Trop J Nat Prod Res, April 2024; 8(4):6814-6819 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Phytochemical Composition, Antibacterial Activity and Acute Toxicity Studies of *Euphorbia resinifiera* O. Berg

Basma Aghoutane[1]*, Amal Naama[1], Imane El Attar[2], Otman El-Gourrami[3], Hanae El Monfalouti[1], Badr Eddine Kartah[1]

[1]*Laboratory of Plant Chemistry and Organic and Bio-Organic Synthesis. Faculty of Sciences, Mohammed-V University of Rabat, Morocco.*
[2]*Microbiology and Molecular Biology Unit, Plant and Microbial Biotechnology, Biodiversity and Environment Center, Faculty of Sciences, University Mohamed V, Rabat, Morocco.*
[3]*Laboratory of Analytical Chemistry and Bromatology, Faculty of Medicine and Pharmacy of Rabat Mohamed V, University of Rabat, Morocco.*

ARTICLE INFO

Article history:
Received 30 December 2023
Revised 12 April 2024
Accepted 15 April 2024
Published online 01 May 2024

ABSTRACT

Euphorbia resinifiera is a medicinal plant exploited by the native population to treat various diseases. The study compared the effects of seven different extracts of *Euphorbia resinifiera* by evaluating their antibacterial activity. The study reported the alkaloid percentage, total protein content, carotenoid content, and tannin content in the aerial part of *Euphorbia resinifiera.* The antibacterial activity of the seven extracts (100 mg/mL/disc) was evaluated by disc diffusion method against six pathogenic bacteria: 2 Gram-positive (*Staphylococcus aureus* and *Microbacteriumresistant*) and 4 Gram-negative (*Klebsiella pneumonia, Escherichia coli, Alcaligenesfaecalis*, and *pseudomonas chloritidismutans*). Aqueous methanol extract obtained by maceration showed the best antibacterial activity against all tested bacteria. Whereas the percentage of total protein content was very low, 1.28%, and the alkaloid yield was 0.08±0.013 g/100 g of dry weight. The results revealed that the lethal dose (LD_{50}) was higher than 300 mg/kg for the aqueous extract obtained by maceration. For other extracts, the LD_{50} was higher than 2000 mg/mL (maceration methanol, maceration Methanol-water, sonication methanol, sonication water, sonication Methanol-water, infusion.). Overall, the results indicated that *Euphorbia resinifiera* possesses antibacterial activity against the isolates studied.

***Keywords*:** *Euphorbia resinifiera*, Extraction Method, Antibacterial Activity, Alkaloids, Acute Toxicity.

Introduction

More than 20,000 plant species are used for medical purposes (WHO).[1] Medicinal plants contain a large number of phytochemicals such as polyphenols, flavonoids, protein, and alkaloids,[2] with a potential for use in the food, pharmaceutical, and cosmetics industries. Nowdays, numerous microscopic organism have become resistant to antibiotic, it is therefore very important to search for an alternative, that will take over in anti-infection medicines, the information of plants and the study of their therapeutic demonstrating that medicinal plants could be an alternative source of new active principals with potential antibiotic effects. [3,4]

Euphorbia resinifiera grows in Morocco in the middle Atlas region. It belongs to the Euphorbiaceae family, a large botanic family with many species utilised as anti-diarrhea, anti-dysentery, and purgative.[5] This plant is a rich source of polyphenols, flavonoids, and antioxidant agents.[6]

This study examined the extraction yield using different methods and extraction solvents. Also, the total protein content, the antibacterial activity, and the toxic effects of the aerial part of *Euphorbia resinifiera* were studied.

*Corresponding author. E mail: basma.agho@gmail.com
Tel: +212654772811

Citation: Aghoutane B, Naama A, El Attar I, El-Gourrami O, El Monfalouti H, Kartah BE. Phytochemical Composition, Antibacterial Activity and Acute Toxicity Studies of *Euphorbia resinifiera* O. Berg. Trop J Nat Prod Res. 2024; 8(4):6814-6819. https://doi.org/10.26538/tjnpr/v8i4.10

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria

Materials and Methods

Plant material

The plant was collectedfrom the Benimellal-khenifera region in November 2021 in Azilal province. It was identified at the Department of Botany and Plant Ecology, Scientific Institute Rabat, Morocco. A voucher specimen (N RAB113340) was deposited at the Herbarium of the same institute.

After removing all impurities, the thorns on the four sides of the plant were removed and the latex was extracted from the stems by automatic micropipette. The stems were cut into small pieces and dried in a laboratory oven fixed at 30°C for ten days. Then, the dried small pieces were ground with an electric grinder into powder and stored in food bags at room temperature until use.

