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Phenolic content, antibacterial, antioxidant, and toxicological investigations of *Erodium guttatum* (Geraniaceae) collected from the Northeast of Morocco

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Abstract: *Erodium* genus contains several medicinal traditionally used and pharmacologically explored. However, *Erodium guttatum* has not been well valorized. Therefore, the aim of the present study was to evaluate the antibacterial and antioxidant activities of *E. guttatum* extracts in addition to their toxicity. To achieve the objectives of this study, methanol and aqueous extracts of *E. guttatum* were prepared. Then, antibacterial activity was evaluated against *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 43816, *Salmonella typhimurium* ATCC 14028, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, and *Listeria monocytogenes* ATCC 13932 by disc diffusion and broth microdilution methods. The antioxidant activity was evaluated by DPPH scavenging assay, scavenging of hydrogen peroxide assay, and xanthine oxidase inhibition assay. The mineral composition was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES). Moreover, the polyphenol, flavonoids, and tannins contents were estimated using colorimetric methods. However, the safety of plant extracts was validated by performing acute and subacute acute toxicity. The results of this study showed that methanolic and aqueous extracts of *E. guttatum* contain important amounts of polyphenols (279.71 ± 0.31 and 142.03 ± 0.81 mg GAE/g extract), flavonoids (118.58 ± 0.14 and 68.25 ± 0.42 mg ER/ g extract), and tannins (61.81 ± 0.25 and 27.47 ± 0.62 mg CE/g extract) as well as a wide range of mineral elements. Additionally, the biological evaluation showed that plant extracts exhibit remarkable antioxidant, and antibacterial activities (MIC ranged between 6.25 and 100 mg/mL for aqueous extract and between 3.12 and 100 mg/mL for methanolic extract). Moreover, our findings showed that *E. guttatum* aqueous extract did not show toxicity. Therefore, *E. guttatum* could be a good source for the identification of antioxidant and antibacterial drugs. In addition, the observed findings could open new horizons on the ethnobotanical usages of *E. guttatum*. However, further investigations are required to identify and isolate bioactive compounds from this plant as well the investigations of their biological effects.

Key words: *Erodium guttatum*, phenolic compound, antibacterial effect, antioxidant effect, toxicity

1. Introduction

For centuries, our ancestors used plants to relieve pain, heal ailments, and heal wounds. Thus, even now, despite the progress of pharmacology, the therapeutic use of medicinal plants is present in some countries of the world, especially in developing countries (Mrabti et al., 2019).

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In recent years, the use of treatment with plants as well as the search for new substances with biological activities constitutes one of the greatest scientific concerns, leading to a thorough search for bioactive compounds, namely plant antioxidants and their importance in medicine, the food industry, and human nutrition (Oliveira et al., 2011).

However, the assessment of the curative properties of plants remains a very useful task, especially for plants of rare or unknown use in medicine and medicinal traditions. These plants constitute a new source of active compounds. Indeed, secondary metabolites are and remain the subject of numerous *in vivo* and *in vitro* researches (Ribeiro Neto et al., 2020). Some secondary metabolites are useful in our diet, such as flavonoids, quinines, and terpenoids, have commercial applications in pharmaceutical, biomedical, and insecticide fields (Gurib-Fakim, 2006).

