the presence of saponins. For this test, 5 mg of the extract was diluted in 5 mL of distilled water before being added to a test tube and violently shaken for approximately 15 s. The formation of a stable foam (greater than 1 cm in height) that persisted for 15 min indicated the abundant presence of saponins.[23]

Determination of total hydroxycinnamic acids content

Total hydroxycinnamic acid (THA) content was determined using the colorimetric method described by Elbouny et al. (2023).[24] The content of THA, expressed as grams of THA per 100 g of powdered plant (PP), was calculated from the following formula: THA (g/100 g PP) = A × 2.5/m in which A represents the absorbance of the test solution at 505 nm and m represents the mass of the powdered sample in grams (THA g/100 g PP, mean ± STD of three determinations).

Determination of liposoluble pigments

The content of lipid-soluble pigments in the plant powder of *T. takoumitense* was assessed based on the protocol established by Barros et al. (2011).[25] After adding 10 mL of a mixture of acetone (40%)/hexane (60%) for 1 min, 150 mg of powder from the aerial parts was shaken vigorously. Next, the mixture was filtered through a syringe filter (0.45 m) and the absorbance was measured using a UV−visible spectrophotometer (PerkinElmer Lambda 35 UV/VIS). Lipid-soluble pigments, were identified at various wavelengths by specific formulae, as shown below.

$$\text{Lycopene (mg/100 mL)} = -0.0458 \times A663 + 0.204 \times A645 + 0.372 \times A505 - 0.0806 \times A453$$

$$\beta\text{-carotene (mg/100 mL)} = 0.216 \times A663 - 1.220 \times A645 - 0.304 \times A505 + 0.452 \times A453$$

$$\text{Chlorophyll A (mg/100 mL)} = 0.999 \times A663 - 0.0989 \times A645$$

$$\text{Chlorophyll B (mg/100 mL)} = -0.328 \times A663 + 1.77 \times A645$$

Determination of total polyphenols

AETT was tested for total polyphenols (TPC) using the method described by Khouya et al. (2022).[26] The concentration of total polyphenols, expressed as milligrams of gallic acid equivalent per gram of extract (mg GAE/g extract), was determined from a gallic acid calibration curve equation.

Determination of total flavonoids

The content of total flavonoids (TFC) in AETT was quantified according to the protocol established by Elbouny et al. (2022).[27] Based on the calibration curve of quercetin, the total amount of flavonoids was expressed as milligram equivalents of quercetin per gram of each extract (mg QE/g extract).

Antioxidant activity

DPPH free radical scavenging test

2,2-Diphenyl-1-picrylhydrazil (DPPH) radical scavenging activity was determined according to the method described by Elbouny et al. (2022).[28] The results were compared to those obtained for quercetin, which was used as a control antioxidant. The test was performed in triplicate, and the percentage of inhibition (I %) of the DPPH free radical by test extracts was calculated as follows:

$$I = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100$$

where I% = percentage of DPPH inhibition, $A_{Control}$ = absorbance of negative control; A_{Sample} = absorbance of the sample.

ABTS free radical scavenging test

AETT was tested for free radical scavenging activity against 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS) radical, as described by Pukalskas et al. (2002).[29] The percentage of inhibition was calculated in the same manner as for DPPH, and the average of the concentrations responsible for 50% of its inhibition (IC_{50}) was determined.

Ferric-reducing antioxidant power (FRAP) test

This test is based on the reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}). The ability of AETT to reduce iron was determined by following the protocol established by Oyaizu (1986),[30] with the modifications described by El-Guourrami et al. (2003).[31] The concentration that converted 50% of Fe^{3+} to Fe^{2+} was determined.

Total antioxidant capacity test

The total antioxidant capacity (TAC) test of AETT was evaluated according to the protocol established by Prieto et al. (1999).[32] The results were expressed in milligrams of ascorbic acid equivalent per gram of extract (mg AAE/g extract).

Animals

Healthy albino Wistar rats of either sexes (weighing 150–180 g) and Swiss mice (weighing 20–30 g) were used in this research. The animals were procured from the Animal House of the Faculty of Sciences and Techniques Errachidia (FSTE) and kept in standard cages with a 12-h light/dark cycle and at a temperature of 24 ± 3 °C. Experiments were conducted according to the guidelines for use of laboratory animals of the pharmacological research committee, FSTE, Moulay Ismail University (AREC-FSTE-12/2020).