Extraction of plant material

The extraction methods used for extracting the biomolecules are sonication, maceration and infusion. Methanol and water, which have different polarities as solvents were used.[9] Also, aqueous methanol (methanol-water 70/30 v/v) was used to test the effects of extraction solvents on extraction yield, antimicrobial activity and acute toxicity of *Euphorbia resinifiera.*

Sonication

Grounded powder (5g) of *Euphorbia resinifiera* was mixed separately with 50 mL of each solvent: Methanol, water, and methanol/water (70-30 v/v). The samples were placed in an ultrasonic bath for 45 minutes with a break of 10 minutes after each 15 minutes. The solvent was eliminated under vacuum with a rotary evaporator to obtain the crude extract. The extract was placed in Glass tubes and stored at 4°C for use in biological analysis.[10]

Maceration
The protocol adopted by Durazzo et al.[11] was used with some modifications. 10 g of the powder was mixed separately with 100 mL of each extraction solvent, Methanol, water, and methanol/water (70-30 v/v). After filtration using Whatman filter paper, the solvent was evaporated using a vacuum with a rotary evaporator to obtain the crude extract. The extract was placed in glass tubes and stored at 4°C for use in biological analysis.

Infusion
Infusion extraction was performed according to the protocol described by Silva-Leite*et al*.[12] 2.5 g of the powdered material was placed in a flask containing 75 mL of boiling water. Then, the mixture was kept at room temperature to cool and filtered. After filtration with Whatman filter paper, the solvent was eliminated under a vacuum with a rotary evaporator to obtain the crude extract. The extract was placed in glass tubes and stored at 4°C for use in biological analysis.
Extraction yield
The extraction yield was calculated by using the following formula:

$\%Yield= (W_1/W_2)*100$
Where:
W_1 is the weight of the dry extract (g)
W_2 is the dry weight of the plant sample (g).

Total protein content determination
Euphoria resinifiera powder (0.75 g) was put in Whatman paper and placed in a test tube. Then, 2 g of the catalyst (copper sulphate, potassium sulphate, and selenium in a ratio of 10:1:0.1) was added.After which, 10 mL of sulfuric acid was added to the tubes and placed in a mineralisation device. Then, the mixture was heated until a greenish colour appeared. After 4 hours, the heating was stopped, and the test tubes were left to cool. Then, 100 mL of distilled water and 80 mL of caustic soda (NaOH 50%) were added. Then, the mixture was placed in the distillation apparatus. The vapour actuates the distillate at the outlet in a beaker already containing boric acid and colour indicator (Tashiro contains methylene blue and methyl red) after the change of colour of the indicator. Finally, 150 mL of the collected distillate was titrated with HCL (0.1N) intel, and the same initial color was obtained as the indicator. A blank was performed in the same conditions as the sample.[7]
The total protein content percentage was calculated using the following formula:

$\%NM=((V_S-V_B)N_{HCL}*14*6.25)/D_W(mg)*100$
Where: V_s: Volume of the sample in mL
V_B: Volume of blank in mL
N_{HCL}: Normality of HCL
Dw: weight of the sample

Total alkaloid extraction
The total alkaloid content of the aerial part of *Euphorbia resinifiera* was extracted according to the method described byHaida *et al*., [8] with some modifications.
100 g of powder of *Euphorbia resinifiera* was extracted with absolute methanol three times at room temperature (25°C). After filtration, the methanol extracts were combined and concentrated to dryness under reduced pressure at 40°C. The remaining residue was acidified with 5% hydrochloric acid solution, filtered, and the aqueous acidic solution was then basified with 25% Ammonium hydroxide and extracted with dichloromethane. The organic phase was filtered, dried over anhydrous sodium sulphate, filtered again, and finally concentrated in vacuo. The acid-base purification procedure was repeated three times to give a dark brown semi-solid extraction of alkaloids. The results are expressed as g of alkaloids equivalent per 100 g of the aerial part of the study plant.

Carotenoids content determination
The total carotenoid content of the powdered plant material was determined using a spectrophotometer, following the method of Barkia*et al*.[13] with some modifications.A methanol solution (1 mg/ml) of each extract was prepared, and then the absorbances were measured at 470, 648, and 664nm using a UV-1800Pc UV-Vis spectrophotometer. Methanol was used as control, and the pigment content (chlorophyll a (Chla), chlorophyll b (Chlb), and total carotenoids) was calculated using the following formula:

Total carotenoids = $(1000*A_{470}-1.63*chla-104.96*chlb)/221$
Where: A_{470} is the absorbance at 470nm
Chla is $13.36*A_{664}-5.19*A_{648,}$ and chlb is $27.43*A648-8.12*A_{664.}$

Total tannin content
The total tannin content was determined using the method described previously by Barhé&Tchouya.[14] Briefly, 50 µL of each extract was added to 1.5 mL of vanillin (4%). After 2 min, 750 µL of HCl (12M) was added to the mixture. Then, after 20 min of incubation in the dark at room temperature, the absorbance was measured at 500nm. In this case, catechin was used as a standard to establish the calibration curve, and the total tannin contents were expressed as mg CE/g dry weight.