Africa is one of the richest continents in the world in terms of biodiversity, with many plants used for medicinal purposes (Farombi, 2003). In fact, North Africa includes a wide range of climatic changes ranging from the Mediterranean in the north to the desert or semi desert in the south, which favors the growth of typical and diversified plant flora. Currently, the interest of contemporary scientific studies on using herbs in the treatment of different diseases increases via ethnobotanical surveys and biological tests in animal models (Olaokun et al., 2014). *Erodium* L'Herit (Geraniaceae) contains 74 species distributed all over the world except Antarctica. Most of these species (62 species) are distributed in the Mediterranean Basin region (Alarcón et al., 2003; Fiz et al., 2006; Fiz-Palacios et al., 2010; Coşkunçelebi et al., 2012). The species *Erodium guttatum* (Desf.) Willd. is a perennial plant and it is widely distributed in Northern Africa, Southern of Spain and Palestine (http://www.plantsoftheworldonline.org/taxon/urn:lsid:ipni.org:names:372280-1). The genus is used in folk medicine to treat several diseases, namely dermatological, gastrointestinal disorders, indigestion and inflammatory diseases, diabetes, cancer, constipation, eczema, hemorrhages as well as carminative agent, astringent, and antiseptic (Fecka and Cisowski, 2002). Similarly, their leaves have been used for the preparation of salads, omelets, sandwiches, sauces and soups and some food products. It's also used in Iraq for treatment of dysentery and abdominal pain, snake and scarpion bites (Güneş et al., 2017; Kawarty et al., 2020; Kawarty et al., 2021), and, in Algeria, against gastro-intestinal disorders (Cheriti et al., 2006). Indeed, few studies have focused on the pharmacological properties and the chemical composition of this species, describing its antibacterial and antioxidant (Hamza et al., 2018). Indeed, in this study, authors showed an important variability between different regions of Tunisia in terms of chemical composition (flavonoids, phenolic acids, and tannins), as well as antioxidant and antimicrobial effects. Moreover, a positive correlation has been established between chemical contents and biological activities (Hamza et al., 2018). In addition, toxicological properties of water and methanol extracts of *E. guttatum* and the influence on biochemical parameters are missing. Concerning the phytochemical composition, studies report the presence of a high content of flavonoids, tannins, and other phenolic compounds. On the other hand, the analyze of the chemical profiles by planar chromatography of certain species of *Erodium* showed the presence of geraniin, dehydrogeranine, corilagine, and isoquercitrin (Munekata et al., 2019). Another study has reported the chemical structure of some phenolic compounds of *E. cicutarium* (L.) L. Hér. species, such as gallic acid, protocatechuic acid, 3- O-galloylshikimic acid, 3-O- (6 ”-O-galloyl) - β-D-galactopyranoside, corilagin, didehydrogeranine (dehydrogeranine), geraniin, hyperine, isoquercitrin, methyl 3-O-β-D-glucopyranoside, and rutin (Fecka and Cisowski, 2005). In another in-depth study, on *E. cicutarium*, by the UHPLC method coupled with the MS technique, has identified either 85 phenolic compounds, mainly derivatives of gallic acid (24 compounds), several derivatives of ellagic acid including ellagitannins (22 compounds), flavonol glycosides (19 compounds), hydroxycinnamic acid derivatives (8 compounds), other hydroxybenzoic acid derivatives (7 compounds), flavonol aglycones (3 compounds), and procyanidins (2 compounds) were determined (Bilić et al., 2020).

However, to the best of our knowledge, the chemical composition and the biological potentials of *Erodium guttatum* remain poorly studied. Therefore, the aim of this work was the evaluation of the antioxidant effect and antibacterial activity of *Erodium guttatum* extracts from Northeast Morocco (Oujda city), as well as the investigation of their toxicity, a screening of the presence of different secondary metabolites, the composition of their mineral content, and the total phenolic, total flavonoids, and total tannins content.

2. Materials and methods

2.1. Plant material

E. guttatum specimen were collected in May 2020 from the province of Oujda, Northern Morocco, from the latitude of 34°40'55.06"N and a longitude of 1°54'0.56"W, at 549m of, 250 mm of average annual rainfall. The plant material was identified and authenticated (Voucher Specimen: **RAB 110970**) by Prof. Mohammed Sghir Taleb from the Department of Botany and Plant Ecology of the Scientific Institute of Rabat, University of Mohammed V Rabat, Morocco. The material was transported to the laboratory, the aerial parts were discarded and cleaned with water and dried in the dark at room temperature for 15 days. The plant material was then powdered and used in the next

two days for phytochemical screening and preparation of extracts.

2.2. Animals

Experiments were performed in healthy, adult Swiss mice weighing from 25 to 30 g. Animals were obtained from the animal center at the Faculty of Medicine and Pharmacy, University Mohammed V in Rabat. Swiss mice were housed under standard environmental conditions 23 ± 2 °C under a 12-h light/dark cycle with access to water and a standard laboratory diet (Mrabti et al. 2018).

2.3. Preparation of extracts

2.3.1. Preparation of aqueous extracts

The aqueous extract of *E. guttatum* was prepared as follow: 100 g of plant powder was boiled in 1L of distilled water for 30 min and then freeze-dried. The lyophilized extract was kept in a desiccator in the dark at room temperature until use.

2.3.2. Preparation of methanol extract

The methanol extract was prepared by the maceration of 100 g of plant powder in 1 L of methanol 90% at room temperature with agitation for 24 h. Then, the resulted product was filtered and dried to remove solvent.

2.4. Phytochemical analysis

2.4.1. Phytochemical screening

The phytochemical screening of *E. guttatum* was conducted following the standard methods (Dib et al., 2013) in order to identify the following groups: alkaloids, flavonoids, tannins, terpenoids, saponosides, free quinones, and anthraquinones. Visual observation of color change or formation of a precipitate after the addition of specific reagents was used for interpretation and analysis of results.

