JoP Journal of Pharmacopuncture
Journal of Pharmacopuncture 2022;25(3):242-249
pISSN: 2093-6966 • eISSN: 2234-6856
https://doi.org/10.3831/KPI.2022.25.3.242
Original Article
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Investigation of Antioxidant, Hypoglycemic and Anti-Obesity Effects of Euphorbia Resinifera L.

Kaoutar Benrahou[1]*, Otman El Guourrami[2], Hanae Naceiri Mrabti[1], Yahia Cherrah[1], My El Abbes Faouzi[1]

[1]Laboratory of Pharmacology and Toxicology, Bio Pharmaceutical and Toxicological Analyzes Research Team, Faculty of Medicine and Pharmacy, Mohammed V University, Rabat, Morocco
[2]Laboratory of Analytical Chemistry and Bromatology, Faculty of Medicine and Pharmacy, Mohammed V University, Rabat, Morocco

Received February 25, 2022
Reviewed May 22, 2022
Accepted June 16, 2022

***Corresponding Author**
Kaoutar Benrahou
Laboratory of Pharmacology and Toxicology, Bio Pharmaceutical and Toxicological Analyzes Research Team, Faculty of Medicine and Pharmacy, Mohammed V University, BP 6203, Rabat 10100, Morocco
Tel: +212-682-577-700
E-mail: benrahoukaoutar@gmail.com

Objectives: The aim of this work is to evaluate the in vitro antioxidant, hypoglycemic, and antiobesity effects of Euphorbia resinifera extracts and investigate the phenolic constituents and the toxicity of these extracts.
Methods: Phytochemical screening was performed to detect polyphenols and flavonoids. Antioxidant activity was evaluated by four methods (DPPH, ABTS, H_2O_2, and xanthine oxidase inhibition). The hypoglycemic effect was determined by the inhibition of α-amylase and α-glucosidase enzymes in vitro and via a starch tolerance study in normal rats. The antiobesity effect was estimated by in vitro inhibition of lipase.
Results: Phytochemical screening revealed that the ethanolic extract was rich in polyphenols (99 ± 0.56 mg GEA/g extract) and tannins (55.22 ± 0.17 mg RE/g extract). Moreover, this extract showed higher antioxidant activity in different tests: the DPPH assay (IC_{50} = 53.81 ± 1.83 μg/mL), ABTS assay (111.4 ± 2.64 mg TE/g extract), H_2O_2 (IC_{50} = 98.15 ± 0.68 μg/mL), and xanthine oxidase (IC_{50} = 10.26 ± 0.6 μg/mL). With respect to hypoglycemic effect, the aqueous and ethanolic extracts showed IC_{50} values of 119.7 ± 2.15 μg/mL and 102 ± 3.63 μg/mL for α-amylase and 121.4 ± 1.88 and 56.6 ± 1.12 μg/mL for α-glucosidase, respectively, and the extracts lowered blood glucose levels in normal starch-loaded rats. Additionally, lipase inhibition was observed with aqueous (IC_{50} = 25.3 ± 1.53 μg/mL) and ethanolic (IC_{50} = 13.7 ± 3.03 μg/mL) extracts.
Conclusion: These findings show the antioxidant, hypoglycemic, and hyperlipidemic effects of E. resinifera extracts, which should be investigated further to validate their medicinal uses and their pharmaceutical applications.

Keywords: euphorbia resinifera, antioxidant activity, hypoglycemic effects, enzyme inhibitory

INTRODUCTION

Diabetes mellitus, obesity, and oxidative stress are metabolic diseases having long-term effects [1]. Due to modern lifestyles and the increased consumption of high-fat and high-carbohydrate foods, the global prevalence of diabetes and obesity has increased dramatically. Hyperglycemia is characterized by abnormally high plasma glucose levels. In type 2 diabetes, insulin resistance can be caused by numerous signal transduction defects, including defects in insulin receptors or glucose transporters. High blood glucose levels will increase free radical production while reducing endogenous antioxidant levels [2]. Obesity is characterized by a large amount of fat preserved in adipose tissue, resulting in weight gain [3]. One strategy to control overweight and hyperglycemia is to inhibit the absorption of dietary carbohydrates and fats. The inhibition of digestive enzymes such as α-amylase and pancreatic lipase responsible for the degradation of complex carbohydrates and lipids into simple molecules is one of the most well-studied therapeutic actions to evaluate the antiobesity and antidiabetic effects of natural products [4].