Antibacterial activity of extracts of Euphorbia resinifiera
The disc-diffusion assay was used to evaluate the antimicrobial activity of the different solvent extracts against clinical pathogens.[15] A total of 6 human pathogenic bacteria were used and divided into gram-positive: *Staphylococcus aureus* ATTC 25923 (Bacteria1), *Pseudomonas chloritidismutans* MW559720 (bacteria 2), *Microbacteriumresistens* lMR1188 (Bacteria 3), and gram-negative: *Klebsiella pneumonia* MW524112 (Bacteria 4), *Escherichia coli dhα* (Bacteria 5), and *Alcaligenes faecalis* 1172 (Bacteria 6). All the extracts were sterilised by filtration using a 0.2 µmmillipore filter. A 100 µL of suspension containing 10^6 cells/mL of overnight bacterial cultures was spread evenly onto Mueller–Hinton agar (MHA) plates. Sterile filter paper discs (about 6 mm in diameter) were pressed onto the surface of the agar plates, and then 100mg/ml of each extract was deposited onto the respective discs. Petri dishes were incubated at 28°C and 37°C for *Escherichia coli* for 24h. Antimicrobial activity was then evaluated by the presence of an inhibition zone against the test microorganisms after the incubation period. Antimicrobial activity was classified into three levels: (3) strong activity, (2) moderate activity, (1) weak activity, and no inhibition activity (0), and the translucent area around the disc was measured. The results are expressed on mm. DMSO solution at a concentration of 10% was included with each test as a negative control.

Oral acute toxicity
The acute oral toxicity assessment for each extract was performed according to the guidelines established by the Organization for Economic Cooperation and Development(OECD 423).[16] For each extract of *Euphorbia resinifiera*, three non-pregnant and nulliparous female mice weighing between 20 and 30 g fasted for 4 hr but had free access to water. The mice were housed separately and individually in sterile polypropylene cages. Each extract (Extract1: infusion, Extract2: Maceration water, Extract3: Maceration mixture, Exttract4: Sonication Water, Extract5: Sonication mixture, Extract 6: Maceration Methanol, Extract 7: Sonication Methanol.) was administered orally at 2000 mg/kg. After the administration of the extracts, the animals were observed for 30 minutes and then for 14 days. During this period, variations in body weight, mortality as well as clinical signs (convulsion, salivation, diarrhoea, lethargy sleep, and coma) were noted.

Statistical analysis
The results obtained are average carried out in triplicates. The values were expressed as mean ± SD analysed using MS Excel 2007 software.

Results and Discussion

Determining the total content of different secondary metabolites in plant materials helps in processing such materials as herbal products and even in determining the isolation of pure compounds.
The extraction yields ranged from 20% to 65% and varied with the solvent and method used (Table 1). The highest yield was obtained by aqueous methanol (70%) followed by methanol, and the lowest yield was obtained with water extract. The latter exhibited a similar and low extraction yield regardless of the method used. The results illustrated

that the maceration was more effective than the ultrasound extraction or infusion. According to Stalikas,[20] the differences in extraction yield are due to the polarity of the solvents, temperature, extraction time, the ratio of plant material to solvents, and the extraction method used.

Results of the study showed that the percentage of total protein content present in the aerial part of *Euphorbia resinifiera*was 1.28%. According to the literature data, this percentage is considered very low.[17]In general, this is due to some interspecies variability. This variability is due to the function of the morphological composition of the plant.[18]

Liquid-liquid extraction of total alkaloid from the aerial parts of *Euphorbia resinifiera* gave an extract with yellowish-brown colour and a yield of 0.08 ± 0.013 g/100g of dry weight. Andriambelason*et al*.[17] reported similar results with a total alkaloids yield of 0.07±0.00245%. The total protein content and the pH.19 influence the production of plant alkaloids

Similarly, carotenoids play an important role in scavenging reactive species of oxygen generated during photosynthesis, especially singlet oxygen.[21] This study determined the total carotenoid content of the methanol, aqueous-methanol (70%), and water extracts obtained by different extraction methods. The results showed that the Aqueous-Methanol (70%) extract obtained by sonication had the highest value (0.35±0.07 mg/g extract weight), followed by the methanol extract (0.25±0.04 mg/g extract weight). In contrast, the water extract had the lowest content (0.19±0.09 mg/g extract weight). Also, the carotenoid content was highest in the Aqueous-Methanol (70%) extract obtained by maceration (0.36±0.03 mg/g extract weight), followed by methanol extract (0.22±0.06 mg/g extract weight), and water extract having the least value (Table 2). The total carotenoid content recorded by infusion was (0.34±0.09 mg/g extract weight) (Table 2). These results were different from the results reported by Barkia*et al.*[13] Those differences in carotenoid content could be due to the variation of species or to the variation of climatic conditions which affect the carotenagenesis.[22]

The total tannin content was calculated using a calibration curve ($y=0.001x+0.031$, $R^2=0.997$) obtained using catechin as standard. The extract obtained by infusion had the highest content of total tannins (0.99±0.01 mg CE/g dry weight). The tannins content of the extract obtained by sonication was (0.625±0.03 mg CE/g dry weight) for the methanol extract, which was higher than the water extract but lower than the Aqueous-Methanol (70%) extract (0.735±0.05 mg CE/g dry weight) (Figure 1). For the maceration, the higher extract value was (0.855±0.04 mg CE/g dry weight) for Aqueous-Methanol (70%), and the lowest extract value was (0.155±0.02 mg CE/ g dry weight) for Water extract (Figure 2). The results are similar to those obtained by Lopez-Fernandez *et al*. [23], who reported that methanol extract obtained by maceration gives the highest total tannin content (1.99±0.11mg CE/g dry weight).