Acute toxicity

The acute oral toxicity of AETT was assessed using the protocol established by the Organization for Economic Cooperation and Development Guideline OECD No. 423.[33] Three non-pregnant and nulliparous female mice weighing between 20 and 30 g were fasted for 4 h; we removed food but not the water. Then, using an esophageal probe, the AETT was given orally at doses of 2000 mg/kg and 500 mg/kg. According to the body weight of each mouse, an estimate of the extract dosage was given. The animals were monitored for 30 min and 14 days following dosing. During this period, we recorded variations in body weight and mortality as well as clinical signs (aggressiveness, agitation, sedation, paralysis, and prostration).

Antihyperlipidemic activity

A saline solution (300 mg/mL) of Triton WR-1339 (tyloxapol, Sigma–Aldrich, USA) was given intraperitoneally to rats (300 mg/kg) to induce acute hyperlipidemia. Then, treatments or distilled water were administered by oral gavage. Overnight-fasted rats were randomly divided into five groups (n = 6). The first group received an intraperitoneal administration of normal saline and distilled water by oral gavage and served as the normal control group (Normal). The hyperlipidemic control group was treated with Triton WR-1339 and gavaged with distilled water (Triton). The third group was treated with Triton WR-1339 and received 20 mg/kg BW of simvastatin (Simva). The fourth and fifth groups were treated with Triton WR-1339 and received 250 mg/kg BW (T250) and 500 mg/kg BW (T500) of AETT, respectively. After 24 h of treatment, the animals were anesthetized, and blood was collected from the retro-orbital sinus using heparinized capillaries. Centrifugation was used to separate the plasma for 5 min at 3000 rpm. The antihyperlipidemic effect was evaluated by measuring the levels of total cholesterol (TC), triglycerides (TGs), high-density lipoprotein (HDL-C), and non-HDL-C in the plasma. The non-HDL-C value was determined using the following formula: Non-HDL-C = TC − HDL-C.

Statistical analysis

Statistical analysis was carried out using GraphPad Prism version 8 software. Data were expressed as mean ± standard deviation (SD). Comparisons between different groups were performed by one-way analysis of variance (ANOVA) followed by a post-hoc Tukey's test. Differences were considered significant at $p < 0.05$.

Results

Phytochemicals

The identified compounds and the chromatogram of the analysis are presented in Table 1 and Figure 1, respectively. Twenty-six compounds were identified, including 2,6a-methano-6aH-indeno[4,5-b]oxirene, octahydro-, (1a.alpha., 2.beta., 3a.alpha., 6a.beta., 6b.alpha.)- (32.42ù)-, benzene, (hexyloxy)- (19.32%), D-fucose (5.47%), 5-hydroxymethylfurfural (5.41%) and guaiacol (3.19%). Five compounds were identified: catechol, coumaran, 2-methyl-3 (2-furyl) acrolein, 1H-pyrrole-3-carboxylic acid, and 4-[2-furanylcarbonyl)amino]. Vanillic acid, 4,5-dihydro-2-methyl-5-oxo-4-(trifluoromethyl), and methyl ester (in a smaller amount). Low levels of levonordefrin, maltol, phenol, 2,6-dimethoxy-, benzoic acid, 3-(5-hydroxy-1-pentenyl)-, and methyl ester (E)- were also detected.

Phytochemical screening

By performing phytochemical screening, we attempted to highlight the qualitative presence or absence of certain families of secondary metabolites in AETT. The results of the phytochemical screening, as based on staining and precipitation reactions, are shown in Table 2. AETT contained tannins, flavonoids, coumarins, free quinones, terpenoids, saponins, and essential oils; neither anthocyanins nor alkaloids were detected.

Determination of phenolic compounds and liposoluble pigments

Next, we quantified total polyphenols and flavonoids in AETT. In addition, the amounts of chlorophyll A and B, lycopene, β-carotene, and hydroxycinnamic acids in the powdered plant material were also quantified (Table 3). We

Table 1: Compounds identified in AETT.