2.4.2. Phenolic and flavonoid contents

The total phenolic and flavonoids contents in the studied extracts were determined based on Folin-Ciocalteu method and colorimetric method, respectively, according to the protocols described previously (Mrabti et al., 2021). The total phenolic content was expressed as mg gallic acid equivalents per gram of dry weight of extract (mg GAE/g extract). However, flavonoid content was determined as the rutin equivalent from the calibration curve of rutin standard solutions and expressed as rutin equivalents per gram of dry weight of extract (mg RE/g extract).

2.4.3. Total tannin content

Condensed tannin contents were determined also using a colorimetric method (Mrabti et al., 2021). Briefly, an aliquot (50 µL) of each diluted extract was mixed with 1.5 mL of 4% vanillin, followed by the addition of 750 µL of concentrated hydrochloric acid. The well mixed solution was incubated at ambient temperature in the dark for 20 min after the solution is well mixed and left for 20 min in the dark at room temperature. The absorbances were measured at 500 nm. The standard curve was performed by using catechin at a concentration of 50–500 µg/mL), and the results were expressed as milligrams catechin equivalents per gram of extract (mg CE/g extract).

2.5. Mineral content

The mineral content of *E. guttatum* was determined using inductively coupled plasma atomic emission spectroscopy (ICP-AES). The *E. guttatum* powder was evaluated according to our previously published protocol (Zaazaa et al., 2021).

2.6. Evaluation of antioxidant activity

2.6.1. DPPH radical assay

The free radical-scavenging activities of solvent extracts were evaluated using the radical using 1,1-diphenyl-2-picrylhydrazyl (DPPH) as reported previously (Huang et al., 2011); antioxidants react with the stable free radical DPPH (deep violet color) and convert it to 1,1-diphenyl-2-picrylhydrazine with discoloration. The studied extracts were mixed with a methanol solution of DPPH (0.02 mM) and incubated in dark for 30 min at room temperature. After incubation, the absorbances of mixtures were measured at 517 nm, and the DPPH scavenging capacity of extracts was calculated using the following formula:

DPPH scavenging effect (%) = $[(Abs_{blank} – Abs_{Sample})/ Abs_{blank}] \times 100$

Ascorbic acid was used as positive control, and the scavenging activity of extracts was expressed as IC_{50}, which refer to the concentrations of extracts required to obtain 50% of DPPH scavenging effect.

2.6.2. Scavenging of hydrogen peroxide assay

The ability to reduce H_2O_2 was determined using a previously described method (Rosen and Rauckman, 1984). A solution of H_2O_2 (40 mmol/L) was prepared in phosphate buffer (pH = 7.4). The H_2O_2 concentration was determined by absorption spectrophotometry at 230 nm. For this, we added 1mL of the extract or standard antioxidant (ascorbic acid) to the H_2O_2 solution (0.6mL, 40mM). The absorption of H_2O_2 at 230nm was determined after 10 min and compared with that of a control solution containing a phosphate buffer without H_2O_2. The percentage inhibition of hydrogen peroxide was determined as follows:

% Scavenging [H_2O_2] = ((A0- A1) / A0)*100

where A0 was the absorbance of the control, and A1 was the absorbance in the presence of the sample or standard.

2.6.3. Xanthine oxidase inhibition assay

The xanthine oxidase (XO) inhibitory activity with xanthine as the substrate was determined spectrophotometrically as described previously (Umamaheswari et al., 2007). Briefly, a mixture consisted of 1.0 mL of extract samples, 1.9 mL phosphate buffer (pH = 7.5), 0.1 mL of enzyme solution (0.2 units/mL) and 1.0 mL of 0.5mM xanthine

solution. This mixture was incubated 25°C for 15 min. Then, the reaction was stopped by adding 1M HCl (1 mL). Afterward, the absorbance was measured at 295 nm against blank solution (the same mixture was prepared without enzyme solution). Thus, the Xanthine inhibitory activity was calculated as follows:

$$I\ (\%) = [((Ac\text{-}Acb)\text{-}\ (As\text{-}Asb)/(Ac\text{-}Acb)) \times 100]$$

2.7. Evaluation of antibacterial activity

The antibacterial activity of *E. guttatum* extracts was evaluated against four gram-negative bacteria, including *E. coli* ATCC 25922, *Klebsiella pneumonia* ATCC 4381, *Salmonella typhimurium* ATCC 14028, and *Pseudomonas aeruginosa* ATCC 27853, and two gram-positive bacteria, including *Listeria monocytogenes* ATCC 13932 and *Staphylococcus aureus* ATCC 25923. The antibacterial activity was performed by disc diffusion method to determine the inhibitory zones created by extracts and broth dilution method to determine the minimum inhibitory concentration (MIC) and minimum bactericide concentration (MBC) according to the protocols described in previous studies (Ed-Dra et al., 2018; Ed-Dra et al., 2020; Ed-Dra et al., 2021; Mrabti et al. 2021).