Since ancient times, medicinal plants have been used for the

treatment of diabetes, especially in developing countries. Currently, scientific research is focused on the control of diabetes [5]. Remedies based on natural products have been found to be more active and cause less adverse effects compared to oral synthetic products [6]. Plants are rich in phenolic compounds that possess insulin-like activity and exert hypoglycemic effects by inhibiting digestive enzymes and lipid peroxidation at the cellular level. These inhibitory effects are associated with their antioxidant activity [7].

Euphorbia is among the most important genera of the family Euphorbiaceae, with approximately 2,000 species identified worldwide, of which 80 species are found in China. Euphorbia plants are characterized by latex that is irritating, and several species are traditionally used for the treatment of skin diseases, edema, and tuberculosis [8]. Among these species, Euphorbia resinifera is one of the oldest "drugs" of the Western medical tradition, frequently used by Moroccan herbalists and therapists. The Arabic name of E. resinifera is "Zaggoume," a species endemic to Morocco generally distributed in the center of the country, in the regions of Azilal and Beni Mellal (Middle Atlas), with some scattered populations in the High Atlas Mountains and the Anti-Atlas [9]. Studies have reported that it contains triterpenoids, diterpenoids, and phenolic acids among other constituents. Moreover, euphane triterpenes, tirucallane triterpenes, tirucallane-type spirotriterpenoids and nortriterpenes have been identified from the latex [10]. In this study, the in vitro antioxidant, hypoglycemic, and antiobesity effects of Euphorbia resinifera extracts were evaluated and the acute toxicity and phenolic content of these extracts investigated.

MATERIALS AND METHODS

1. Plant material and extraction

The aerial part of E. resinifera was collected from the regions of Beni-Mellal, Morocco (geographic coordinates: 32°12'16.6°33'36.6" W). The specimens of E. resinifera have been deposited in herbarium HUMPO at University Mohammed First, Oujda, Morocco, under the number HUMPOM 10052. The aerial part of E. resinifera was dried at room temperature, powdered, and stored in a dark and humid place until use.

The aqueous extract was prepared by infusion, wherein 30 g of E. resinifera was mixed with 300 mL of distilled water for 1 hour. The mixture was filtered and evaporated at 50℃ using a rotary evaporator. The extract was finally freeze-dried to remove traces of water and stored for further use. To prepare the ethanolic extract, 30 g of the plant material was macerated for 48 hours under stirring and at room temperature. The extract was filtered and evaporated at 40℃ using a rotary evaporator.

2. Determination of phenolic content

The phenolic content was determined by the method described by Spanos and Wrolstad [11], and the flavonoid content according to the method of Dewanto et al. [12].

3. Antioxidant activity

1) DPPH, ABTS, H_2O_2, and xanthine oxidase assays

The antioxidant capacity by the DPPH method was determined according to the protocol described by Huang et al. [13], the ABTS test by the protocol of Tuberoso et al. [14] H_2O_2 by Muruhan et al. [15], and xanthine oxidase by Umamaheswari et al. [16].

4. Enzyme inhibitory activities

1) α-Amylase, α-glucosidase, and lipase inhibition tests

The method described by Chakrabarti et al. [17] was used to determine the percentage inhibition of α-amylase, the protocol described by Kee et al. [18] for the determination of α-glucosidase, and the protocol described by Hu et al. [19] for the inhibition of lipase.

2) Oral starch tolerance in normal rats

The evaluation of oral starch tolerance in normal rats was performed according to the protocol described by Beejmohun et al. [20].

5. HR-MS analysis

Analysis was performed by using electrospray ionization (ESI) mass spectrometry (MS). The samples were dissolved in methanol to a final concentration of 1-2 pmol/μL. All compounds were then measured in negative and positive modes by total mass scanning (m/z 50-1,000) using a Thermo Scientific Orbitrap Exactive mass spectrometer with a heated ESI source (HESI-II). Mass spectra were collected at a resolution of 100,000. The instrument parameters were as follows: sheath gas

10 in positive mode and 20 in negative mode (arbitrary units), sputtering voltage 3.5 kV in positive mode (3 kV in negative), capillary temperature 275℃. Data processing was performed using the associated software Xcalibur 2.2 and Exactive 1.1.

6. Acute toxicity

Acute oral toxicity was investigated according to the method described in OECD-423 [21]. Swiss albino mice weighing 25-35 g were used in this study. The groups received the extracts orally at the dose of 2 g/kg. After administration, the animals were observed for 14 days to assess the toxic and behavioral effects.