Number	RT (min)	Area (%)	Chemical formula	Name
1	11.078	3.19	$C_7H_8O_2$	Guaiacol
2	11.270	0.93	$C_6H_6O_3$	Maltol
3	11.439	1.26	$C_6H_{10}Cl_2O_3$	2,2-Dichloroethyl propyl carbonate
4	11.529	1.11	$C_6H_8O_4$	4H-Pyran-4-one, 2,3-dihydro-3,5-di hydroxy-6-methyl-
5	11.935	1.59	$C_3H_8S_2$	2,4-Dithiapentane
6	12.048	2.54	$C_6H_6O_2$	Catechol
7	12.307	2.65	C_8H_8O	Coumaran
8	12.487	5.41	$C_6H_6O_3$	5-Hydroxymethylfurfural
9	13.918	1.63	$C_9H_{10}O_2$	3-Methoxyacetophenone
10	14.662	0.93	$C_8H_{10}O_3$	Phenol, 2,6-dimethoxy-
11	16.871	2.54	$C_8H_8O_2$	2-Methyl-3(2-furyl)acrolein
12	19.845	1.47	$C_8H_8O_4$	Vanillic acid
13	21.694	5.47	$C_6H_{12}O_5$	D-Fucose
14	41.639	0.75	$C_8H_8O_3$	Benzoic acid, 3-(5-hydroxy-1-pentenyl)-, methyl ester, (E)-
15	41.966	1.29	$C_{11}H_8O_3$	2-Furancarboxylic acid, phenyl ester
16	42.338	1.91	$C_{11}H_{10}O_2S$	(7-Methylbenzo(b)thien-3-yl)acetic acid
17	43.172	1.90	$C_{12}H_{15}F_3O$	2,3,6-Trifluorobenzyl alcohol,2-methylbutyl ether
18	43.712	19.32	$C_{12}H_{18}O$	Benzene, (hexyloxy)-
19	44.997	1.53	$C_{13}H_{12}O_2S$	Benzoic acid, 4-(2-thienyl)-, ethyl ester
20	45.369	32.42	$C_{10}H_{14}O$	2,6a-Methano-6aH-indeno[4,5-b]oxirene, octahydro-, (1a.alpha.,2.beta.,3a.alpha.,6a.beta.,6b.alpha.)-
21	45.470	2.00	$C_{15}H_{24}N_2$	5,17-Dehydroallomatridine, (6.beta.)-
22	45.583	1.60	$C_{18}H_{24}N_2O_4$	Octane-1,8-diamine, N,N'-bis(2-furoyl)-
23	45.808	1.24	$C_8H_{12}O_4$	1,2-Cyclohexanedicarboxylic acid,
24	46.056	0.78	$C_9H_{13}NO_3$	Levonordefrin
25	47.735	2.89	$C_{13}H_{11}F_3N_2O_5$	1H-Pyrrole-3-carboxylic acid, 4-[(2-furanylcarbonyl)amino]-4,5-dihydro-2-methyl-5-oxo-4-(trifluoromethyl)-, methyl ester
26	48.130	1.63	$C_{10}H_9NO_4$	Ethyl 5-(furan-2-yl)-1,2-oxazole-3-carboxylate
Total		**99.98**	-	-

RT, retention time.