2.8. Toxicological investigation

2.8.1. Acute oral toxicity

The toxicological study is carried out according to the guidelines 423(OECD, 2001). The acute oral toxicity of *E. guttatum* extract was tested on female Swice mice. The animals were divided into 3 groups containing 6 mice in each and subjected to a fast for 18 h. Then, a dose of 2000 mg/kg and 5000 mg/kg were chosen to be administered orally to the treated mice. The control group received distilled water instead of extracts. Animals were monitored to record immediate clinical symptoms and then daily for 14 days (Prasanth et al., 2015).

2.8.2. Sub-acute oral toxicity

The assay of subacute toxicity was performed according to OECD, 1998, Test Guideline No. 407 (OECD, 2008). A total of 18 mice were randomly divided into three experimental groups of 6 mice each. After fasting overnight, the treated group received daily by gastric gavage at dose of 2000 mg/kg and 5000 mg/kg of the aqueous extract of the plant tested at the time the control groups were treated with the same volume of distilled water (vehicle) for 28 days. Signs and symptoms of toxicity were observed during the experimental period, and the body weight was measured weekly.

2.8.3. Determination of biochemical parameters

On completion of the treatment, animals were sacrificed, and blood samples were collected; biochemical parameters such as creatinine, urea, uric acid, aspartate aminotransferase (ASAT), and alanine aminotransferase (ALAT) for all groups of animals were measured using an auto-analyzer "Architect C8000" (Abbott Laboratories) (Mrabti et al., 2018).

3. Statistical analysis

All experiments were conducted in triplicates, and results were presented as means ± standard deviations. However, statistical difference of total bioactive contents in the extracts were determined by the Student t-test ($p < 0.05$) using Microsoft Excel. Also, the differences of biological activity assays were tested by ANOVA (by Tukey's test) in Statistica version 13.0.

4. Results and discussion

4.1. Phytochemical analysis

The phytochemical tests carried out on the dry plant of *E. guttatum*, allowed to detect the different families of existing compounds by qualitative characterization reactions. The results showed dominance of flavonoids, followed by tannins, then anthraquinones. We also note the absence of saponins, alkaloids, free quinones, and terpenes. Additionally, the content of total polyphenols, flavonoids, and tannins of the aqueous and methanolic extracts of *E. guttatum* were determined, and the obtained results were summarized in Table 1. The results showed that polyphenolic compounds, flavonoids, and tannins were highly abundant in the methanolic extract compared to the aqueous extract ($p < 0.05$) (Table 1).

This difference could be related to the harsh climatic conditions of the places where they grow like temperature, the exposure to the sun, salinity, and drought, which

Table 1. Total phenolic, flavonoids contents, and tannins contents of different solvents.

E. guttatum	Phenolic content (mg GAE/g extract)	Flavonoid content (mg ER/ g extract)	Tannin content (mg CE/g extract)
Aqueous extract	142.03 ± 0.81[a]	68.25 ± 0.42[a]	27.47 ± 0.62[a]
Methanolic extract	279.71 ± 0.31[b]	118.58 ± 0.14[b]	61.81 ± 0.25[b]

Data are expressed as mean ± SD ($n = 3$). Different letters in the same column represent significant differences at $p < 0.05$ (by Student's t test).

stimulate the biosynthesis of secondary metabolites such as polyphenols (Falleh et al., 2008). The methanolic extract is richer in polyphenols than the aqueous extract, which most probably refers to the relative solubility of the polyphenols present in the plant in methanol and water, respectively. In fact, the solubility of polyphenols is influenced by the solvent, their degree of polymerization as well as their interaction with other constituents and the formation of insoluble complexes (Falleh et al., 2008). For higher polyphenol recovery, methanol is the appropriate solvent (Brglez Mojzer et al., 2016). However, despite the several works interests in the polyphenols extraction, there is no standard solvent that allows the extraction of a high polyphenol content, and this may depend also on the plant matrices (Thouri et al., 2017). According to Novak et al. (2008), water and methanol are both polar solvents that particularly extract glycosylated flavonoids and tannins, while aglyconic flavonoids are extracted by alcohols or water-alcohol mixtures (Marston and Hostettmann, 2006). This largely explains the richness in flavonoids of the methanolic extract of *E. guttatum* (118.58 ± 0.14 mg ER/g extract) compared to the aqueous extract (68.25 ± 0.42 mg ER/g extract). On the other hand, the catechic tannin content for the methanolic extract (61.81 ± 0.25 mg CE/g extract) is much higher than the aqueous extract (27.47 ± 0.62 mg CE/g extract).