RESULTS

1. Phytochemical analysis

The results of the phytochemical analysis are summarized in Table 1; the ethanolic extract was found to be richer in polyphenols and flavonoids than the aqueous extract. The TPC and TFC were 99 ± 0.56 mg GEA/g extract and 55.22 ± 0.17 mg RE/g extract in the ethanolic extract and 89.31 ± 0.42 and 35.24 ± 0.89 mg RE/g extract in the aqueous extract, respectively.

2. Antioxidant activity

1) DPPH, ABTS, H_2O_2, and xanthine oxidase activity

The results of the antioxidant activity of the aqueous and ethanolic extracts using the DPPH, ABTS, H_2O_2, and xanthine oxidase methods are summarized in Table 2. Indeed, the ethanolic extract showed higher antioxidant activity in all the methods. In the DPPH assay, both the aqueous and ethanolic extracts showed inhibitory activity with IC_{50} = 149 ± 1.55 μg/mL and IC_{50} = 53.81 ± 1.83 μg/mL, respectively. Similarly, the $ABTS^+$ radical-scavenging activity of the extracts revealed that the aqueous and ethanolic extracts exhibited inhibitory values of 86.6 ± 1.23 mg TE/g extract and 111.4 ± 2.64 mg TE/g extract, respectively. The inhibitory effects of E. resinifera extracts on H_2O_2 were evidenced by IC_{50} = 98.15 ± 0.68 μg/mL for the ethanolic extract compared with IC_{50} = 224.6 ± 0.7 μg/mL for the aqueous extract. Regarding the inhibition of xanthine oxidase, the ethanolic extract showed a significantly greater inhibitory effect (IC_{50} = 10.26 ± 0.6 μg/mL) than the aqueous extract (IC_{50} = 69.83 ± 1 μg/mL).

3. Enzyme inhibitory activity

1) α-Amylase, α-glucosidase, and lipase inhibition

The results of in vitro antihyperglycemic activity against α-amylase and α-glucosidase enzymes are summarized in Table 3. Both extracts possessed inhibitory activity against α-amylase and α-glucosidase, showing higher activity than acarbose (used as a reference compound), which showed inhibitory values of IC_{50} = 44.75 ± 0.54 μg/mL and IC_{50} = 18.01 ± 2.00 μg/mL against α-amylase and α-glucosidase, respectively. The ethanolic extract showed an IC_{50} of 102 ± 3.63 μg/mL against α-amylase and an IC_{50} of 56.6 ± 1.12 μg/mL against α-glucosidase. The aqueous extract had lower inhibitory activity, with an IC_{50} of 119.7 ± 2.15 μg/mL against α-amylase and an an IC_{50} of 121.4 ±

Table 1. Total phenols and flavonoids content of *E. resinifera*

	Aqueous extract		Ethanol extract	
	TPC (mg GEA/g extract)	TFC (mg RE/g extract)	TPC (mg GEA/g extract)	TFC (mg RE/g extract)
Euphorbia resinifera	89.31 ± 0.42	35.24 ± 0.89	99 ± 0.56	55.22 ± 0.17

Table 2. Antioxidant activity by DPPH, ABTS, H_2O_2 and xanthine oxidase (XO) methods of *E. resinifera* extracts

	DPPH (IC_{50})	ABTS (mg TE/g extract)	H_2O_2 (IC_{50})	Xanthine oxidase (IC_{50})
Aqueous extract	149 ± 1.55	86.6 ± 1.23	224.6 ± 0.7	69.83 ± 1
Ethanol extract	53.81 ± 1.83	111.4 ± 2.64	98.15 ± 0.68	10.26 ± 0.6
BHT	3.28 ± 0.79	-	-	-
Ascorbic acid	-	-	5.98 ± 0.47	-
Allopurinol	-	-	-	0.78 ± 0.01

mg TE/g extract: mg Trolox equivalent per gram of extract, IC_{50}: μg/mL.

 https://doi.org/10.3831/KPI.2022.25.3.242

Table 3. IC_{50} values of *E. resinifera* extract on α-amylase, α-glucosidase and lipase inhibition

	IC_{50} (μg/mL)		
	α-amylase	α-glucosidase	Lipase
ERA	119.7 ± 2.15	121.4 ± 1.88	25.3 ± 1.53
ERE	102 ± 3.63	56.6 ± 1.12	13.7 ± 3.03
Acarbose	44.75 ± 0.54	18.01 ± 2.00	-
Orlistat	-	-	12.55 ± 4.17

ERA, aqueous extract of *E. resinifera*; ERE, ethanolic extract of *E. resinifera*.