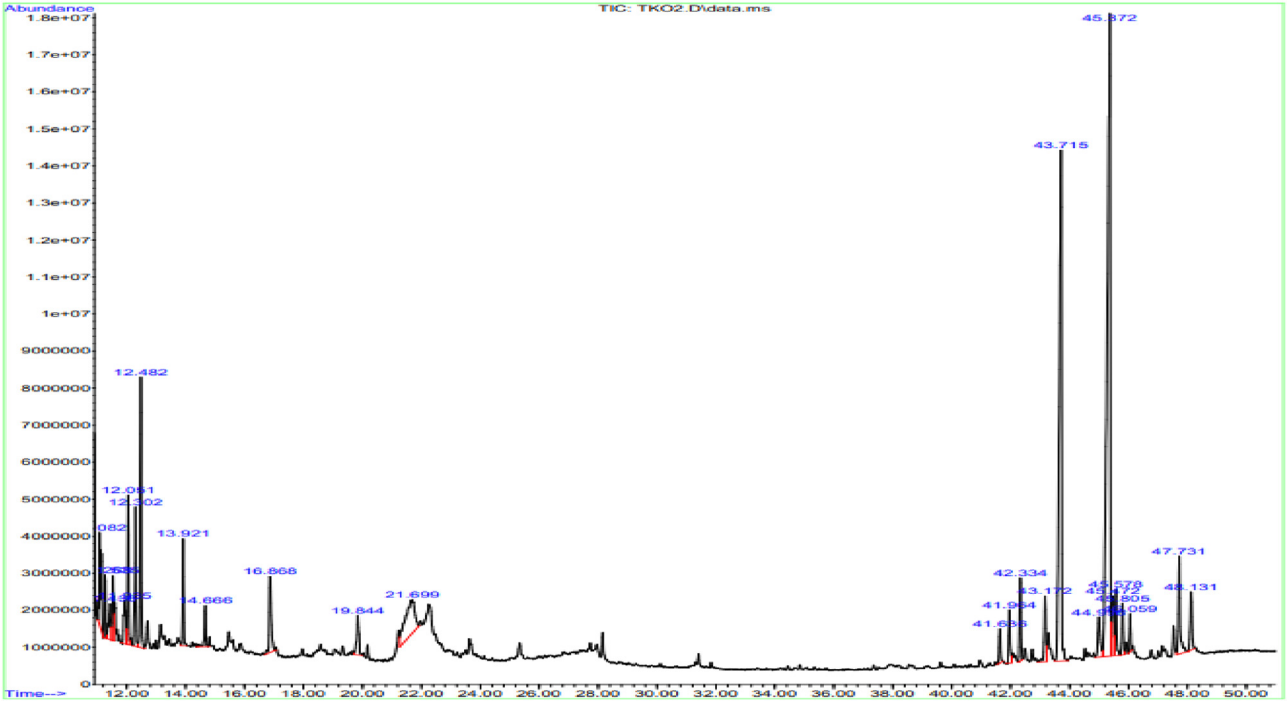

Figure 1: GC/MS chromatograms of an aqueous extract made from the aerial parts of *T. takoumitense*.

Table 2: Phytochemical screening of AETT.

Secondary metabolites	(+) Presence or (−) Absence
Flavonoids	+
Tannins	+
Coumarins	+
Anthocyanins	−
Free quinones	+
Terpenoids	+
Alkaloids	−
Saponins	+
Essential oils	+

Table 3: Total polyphenols, flavonoids, hydroxycinnamic acids, and liposoluble pigments of *T. takoumitense*.

Metabolite	Amount
THA (g/100 g PM)	2.28 ± 0.1
TPC (mg GAE/g extract)	87.01 ± 0.31
TFC (mg QE/g extract)	2.99 ± 0.16
Chlorophyll A (µg/100 mL)	746.35 ± 2.73
Chlorophyll B (µg/100 mL)	354.50 ± 3.53
Lycopene (µg/100 mL)	ND
β-Carotene (µg/100 mL)	192.00 ± 1.83

Data represent the mean ± standard deviation of three independent experiments values.
GAE, gallic acid equivalent; ND, not detected; QE, quercetin equivalent; TFC, total flavonoid content; THA, total hydroxycinnamic acids; TPC, total phenolic content PM: Powdered material.

found that AETT revealed a high content of polyphenols (87.01 ± 0.31 mg GAE/g extract) and a low content of flavonoids (2.99 ± 0.16 mg QE/g extract). Chlorophyll A was the most abundant fat-soluble pigment in vegetable powder (746.35 ± 2.73 g/100 mL), followed by chlorophyll B (354.50 ± 3.53 g/100 mL), and β-carotene (192.00 ± 1.83 g/100 mL). Lycopene was not detected in this plant. Total hydroxycinnamic acids were detected (2.28 ± 0.1 g/100 g Powdered Material) in the plant powder.

Antioxidant activity

Table 4 shows the antioxidant activity of AETT obtained by DPPH, ABTS, FRAP, and TAC methods. Our findings revealed that AETT exhibited an antioxidant effect in a dose-dependent manner. The IC_{50} value of the scavenging DPPH radical was 76.67 ± 0.50 µg/mL followed by those of ABTS (89.65 ± 0.27 µg/mL) and FRAP with a ferrous reduction value of 296.32 ± 0.86 µg/mL; the TAC method exhibited a value of 43.87 ± 0.27 mg EAA/g extract.

Acute toxicity

The aim of the present study was to evaluate the acute toxicity of AETT. The investigated extract did not exhibit any clinical toxicity at 500 mg/kg. All of the tested animals survived over the 14 days of observation, and their behaviors remained normal, thus implying that the lethal dose (LD_{50}) was higher than 500 mg/kg. Based on these findings and the OECD 423 guidelines, the body weight of experimental animals did not vary considerably throughout the 14 days of follow-up (Figure 2). We can conclude that this extract is considered a non-toxic substance for a single oral administration at 500 mg/kg. Interestingly, at 2000 mg/kg, the administration of the extract revealed toxicological signs, most notably the death of more than half of the animals (animals died within 24 h of administration), thus demonstrating that AETT is toxic at 2000 mg/kg.