4.2. Mineral content

Mineral elements in plants are divided into macroelements, heavy metals, and microelements, which are involved in important biological functions of the cell. The results of the mineral analysis showed that the macroelements (Ca, Fe, Mg, P and Na) were concentrated in the aerial part of *E. guttatum* with concentrations of 10.84 g/kg, 7.20 g/kg, 4.16g/kg, 0.47g/kg, and 0.26 g/kg, respectively (Table 2). To our knowledge, these are the first reports of mineral contents of the aerial part of *E. guttatum*. Due to their high content of macroelements, appropriate amounts of microelements and the absence or very low amount of heavy metals can be a valuable addition to human diet and therapy (Zaynab et al., 2021).

4.3. Antioxidant capacity

The antioxidant activity of the studied extract was evaluated by using DPPH scavenging assay, and the results were presented in Table 3. Our results showed that the studied extracts present a considerable antioxidant activity, especially methanol extract (IC_{50}=39.11 ± 3.28 µg/mL), which was higher than that of aqueous extract (IC_{50}=52.13 ± 0.02 µg/mL). However, ascorbic acid presents a great antioxidant activity with an IC_{50} of 4.25 ± 0.31 µg/mL. The methanolic extract is rich in polyphenols compared to the aqueous extract, which increase its ability to trap DPPH radicals. This shows that there is a correlation between the polyphenol content and the antioxidant activity of *E. guttatum* extracts and could indicate that polyphenols are responsible for this activity.

Table 2. The levels of mineral contents in the aerial part of *E. guttatum*.

Mineral elements	Content (mg/kg dw)
Macroelements:	
Ca	10845.83
Fe	7205.56
Mg	4166.51
P	473.52
Na	261.64
Microelements:	
Se	0.0001
B	30.90
Cu	5.56
Mn	253.60
Zn	20.40
V	5.49
Co	2.64
Heavy metals:	
Cd	0.10
Pb	3.97
Ni	5.15
Mo	0.25
Cr	3.11
As	2.26

The xanthine oxidase is a cytosolic enzyme involved in the conversion of hypoxanthine into xanthine and the reduction of O_2 into superoxide $O_2^{\cdot-}$. This superoxide anion radical ($^{\bullet}O_2^-$) can be converted to hydrogen peroxide (H_2O_2). Superoxide $O_2^{\cdot-}$ hydrogen peroxide is two free radical mainly involved in the oxidation of macromolecules and therefore the genesis of some diseases including cancer (Bouyahya et al., 2021). Furthermore, the inhibition of xanthine oxidase is a promising strategy to eliminate oxidative stress resulting from superoxide anion radical $O_2^{\cdot-}$ and therefore to prevent oxidative stress and therefore reducing risk factors of some complex pathologies. As showed in Table 3, the inhibitory effect of xanthine oxidase by *E. guttatum* extracts showed that methanolic extract reveals an important inhibitory activity ($IC_{50=}$86.72 ± 0.46 µg/mL) compared with Allopurinol ($IC_{50=}$51.14 ± 0.47 µg/mL), used as positive control. Moreover, the H_2O_2 production showed that methanolic extract reduced remarkably the production of H_2O_2 ($IC_{50=}$6.95 ± 0.32 µg/mL) compared to ascorbic acid ($IC_{50=}$5.983 ± 0.45µg/mL).

These results confirm that *E. guttatum* extracts act as antioxidant agents at different levels by inhibition of DPPH free radical, inhibition xanthine oxidase activity and reducing the production of H_2O_2. The works evaluated the antioxidant activities of our species are rare. Recently, Hamza et al. (2018) showed that *E. guttatum* extracts have an important antioxidant with similar values of inhibition of our results with some variabilities, which certainly due to the origin of species.

Some researchers examined the antioxidant properties of some members of the genus *Erodium*. For example, Bilić et al. (2020) tested the antioxidant properties of the methanol and water extracts of *Erodium cicutarium,* and the methanol extracts had stronger antioxidant properties than the water extracts. In addition, the authors reported that gallic acid was the main component of the tested extracts and that the observed ability could be attributed to presence of gallic acid. Ellagitannins were reported by Graça et al. (2018) as the main component in *Geranium molle* L. In another study conducted by Pineda-Ramirez et al. (2020), two *Geranium* species (*G. mexicanum* H. B & K and *G. niveum* S. Watson) were examined for antioxidant properties, and their ethanol extracts showed strong radical scavenging abilities. In addition, some authors reported good antioxidant properties of the members of the Geraniaceae family, as well as important phenolic constituents (Cohen et al., 2020; Zeljković et al., 2020).