The results are similar to those of Mansouri *et al*.[24], who found that the methanol extract obtained by maceration gives the highest total tannins content (1.99±0.11 mg CE/g dry weight). In another study, Souhila and Mustapha,[25] reported that the Aqueous extract had the best tannin content of 4.09 mg CE/g dry weight. Also, the infusion extract had the highest content of tannins, which could be due to the use of high temperature in infusion extraction, which contributed to the good diffusion and solubility of the extract.[15]

In the antimicrobial activity screening, the extracts showed antibacterial activity with variable degrees of inhibition (Figure 3). The extracts from maceration with either water or methanol showed low inhibition activity. In contrast, their mixture produced the maximum zone of inhibition among all extracts tested against Gram-positive and Gram-negative bacteria (Table 3).

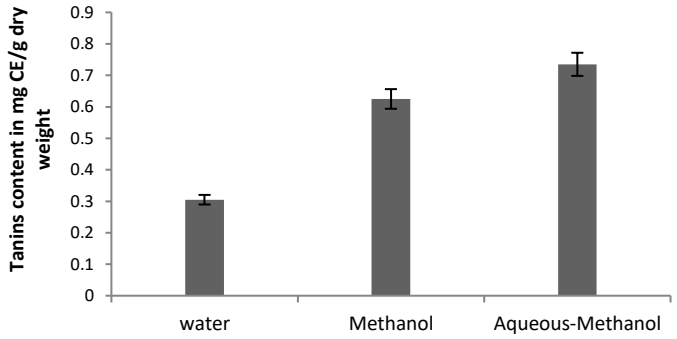

Figure 1: Tanins content of *Euphorbia resinifiera* extract obtained by sonication.

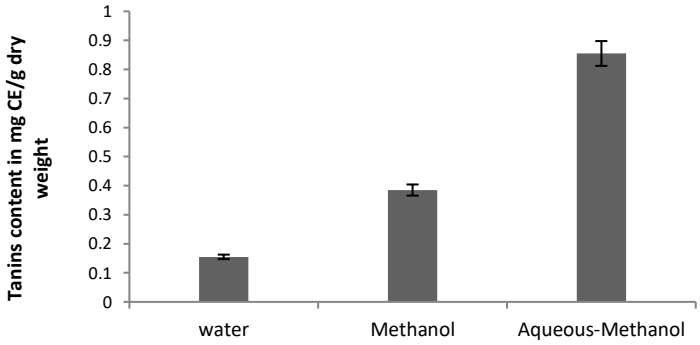

Figure 2: Tanins content of *Euphorbia resinifiera* extract obtained by maceration.

Table 1: Results of extraction yield

Extraction method	Maceration Water	Maceration Aqueous methanol	Maceration Methanol	Sonication Water	Sonication Aqueous methanol	Sonication Methanol	Infusion
Yield (%)	27.5 ± 0.004	63.5 ± 0.004	62.13 ± 0.004	23.6 ± 0.004	58.94 ± 0.02	32.03 ± 0.05	24.4 ± 0.4

Table 2: Carotenoids content of *Euphorbia resinifiera* extract obtained by sonication, maceration, and infusion

Extraction method	Extraction solvent	Total Carotenoids content(mg/g extract weight)
	Aqueous-Methanol (70%)	0.35 ± 0.07
Sonication	Methanol	0.25 ± 0.04
	Water	0.19 ± 0.09
	Aqueous-Methanol(70%)	0.36 ± 0.03
Maceration	Methanol	0.22 ± 0.06
	Water	0.19 ± 0.04
Infusion	Water	0.43 ± 0.09

It has been reported that extracts obtained through maceration present high bactericidal activity against various bacterial pathogens, including *Staphylococcus*, *E. coli*, and *Pseudomonas*, which were also used in this study.[26] Moreover, the strains used in this study were less susceptible to water extract obtained by maceration. Indeed, several studies have documented that plant water extracts showed less antibacterial inhibitory action.[27] It is suggested that low polar compounds present in the active plant extracts may be responsible for this antimicrobial activity.[28]