Table 4: The concentrations (µg/mL) of AETT and the standard controls that led to 50% inhibition of DPPH and ABTS radicals and 50% transformation of Fe^{3+} into Fe^{2+} for the FRAP test. Total antioxidant capacity is represented in mg AAE/mg of extract.

Method	Extract	Standards		
	AETT	Quercetin	Ascorbic acid	Catechin
DPPH	76.67 ± 0.56	5.49 ± 0.02		−
ABTS	89.65 ± 0.27	−	2.52 ± 0.02	−
FRAP	296.32 ± 0.86	−	−	13.94 ± 0.03
TAC	43.87 ± 0.27	−	−	−

Data values represent the mean ± standard deviation of three independent experiments values.
ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid; AETT, aqueous extract of *T. takoumitense*, DPPH, 2,2-diphenyl-1-picrylhydrazil; FRAP, ferric-reducing antioxidant power; TAC, total antioxidant capacity.

Antihyperlipidemic activity

The results of the antihyperlipidemic effects of AETT are shown in Figure 3. The injection of tyloxapol induced a significant ($p < 0.0001$) increase in the levels of TC (9.55 mmol/L), TGs (17.54 mmol/L) and non-HDL-C (9.10 mmol/L) in the hyperlipidemic group when compared with the normal control group. Moreover, the Triton injection and different treatments did not affect the levels of HDL-C. The levels of lipids were significantly reduced ($p < 0.01$ to $p < 0.0001$) by AETT administration in a dose-dependent manner. At 250 mg/kg, TC, TGs, and non-HDL-C were reduced by 17.17%, 19.66%, and 24.58%, respectively. However, at higher doses (500 mg/kg), AETT exerted more extensive lipid-lowering effects by reducing TC, TGs, and non-HDL-C by 34.03%, 42.02%, and 41.16%, respectively. The administration of simvastatin at a dose of 20 mg/kg had better hypolipidemic efficacy than AETT at both doses ($p < 0.01$).

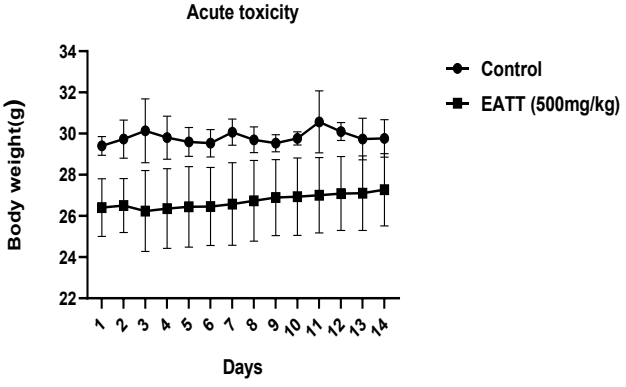

Figure 2: Changes of body weight in the group of rats treated with AETT (500 mg/kg) when compared to the control group.