4.4. Antibacterial activity

The antibacterial activity of aqueous and methanol extracts of *E. guttatum* was conducted by disc diffusion and broth dilution methods, and results were summarized in Table 4 and Figure 1. The results of disc diffusion method against *E. coli*, *S. typhimurium*, *P. aeruginosa*, *K. pneumonia*, *S. aureus*, and *L. monocytogenes* showed that methanol extract was more effective on the tested bacteria with inhibition diameters of 14.6 ± 0.3 mm, 12.9 ± 0.2 mm, 8.1 ± 0.1 mm, 13.7 ± 0.2 mm, 15.3 ± 0.3 mm, and 16.2 ± 0.3 mm, respectively; while aqueous extract presents inhibition diameters of 12.4 ± 0.2 mm, 11.3 ± 0.3 mm, 8.1 ± 0.1 mm, 11.9 ± 0.3 mm, 13.7 ± 0.5 mm, and 14.1 ± 0.4 mm, respectively (Figure 1). Additionally, broth dilution method showed that MIC of methanol extract ranged between 3.12 mg/mL for *S. aureus* and 100 mg/mL for *P. aeruginosa*, and those of aqueous extract ranged between 6.25 mg/mL for both *S. aureus* and *L. monocytogenes* and 100 mg/mL for *P. aeruginosa*. Moreover, the results of our study showed that methanol and aqueous extracts had a bactericidal effect, with a ratio of MBC/MIC≤2 (Table 4). The difference in the antibacterial activity of the studied extracts might be correlated to the phenolic contents. According to the phytochemical analysis, methanol extract has the higher phenolic content, which demonstrates its effectiveness against the tested bacteria compared to aqueous extract. In fact, previous studies

Table 3. Antioxidant activities (IC_{50} µg/mL) of *E. guttatum.*

Extracts	DPPH assay	H_2O_2	Xanthine oxidase
Aqueous extract	52.10 ± 0.02^c	nd	nd
Methanolic extract	39.10 ± 3.28^b	6.95 ± 0.32^b	86.72 ± 0.46^b
Ascorbic acid	4.25 ± 0.31^a	5.98 ± 0.45^a	-
Allopurinol	-	-	51.14 ± 0.47^a

Data are expressed as mean ± SD (n = 3). Different letters in the same column represent significant differences at $p < 0.05$ (by Tukey's test). nd: not determined

Table 4. Results for MIC and MBC for aqueous and methanol extracts of *E. guttatum.*

Bacteria	Gram	Aqueous extract (mg/mL)			Methanol extract (mg/mL)		
		MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC
E. coli ATCC 25922	-	12.5	12.5	1	6.25	6.25	1
S. typhimurium ATCC 14028	-	25	25	1	12.5	12.5	1
P. aeruginosa ATCC 27853	-	100	200	2	100	100	1
K. pneumonia ATCC 43816	-	12.5	25	2	6.25	12.5	2
S. aureus ATCC 25923	+	6.25	6.25	1	3.12	3.12	1
L. monocytogenes ATCC 13932	+	6.25	6.25	1	3.12	6.25	2

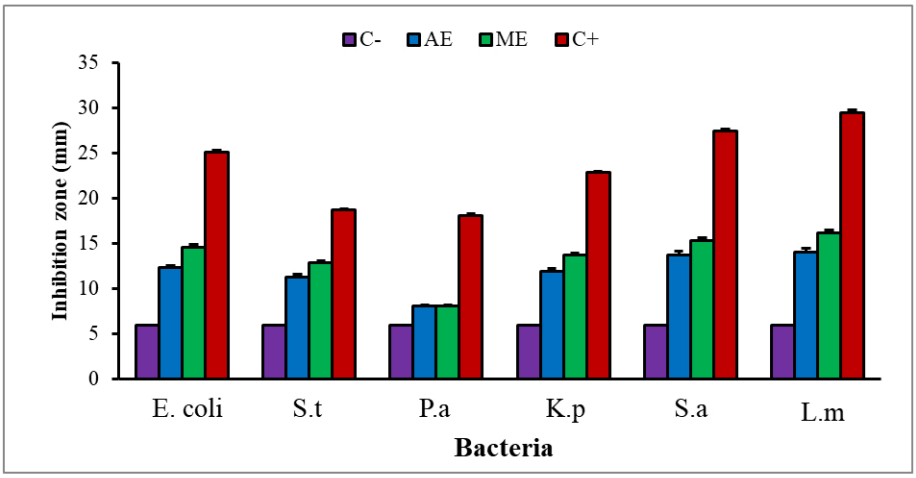

Figure 1. Inhibition zones (the vertical bars represent standard deviation of the means) created by aqueous and methanol extracts of *E. guttatum* against the studied bacteria. *E. coli*: *Escherichia coli* ATCC 25922; K.p: *Klebseila pneumonia* ATCC 43816; P.a: *Pseudomonas aeruginosa* ATCC 27853; S.t: *Salmonella typhimurium* ATCC 14028; L.m: *Listeria monocytogenes* ATCC 13932; S.a: *Staphylococcus aureus* ATCC 25923; C-: negative control (methanol); C+: positive control (Gentamicin, 30 µg).