On the other hand, extracts produced by sonication exhibited moderate to high levels of growth inhibition of all tested bacteria. Unlike the maceration mixture, the combination of methanol and water sonication extracts did not exhibit a potent inhibitory effect. The best effect was observed by extract obtained from water and methanol maceration mixture, which efficiently suppressed the growth of all pathogenic strains, producing larger inhibition zones. Methanol extracts of different plant species have been reported to show strong antibacterial activity against a wide range of bacteria since they possess the highest antibacterial and significant antioxidant activities.[29-30]Moreover, it has been indicated in previous studies that sonication-assisted extracts exhibited the best antimicrobial properties.[31-32]Results also showed that the lowest inhibitory effect was recorded with the extract obtained by infusion (extract 7) against *E. coli*, *Mycobacterium*, and *Pseudomonas*. In contrast, no inhibition effect was shown on *Klebsiella*, *Staphylococcus*, and *Alcaligenes*.

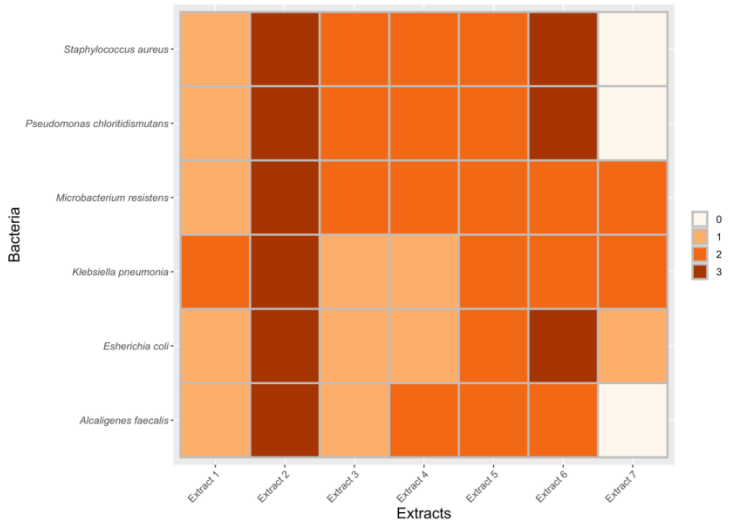

Figure 3: Heatmap representation of the antimicrobial activity of water and methanol extracts of *Euphobiaresinefera* against test bacteria. Dark colours indicate effective inhibition, whereas light colours indicate little to no inhibition, with 3 being the most effective and 0 being the least effective growth inhibitors.

Table 3: Antibacterial Activity of the extract of *Euphorbia Resinfiera* by the disc diffusion method

Microorganisms	Inhibition zone diameter (mm)						
	Extracts						
	Maceration water: Extract 1	Maceration Mixture: Extract 2	Maceration Methanol: Extract 3	Sonication Water: Extract 4	Sonication Mixture: Extract 5	Sonication Methanol: Extract 6	Infusion: Extract 7
Bacteria1	2.1 ± 0.3	15.3 ± 0.0	6.7 ± 0.4	7.6 ± 0.2	5.8 ± 0.1	29.7 ± 0.4	Negatif
Bacteria2	3.4 ± 0.4	16.4 ± 0.3	5.3 ± 0.1	7.4 ± 0.3	9.3 ± 0.1	23.5 ± 0.3	Negatif
Bacteria3	4.5 ± 0.4	17.2 ± 0.1	6.2 ± 0.1	8.9 ± 0.4	8.7 ± 0.1	6.1 ± 0.3	9.3 ± 0.0
Bacteria4	6.7 ± 0.3	18.9 ± 0.2	2.6 ± 0.0	3.1 ± 0.4	6.9 ± 0.2	5.9 ± 0.4	8.9 ± 0.0
Bacteria5	3.2 ± 0.2	23.1 ± 0.1	3.7 ± 0.4	2.4 ± 0.3	7.4 ± 0.2	27.4 ± 0.3	4.3 ± 0.3
Bacteria6	4.1 ± 0.2	29.3 ± 0.3	4.9 ± 0.1	7.6 ± 0.0	9.3 ± 0.2	6.7 ± 0.4	Negatif

Kirbag*et al*.,[33] researched 8 *Euphorbia* species and reported that the tested extracts inhibited the growth of several pathogenic microorganisms in different ratios. These authors suggested that *Euphorbia* extracts may possess compounds with antibacterial and antifungal properties that can be used as antimicrobial agents in developing new drugs to treat infectious diseases.

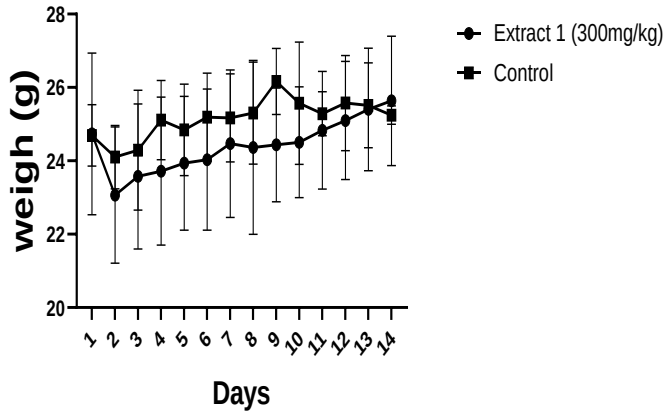

Figure 4: Changes in the body weight of the animals treated with extract 1 and of the control group.