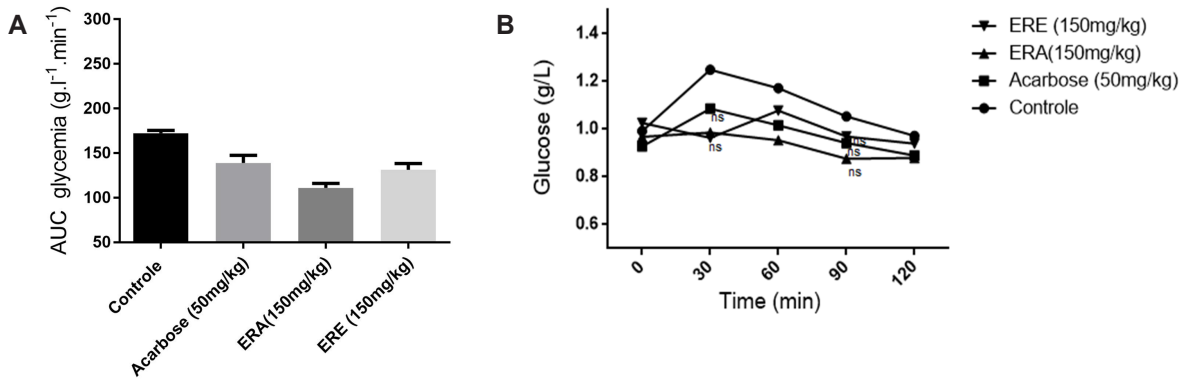

Figure 1. Effect of *Euphorbia resinifera* on blood glucose after starch loading in normal rats (A) and with presentation in the area under curve (B). Values are means ± SEM (n = 5). Ns, not significant to the normal controls; ERA, aqueous extract of *E. resinifera*; ERE, ethanolic extract of *E. resinifera*; AUC, area under the curve.

1.88 μg/mL against α-glucosidase. In addition, the ethanolic extract displayed an IC_{50} = 13.7 ± 3.03 μg/mL against lipase, which was higher than that of the aqueous extract.

2) Oral starch tolerance in normal rats

The results of the effects of E. resinifera extracts in an oral starch tolerance test in normal rats are shown in Fig. 1. Acarbose and ERA decreased blood glucose after 30 minutes; however, ERE reduced blood glucose only after 60 minutes at 1.07 g/L. However, at 30 minutes, acarbose and ERA ($p > 0.05$) reduced blood glucose to 0.93 and 0.81 g/L, respectively, while ERA ($p > 0.05$) reduced blood glucose to 0.88 g/L only after 120 min. Similarly, ERA reduced blood glucose compared with acarbose and control.

3) HR-MS analysis

ESI-HRMS analysis was able to identify saponarin in the aqueous extract with a molecular formula $C_{27}H_{29}O_{15}$, m/z 593.15, and an RDB unsaturation value of 13.5 (Fig. 2).

4) Acute toxicity

The oral administration of aqueous and ethanolic extracts of E. resinifera at a dose of 2 g/kg showed no mortality in the treated mice. Further, the treatment with the two extracts did not induce any behavioral disorder during the 14 days of observation. Therefore, we concluded that the oral LD_{50} of E. resinifera is greater than 2,000 mg/kg.

DISCUSSION

The phytochemical analysis revealed that the extracts of E. resinifera, particularly the ethanolic extract, are rich in polyphenols and flavonoids. However, a study by Farah et al. [22] on the same species reported lower content of polyphenols and flavonoids than that obtained in our study. The methanolic extract of the stems showed TPC of 94.83 ± 1,027 μg GAE/g extract and TFC of 39.14 ± 0.48 μg RE/g extract. These results can be influenced by several factors such as the type of extraction, the solvent used, the extraction time, the extraction temperature, the particle size of the plant materials, the solvent/solid ratio, the environment, and the harvest period [23]. Therefore, ex-