have been demonstrated the correlation between the antibacterial activity of plant extracts and its phenolic content (Rodríguez Vaquero et al., 2010; Tomás-Menor et al., 2013; Elsharkawy et al., 2018).

For our knowledge, this is the first report of antimicrobial activity of Moroccan *E. guttatum* extracts. However, only one study performed in Tunisia has described the antimicrobial activity of water-methanol extract of *E. guttatum* (Hamza et al., 2018). This study showed that the studied extract had an inhibitory diameter of 8.2 ± 1.7 mm against *E. coli* ATCC25922, 5.7 ± 1 mm against *E. coli* ATCC8739, 6.4 ± 2.6 mm against *S. aureus* ATCC 25923, 3.9 ± 1.2 mm against *S. marcescens* ATCC13880, 6.7 ± 2.3 mm against *K. aerogenes* ATCC 13048, 8.1 ± 2 mm against *E. faecalis* ATCC29212, while it was ineffective against *P. aeruginosa* ATCC27853. Moreover, the results of our study showed that the studied extracts were more effective against gram-positive bacteria; this finding could be explained by the difference in the composition of bacterial cell wall. In fact, during the penetration through the bacterial cell wall, antibacterial components of extracts create damage in the cell wall by degrading the cytoplasmic membrane, altering membrane proteins, leaking cellular contents, coagulating cytoplasm, and exhausting the proton movement force (Burt, 2004; Calo et al., 2015; Gonelimali et al. 2018).

4.5. Toxicological investigations

4.5.1. Acute toxicity

Oral administration of *E. guttatum* extract at doses of 2000 mg/kg and 5000 mg/kg for 14 days did not interfere with the growth of the animals and showed no lethal effect (Figure 2). It appears that the aqueous extract of *E. guttatum* exhibits no lethal effect, as no mortality or change in general condition was observed in mice subjected to oral treatment in both the tested doses for 14 days according to the OECD n° 420. LD_{50} value of *E. guttatum* was found to be greater than 5000 mg/kg.

4.5.2. Subacute toxicity

The body weight and physical appearance of the animals are preliminary factors that could be used to identify the toxic effects that occurred in mice treated with an extract (Traesel et al., 2014). During the entire experimental dosing period (28 days), no observed adverse effects or behavioral changes appeared in the mice treated at doses of 2000 mg/kg/day and 5000 mg/kg/day. All the treated animals gained weight throughout the treatment period. Therefore, this could confirm the safety of the extract tested on treated mice since no change in weight was observed (Figure 3).

4.5.3. Biochemical parameters

Sub-acute administration of *E. guttatum* extract did not cause any significant disturbance of the biochemical parameters such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, creatinine, cholesterol, triglycerides, and blood glucose, compared to the control group (Table 5). In several organs, cell damage is followed by the release of a number of cytoplasmic enzymes into the blood, a phenomenon which forms the basis for clinical diagnosis (Dolai et al., 2012). Damage to the structural integrity of the liver is known to lead to an increase in specific liver enzymes (ALT and AST) in serum, because they are cytoplasmic enzymes and are released after cellular damage (Metushi et al., 2016; Wang et al., 2016). The results did not show a significant change in the

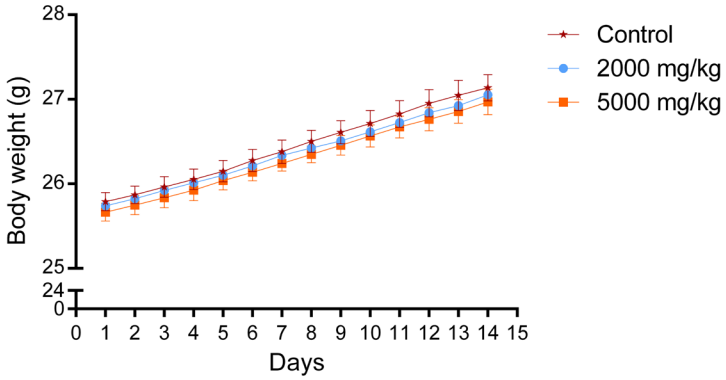

Figure 2. Acute toxicity of *E. guttatum* aqueous extract. The vertical bars represent standard deviation of the means.