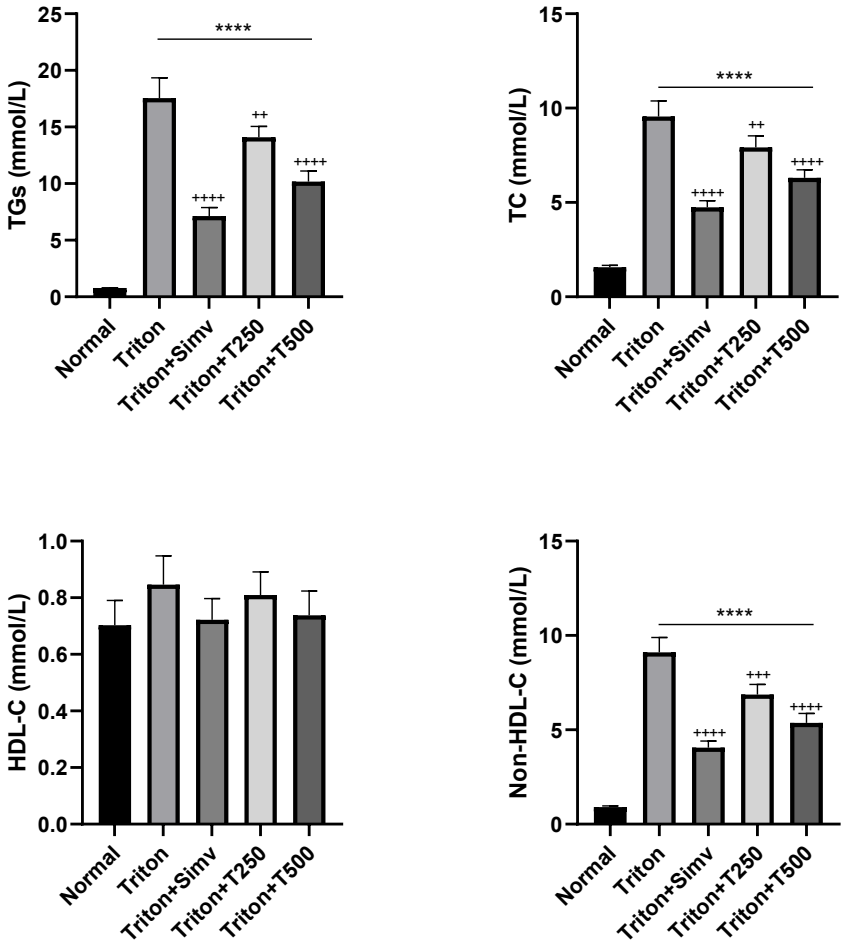

Figure 3: The levels of plasma lipids in different groups. (Simv) simvastatin 20 mg/kg; (T250 and T500) AETT 250 and 500 mg/kg respectively. ****: $p < 0.0001$ when compared to normal group. ++: $p < 0.01$, +++: $p < 0.001$, and ++++: $p < 0.0001$ when compared to Triton group.

Discussion

Our phytochemical analysis revealed the presence of various phytochemicals in AETT. These findings, and those of previous reports, confirmed the presence of similar compounds in other *Teucrium* plants, including vanillic acid in the n-butanol extract of *Teucrium montanum*[34] and in methanolic extracts from Serbian *Teucrium polium* and *Teucrium scordium*.[35] Moreover, catechol was also reported in *T. polium* from Austria.[36] However, we suggest to carry out more phytochemical investigations on the extracts of *T. takoumitense*; for example, by performing analysis of extracts by other techniques, such as HPLC coupled with mass spectroscopy. This is because GC/MS analysis only detects volatile compounds. Moreover, our phytochemical screening revealed the presence of tannins, flavonoids, coumarins, free quinones, terpenoids, saponins, and essential oils. These findings are consistent with those reported for *Teucrium stocksianum*[37] and *Teucrium buxifolium*,[38] which both reported the presence of the same phytochemicals in these two species but with the absence of alkaloids and anthocyanins. In addition, AETT had a higher content of total polyphenols when compared to those of an aqueous extract of *T. polium* from Turkey (59 mg GAE/g extract) and a hydroethanolic extract of *T. polium* from Iran (65.74 mg GAE/g extract).[39] On the other hand, several species were shown to possess higher amounts than that of AETT including, *Teucrium chamaedrys* and *T. montanum* from Serbia (143.42–190.20 mg GAE/g of extract),[40] and *Teucrium arduini* and *Teucrium flavum* from Serbia (200.35 and 171.08 mg GAE/g of extract, respectively).[41] *T. takoumitense* contained a sufficient amount of total hydroxycinnamic acids (2.28%). However, this proportion was low when compared to that of *T. montanum* from Croatia (8.20%).[42]

Analysis of the antioxidant potential of AETT showed that this plant exhibits different antioxidant properties. Compared to the antioxidant effect of the extracts of other *Teucrium* species, AETT had a lower DPPH radical scavenging effect when compared to that of ethyl acetate extracts of *T. polium* and *T. chamaedrys*, as well as ether extracts of *T. montanum* from Macedonia ($IC_{50} = 10–70$ mg/mL).[43] In addition, similar findings were reported for the aqueous extract of *T. polium* from Morocco ($IC_{50} = 0.61$ mg/mL).[44] However, AETT had a DPPH radical scavenging effect that was comparable to that of *T. polium* from Palestine

($IC_{50} = 73.1 \pm 5.2$ mg/mL).[45] Overall, our finding revealed that AETT is rich in phenolic compounds and exerts significant and varied antioxidant effects. Although the obtained results were almost insignificant when compared to the reference antioxidant standards (ascorbic acid, quercetin, and catechin), the isolation of bioactive compounds from this plant, along with our evaluation of their antioxidant activities, provides encouraging results that may help us to identify antioxidant substances in this species.