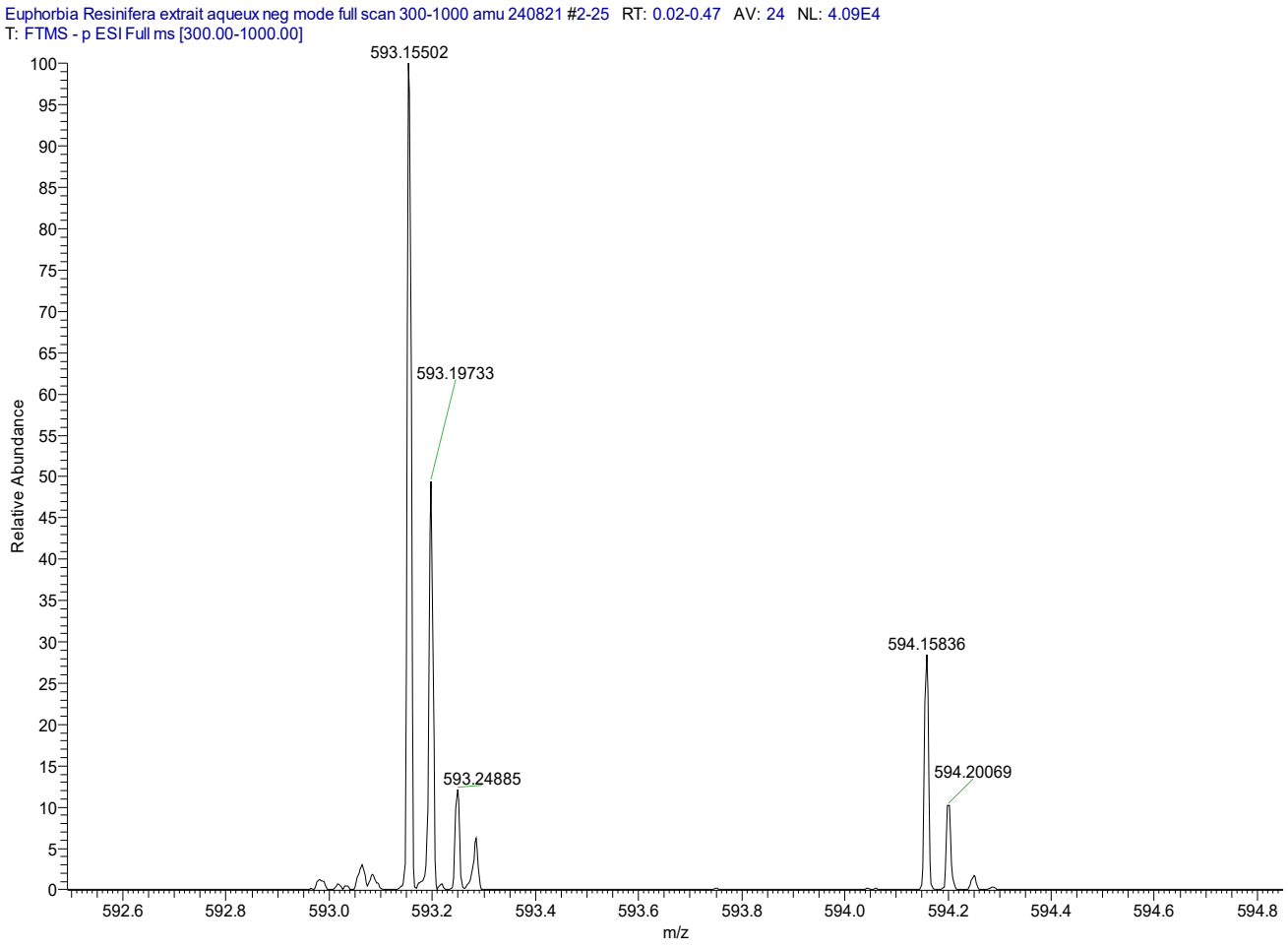

Figure 2. HRMS mass spectrum obtained in negative mode for Saponarin in aqueous extract.

traction methods such as infusion and maceration are popular methods used in traditional herbal preparations due to their short times, simplicity, and high yields of bioactive compounds.

The study of the antioxidant activity of the E. resinifera extracts by four methods (DPPH, ABTS, H_2O_2, and xanthine oxidase) showed that the two extracts have both antioxidant and antiradical effects. The wavelength absorption of the $ABTS^+$ at 734 nm eliminated color interference by the extracts [24]. H_2O_2 is an oxidant, weakening the activity of enzymes by the oxidation of thiol groups (-SH); it can also react with Fe^{2+} and or Cu^{2+} to form a hydroxyl radical, causing cellular poisoning. Therefore, the inactivation of hydrogen peroxide is essential [25]. Compared to another study performed on the same species, the ethanolic extract in our study showed higher antioxidant activity against H_2O_2 than that reported (IC_{50} = 65.01 ± 0.32 μg/mL) for DPPH [26]. This may be due to many factors such as the presence of several compounds or the synergistic effect of bioactive compounds and the position of hydroxyl groups and binding of phenolic compounds [27]. Indeed, the estimated phenolic content indicates that there may be a correlation of phenolic constituents with this antioxidant activity.