As for *Euphorbia resinifiera*, only a few studies investigated the antimicrobial effect of plant extracts; namely, Benmehdi*et al*.,[34] studied the effect of the aerial part extracts, and Zarshenas*et al*.,[35] investigated the root extracts effect. Both studies reported significant effects in suppressing the growth of pathogenic microorganisms with variable potency, which aligns with the results obtained in the present study. Moreover, according to the results, mixed methanol, water maceration extract, and methanol extracts obtained by sonication showed effective growth inhibition. Hence, these extracts could be exploited in medicinal chemistry as broad-spectrum antimicrobial agents.

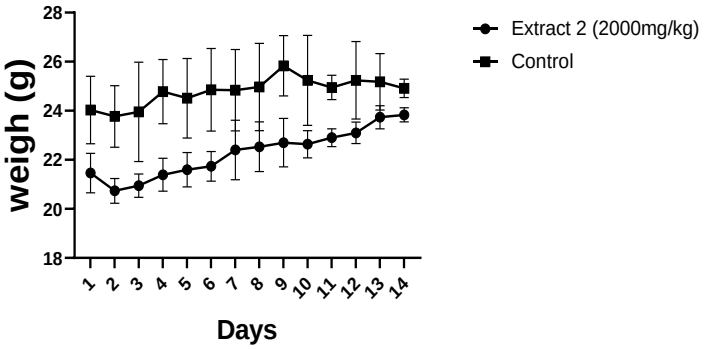

Figure 5: Changes in the body weight of the animals treated with extract 2 and the control group.

It could also be stated that methanol sonication and mixed maceration extractions have proven to be efficient extraction procedures for better antimicrobial efficiency.

The results of acute toxicity at the dose of 2000 mg/kg showed no clinical signs of toxicity. All animals tested survived during the 14 days of observation, and their behavior remained normal except for the extract obtained by water maceration, which showed a reduction in body weight and sedation. Therefore, the dose was lowered to 300 mg/kg, and the study was repeated. According to OECD No. 423, increasing the dose above 2000 mg/kg for animal protection is not permitted, except in justified cases. These results show that the lethal dose (LD_{50}) is higher than 2000 mg/kg for maceration aqueous-methanol (Figure 5), maceration methanol (Figure 6), sonication water (Figure 7), sonication aqueous-methanol (Figure 8), sonication methanol, infusionextracts and for water maceration extract(Figure 4), the LD_{50} is higher than 300 mg/kg. During the 14 days of follow-up, the mean body weight of each group did not change, especially after 10 days (Figure 4). Therefore, based on these results and EOCD No. 423 guidelines, the extracts are considered non-toxic for single oral administration at 2000 mg/kg. No study of the oral toxicity of *Euphorbia resinifiera* has been reported in the literature.

Conclusion

The extract obtained by maceration with aqueous methanol was the most efficient in inhibiting the growth of all tested bacteria (gram-positive and gram-negative). Also, it exhibited the highest extraction yield and the best results for acute oral toxicity. This demonstrates that *Euphorbia resinifiera* extracts could be a great natural remedy for treating infections caused by the studied bacteria. Further in vitro and in vivo studies are required to identify the active compounds for possible utilization as new natural products with potential antibacterial activity.

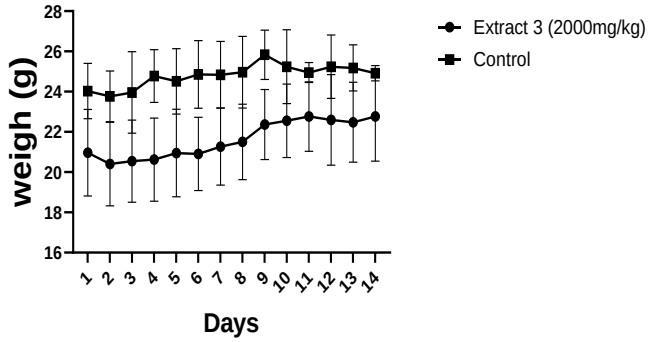

Figure 6: Changes in the body weight of the animals treated with extract 3 and of the control group

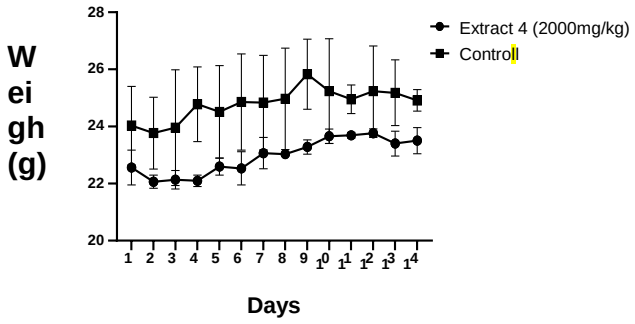

Figure 7: Changes in the body weight of the animals treated with extract 4 and the control group.