Our acute toxicity study revealed that administration of the extract at a dose of 2000 mg/kg induced obvious toxicological signs, most notably the death of more than half of the animals. Puntero et al. (1997) previously reported that the lethal dose of an Iranian hydroethanolic extract of *T. polium* was greater than 2000 mg/kg.[46] In addition, the extracts of other *Teucrium* plants were reported to be safe at a dose greater than 2 g/kg, including *T. polium* subsp. *geyrii* from Algeria (Albino mice)[47] and *T. stocksianum* from Pakistan (Albino mice).[48] Based on the available literature, there has been no aqueous extract obtained from a *Teucrium* plant with an LD_{50} value lower than 2000 mg/kg. Thus, AETT is the first *Teucrium* species that produces a toxic aqueous extract at a dose of 2000 mg/kg.

Hyperlipidemia is a leading risk factor for cardiovascular diseases, which represent a primary cause of global mortality. Thus, the development of new hypolipidemic agents is very important. Scientists are becoming increasingly interested in natural agents as they seek safer and more effective lipid-lowering medications. Moreover, medicinal plants are a potent source of bioactive phytochemicals that exhibit a wide variety of pharmacological and biological properties, including antihyperlipidemic activity. The effects of several Lamiaceae herbs on hypolipidemia have been investigated. In our study, we found that AETT had the ability to manage tyloxapol-induced hyperlipidemia by significantly reducing the levels of TC, TGs, and non-HDL-C. This effect can be explained by the richness of the aqueous extract of this plant in bioactive lipid-lowering compounds. Indeed, our findings revealed that *T. takoumitense* possesses significant amounts of phenolic compounds, including flavonoids, tannins, and hydroxycinnamic acids. According to numerous reports, these compounds have strong hypolipidemic potential.[49,50] Furthermore, other *Teucrium* species have been reported to exhibit antihyperlipidemic effects. For example, a hydroalcoholic extract of *T. polium* from Iran was tested on dexamethasone-induced hyperlipidemic rats.[51] The results of this investigation demonstrated that *T. polium* had a potent antihyperlipidemic effect by reducing the levels of TG, TC, and LDL-C and by increasing HDL-C levels. In addition, the hypolipidemic impact of streptozotocin-induced diabetic rats in Palestinian *Teucrium leucocladum* was also studied by Bassalat et al. (2020).[52] The findings of their study revealed that an aqueous extract of *T. leucocladum* exerted significant lipid-lowering effects. Collectively, these previous reports, and our own findings, demonstrated that the *Teucrium* genus contains potent hypolipidemic species. However, more studies should be performed to determine the possible mechanisms of the lipid-lowering actions and the compounds responsible for this activity.

Conclusion

The main objective of the present work was to determine the chemical composition, and evaluate the acute toxicity, antioxidant, and antihyperlipidemic activities of an aqueous extract of *T. takoumitense*. This study demonstrated that the aerial parts of this Moroccan endemic plant could be used as a source of bioactive substances such as polyphenols. The extract of this plant was found to be toxic at a dose of 2000 mg/kg. Investigations of the biological activities of the tested extract showed that this species of *Teucrium* can exert considerable effects against oxidants and represents a promising source of antihyperlipidemic compounds. Taking into consideration the results reported previously, it would seem possible to use the areal parts of *T. takoumitense* as a source of natural compounds that could be incorporated into foods, cosmetics, or pharmaceutical products.

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Conflict of interest

The authors have no conflict of interest to declare.

Ethical approval

Experiments were conducted according to the guidelines for use of laboratory animals of the pharmacological research committee, FSTE, Moulay Ismail University (AREC-FSTE-12/2020).

Consent

All authors have read and agreed to the published version of the manuscript.

Authors' contributions

HE and BO were responsible for conceptualization, as well as project administration, and HB, CA, AD provided supervision. AA and SD were responsible for data curation and formal analysis and, conducted investigations. OE and HE were responsible for validation, and contributed to visualization. OE and HE were responsible for writing the original draft, while HB, CA and AD reviewed and edited the manuscript. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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