E. resinifera aqueous and ethanolic extracts were also tested for their inhibitory activity against the enzymes α-amylase, α-glucosidase, and lipase. Both extracts exhibited an inhibitory effect against α-amylase, α-glucosidase, and lipase, with higher activity obtained with the ethanolic extract. Additionally, extracts of E. resinifera lowered blood sugar in normal starch-loaded rats. These effects could be attributed to the phytochemical constituents in the plant. Ameer et al. [28] and Birari and Bhutani [29] showed that polyphenols, terpenoids, and their derivatives have great promise as antidiabetic and antiobesity agents. Indeed, it has been reported that plants acting on digestive enzymes such as α-amylase and α-glucosidase, that react with proteins, blocking enzymatic activity [3], or that act on

 https://doi.org/10.3831/KPI.2022.25.3.242

hepatic enzymes by stimulating glycogenesis or inhibiting glycogenolysis, can also inhibit glucose transporters at the level of the intestinal barrier to limit the intestinal absorption of glucose [30]. However natural products can also inhibit the pancreatic lipase responsible for the degradation of triglycerides by physically blocking the interaction between triacylglycerol (in the oily phase) and the enzyme (in the aqueous phase) at the water-oil interface. Binding of a lipase substrate and its inhibitor will prevent the substrate from entering the active site of the lipase [31]. However, our results suggest that the E. resinifera extracts inhibit enzymes (α-amylase, α-glucosidase, and lipase) and glucose transporters. Similarly, our study showed the high levels of phenolic compounds in E. resinifera extracts with a major proportion consisting of terpenoids [8, 32]. Terpene compounds such as carvacrol, thymol, α-pinene, β-pinene, and α-terpineol have shown antioxidant power and effects on enzyme expression [33, 34]. In addition, certain terpene derivatives have been identified and isolated from plants and their mechanisms of action elucidated. These terpene derivatives have been used as activators to facilitate permeability and transdermal absorption of drugs [35]. Indeed, Ćavar Zeljković et al. [36] reported that the phenolic and volatile terpenoid content is closely related to the phenological stage of the plant. Similarly, studies have reported the antioxidant and antidiabetic activities of natural products [37, 38]. Thus, bioactive compounds isolated from natural products offer great promise for developing new therapies.

ESI-HRMS analysis identified the saponarin compound in the aqueous extract but not the terpenoid compounds cited by other studies. Simeonova et al. [39] and Sengupta et al. [40] have shown strong antioxidant and antidiabetic effects of the saponarin molecule. Treatment with aqueous and ethanolic extracts of E. resinifera for 14 days showed no signs of intoxication or weight loss. Therefore, we concluded that the oral LD_{50} of E. resinifera is greater than 2,000 mg/kg.

CONCLUSION

Euphorbia resinifera was found to be rich in phenolic compounds, possessing antioxidant, antiobesity, and hypoglycemic activities. Indeed, the aqueous and ethanolic extracts showed antioxidant activity in DPPH and ABTS assays and against xanthine oxidase enzyme and H_2O_2. Similarly, the extracts inhibited digestive enzymes (α-amylase, α-glucosidase, and lipase) and improved postprandial glycemia in normal rats. However, further antioxidant and hypoglycemic studies must be performed in vivo to confirm these effects.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

FUNDING

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

AUTHORS' CONTRIBUTION

Authors are expected to present author contributions statement to their manuscript such as; Kaoutar Benrahou: Investigation, Resources, and Writing – original draft. Otman El Guourrami: Methodology, Supervision, and Validation. Hanae Naceiri Mrabti:Visualization, Software, Formal Analysis. Yahia Cherrah: Software, Formal Analysis. My El Abbes Faouzi: Software, Formal Analysis.

ORCID

Kaoutar Benrahou, https://orcid.org/0000-0002-1391-6232
Otman El Guourrami, https://orcid.org/0000-0002-5315-6063
Hanae Naceiri Mrabti, https://orcid.org/0000-0002-8145-2572
Yahia Cherrah, https://orcid.org/0000-0001-7890-9028
My El Abbes Faouzi, https://orcid.org/0000-0003-3863-4677

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