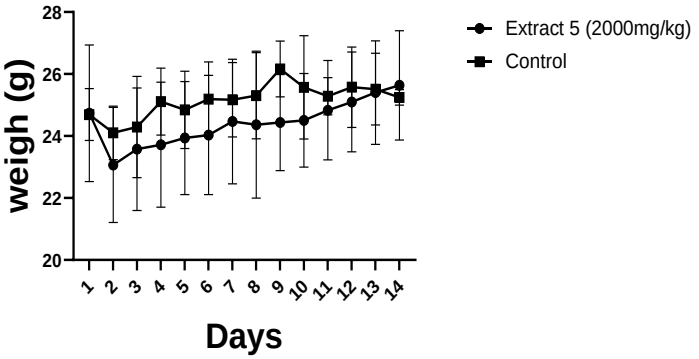

Figure 8: Changes in the body weight of the animals treated with extract 5 of the control group.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

References

1. Zhao Y, Wang M, Xu B. A comprehensive review on secondary metabolites and health-promoting effects of edible lichen. J Funct Foods. 2021;80:104283.
2. Laksemi DAAS, Sukrama DM, Sudarmaja M, Damayanti PAA, Swastika K, Diarthini NLPE, Astawa NM, Tunas K. Medicinal Plants as Recent Complementary and Alternative Therapy for COVID-19: A Review. Trop J Nat Prod Res TJNPR.2020;4(12):1025-32.
3. Liu J, Du C, Beaman HT, Monroe MBB. Characterisation of Phenolic Acid Antimicrobial and Antioxidant Structure–Property Relationships. Pharmaceutics. 2020;12(5):419.
4. Amro BI, Hajleh MNA, Afifi F. Evidence-Based Potential of some Edible, Medicinal and Aromatic Plants as Safe Cosmetics and Cosmeceuticals. Trop J Nat Prod Res TJNPR. 2021;5(1):16-48.
5. Crepaldi CG, Campos JLA, Albuquerque UP, Sales MF. Richness and ethnobotany of the family Euphorbiaceae in a tropical semiarid landscape of Northeastern Brazil. South Afr J Bot. 2016;102:157-65.
6. Aghoutane B, Talbi H, Naama A, EL Monfalouti H, Kartah BE. Effect of Extraction Solvent on Total Phenol Content, Total Flavonoid Content, and Antioxidant Activity of *Euphorbia resinifiera* O. Berg. Trop J Nat Prod Res. 2023;7(3):2530-5.
7. Walsh KB, Blasco J, Zude-Sasse M, Sun X. Visible-NIR 'point' spectroscopy in postharvest fruit and vegetable assessment: The science behind three decades of commercial use. Postharvest Biol Technol. 2020;168:111246.
8. Haida S, Kribii A, Daoud NA, Belakhmima RA, Kribii A. Antioxidant activity of *Haloxylon scoparium* alkaloid extracts from Figuig region (southeastern of Morocco). Braz J Pharm Sci. 2022;58:19494.
9. Altemimi A, Lakhssassi N, Baharlouei A, Watson DG, Lightfoot DA. Phytochemicals: Extraction, Isolation, and Identification of Bioactive Compounds from Plant Extracts. Plants. 2017;6(4):42.
10. Carrera Fernández CA, Ruiz Rodríguez A, Palma Lovillo M, García Barroso C. Ultrasound assisted extraction of phenolic compounds from grapes. Anal Chim Acta .2012. 732-30.