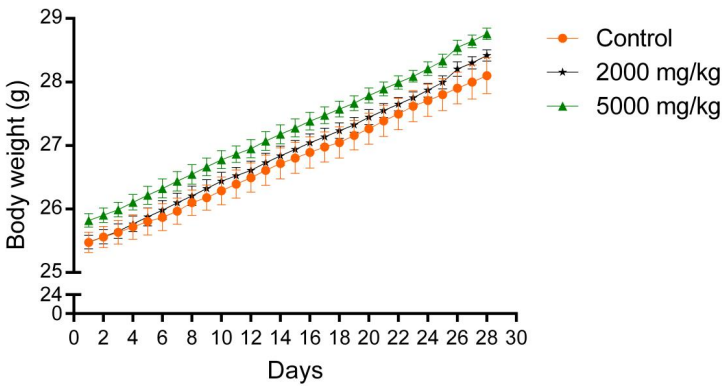

Figure 3. Subacute toxicity of *E. guttatum* aqueous extract. The vertical bars represent standard deviation of the means.

Table 5. Effects of *E. guttatum* aqueous extract on biochemical blood parameters of blood of mice after 28-day period of oral administration.

Biochemical parameters	**Control**	***E. guttatum* (2000mg/kg)**	***E. guttatum* (5000mg/kg)**
Liver analysis:			
AST (U/L)	94.11 ± 0.13[a]	89.71 ± 0.09[b]	99.31 ± 0.39[c]
ALT (U/L)	40.25 ± 1.39[a]	41.67 ± 2.72[a]	42.83 ± 1.92[a]
Renal Analysis:			
Creatinine (mg/L)	3.91 ± 0.21[a]	3.50 ± 1.00[a]	4.01 ± 0.11[a]
Urea (g/L)	0.26 ± 0.13[a]	0.27 ± 0.08[a]	0.29 ± 0.25[a]
Blood biochemistry:			
Total protein (g/L)	67.23 ± 6.02[a]	64.15 ± 4.32[a]	62.85 ± 1.53[a]
Glucose (g/L)	0.93 ± 0.45[a]	0.94 ± 0.37[a]	0.98 ± 1.21[a]
Cholesterol (g/L)	1.02 ± 0.09[a]	1.03 ± 0.05[a]	1.17 ± 1.03[a]
Triglycerides (g/L)	0.59 ± 0.07[a]	0.56 ± 0.02[a]	0.58 ± 0.04[a]

Different letters in the same lines represent significant differences at $p < 0.05$ (by Tukey's test).

levels of AST and ALT transaminases after administration, suggesting that the extract is not hepatotoxic. Renal function has been assessed in this study by measuring plasma creatinine and urea concentrations, which are known to be important markers of renal dysfunction (Mukinda and Eagles, 2010). Any rise in creatinine levels is only observed if there is marked damage to functional nephrons (Lameire et al., 2005). In contrast, the two doses 2000 mg / kg / day and 5000 mg / kg / day produced no disturbance, strongly suggesting that renal function was not impaired after treatment with the extract of *E. guttatum* in subacute administration for 28 days. Thus, total protein was not affected in the experimental group. Therefore, we conclude that treatment with *E. guttatum* extract had no significant impact on protein metabolism. Nandy and Datta say the liver is the site of cholesterol elimination or degradation and its major site of synthesis. Thus, it controls the synthesis of glucose and generates free glucose from hepatic glycogen reserves (Nandy and Datta, 2012). In this study, no changes were seen in glucose, cholesterol, and triglycerides levels, it suggests that *E. guttatum* had no effect on lipids and carbohydrate metabolism in mice.

5. Conclusion and perspectives

In this study, *E. guttatum* extracts showed antibacterial and antioxidant activities as well as toxicological investigations. Despite that, this species presented important results that were not explored in traditional medicine systems. Therefore, ethnobotanical investigations about *E. guttatum* should continue to explore more of its potentialities in traditional medicines in different populations. It was noticed that extracts presented important charges of phenolic and flavonoids contents. In vitro antioxidant effects revealed that the methanolic extract exhibits remarkable potential against DPPH radical, oxygen superoxide, and H_2O_2 production. Moreover, some extract inhibited importantly the growth of gram negative and gram-positive strains. Ina addition, toxicological tests confirmed the safety of this species. The chemical compounds of this species can be used as antibacterial and anti-oxidative stress disease agents. However, further investigations should be carried out to determine and isolate bioactive chemical compounds responsible for these effects and to evaluate their mechanism of action as well as their toxicological validations.

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