11. Durazzo A, Lucarini M, Souto EB, Cicala C, Caiazzo E, Izzo AA, Novellino E, Santini A. Polyphenols: A concise overview on the chemistry, occurrence, and human health. Phytother Res. 2019;33(9):2221-43.
12. Silva-Leite KES da, Assreuy AMS, Mendonça LF, Damasceno LEA, Queiroz MGR de, Mourão PAS, Pires AF, Pereira MG. Polysaccharide rich fractions from barks of *Ximenia americana* inhibit peripheral inflammatory nociception in mice Antinociceptive effect of *Ximenia americana* polysaccharide rich fractions. Rev Bras Farmacogn. 2017;27:339-45.
13. Barkia I, Saari N, Manning SR. Microalgae for High-Value Products Towards Human Health and Nutrition. Mar Drugs. 2019;17(5):304.
14. Barhé TA, Tchouya GF. Comparative study of the antioxidant activity of the total polyphenols extracted from *Hibiscus Sabdariffa* L., *Glycine max* L. *Merr*., yellow tea and red wine through reaction with DPPH free radicals. Arab J Chem. 2016;9(1):1-8.
15. Mohamed EAA, Muddathir AM, Osman MA. Antimicrobial activity, phytochemical screening of crude extracts, and essential oils constituents of two Pulicaria spp. growing in Sudan. Sci Rep. 2020;10(1):17148.
16. Walum E. Acute oral toxicity. Environ Health Perspect.1998;106:497-503.
17. Andriambeloson OH, Noah RMA, Rigobert A, Jean-Marc C, Luciano R, Rado R. Isolation of Novel Vincristine and Vinblastine Producing Streptomyces Species from *Catharanthus Roseus* Rhizospheric Soil. Research Square. 2021; 1-17. DOI: 10.21203/rs.3.rs-1082130/v1
18. Cabiddu A, Dattena M, Decandia M, Molle G, Lopreiato V, Minuti A, Trevisi E. The effect of parity number on the metabolism, inflammation, and oxidative status of dairy sheep during the transition period. J Dairy Sci.2020;103(9):8564-75.
19. Amdoun R, Khelifi L, Khelifi-Slaoui M, Amroune S, Benyoussef EH, Thi DV, Assaf-Ducrocq C, Gontier E. Influence of minerals and elicitation on *Datura stramonium* L. tropane alkaloid production: Modelisation of the *in vitro* biochemical response. Plant Sci.2009;177(2):81-7.
20. Stalikas CD. Extraction, separation, and detection methods for phenolic acids and flavonoids. J Sep Sci. 2007;30(18):3268-95.
21. Ali MY, Sina AAI, Khandker SS, Neesa L, Tanvir EM, Kabir A, Khalil MI, Gan SH. Nutritional Composition and Bioactive Compounds in Tomatoes and Their Impact on Human Health and Disease: A Review. Foods.2021;10(1):45.
22. Lezoul NEH, Belkadi M, Habibi F, Guillén F. Extraction Processes with Several Solvents on Total Bioactive Compounds in Different Organs of Three Medicinal Plants. Molecules. 2020;25(20):4672.
23. López-Fernández O, Domínguez R, Pateiro M, Munekata PES, Rocchetti G, Lorenzo JM. Determination of Polyphenols Using Liquid Chromatography–Tandem Mass Spectrometry Technique (LC–MS/MS): A Review. Antioxidants. 2020;9(6):479.
24. Mansouri FE, Silva JCGE, Cacciola F, Asraoui F, Tayeq H, Ben Amar YM, Lovillo MP, Chouaibi N, Brigui J. Evaluation of Different Extraction Methods on the Phenolic Profile and the Antioxidant Potential of Ceratonia siliqua L. Pods Extracts. Molecules.2022;27(19):6163.
25. Souhila M, Mustapha K. Study of the extraction of phenolic compounds from different parts of the artichoke flower (*Cynara scolymus* L.). J Nature and Tech. 2013
26. Bin C, Al-Dhabi NA, Esmail GA, Arokiyaraj S, Arasu MV. Potential effect of *Allium sativum* bulb for the treatment of biofilm forming clinical pathogens recovered from periodontal and dental caries. Saudi J Biol Sci. 2020;27(6):1428-34.
27. Silva E, Fernandes S, Bacelar E, Sampaio A. Antimicrobial activity of aqueous, ethanolic and methanolic leaf extracts from Acacia Spp. And Eucalyptus nicholii. Afr J Tradit Complement Altern Med. 2016;13(6):130-4.
28. De Zoysa MHN, Rathnayake H, Hewawasam RP, Wijayaratne WMDGB. Determination of In Vitro Antimicrobial Activity of Five Sri Lankan Medicinal Plants against Selected Human Pathogenic Bacteria. Int J Microbiol. 2019;2019:7431439.
29. Liu Q, Meng X, Li Y, Zhao CN, Tang GY, Li HB. Antibacterial and Antifungal Activities of Spices. Int J Mol Sci. 2017;18(6):1283.
30. Nguyen TVL, Nguyen QD, Nguyen NN, Nguyen TTD. Comparison of Phytochemical Contents, Antioxidant and Antibacterial Activities of Various Solvent Extracts Obtained from 'Maluma' Avocado Pulp Powder. Molecules. 2021;26(24):7693.
31. Pobiega K, Kraśniewska K, Derewiaka D, Gniewosz M. Comparison of the antimicrobial activity of propolis extracts obtained by means of various extraction methods. J Food Sci Technol. 2019;56(12):5386-95.
32. Safarpoor M, Ghaedi M, Asfaram A, Yousefi-Nejad M, Javadian H, Zare Khafri H, Bagherinasab M. Ultrasound-assisted extraction of antimicrobial compounds from *Thymus daenensis* and *Silybum marianum*: Antimicrobial activity with and without the presence of natural silver nanoparticles. Ultrason Sonochem. 2018;42:76-83.
33. Kirbag S, Erecevit P, Zengin F, Guvenc AN. Antimicrobial activities of some Euphorbia species. Afr J Tradit Complement Altern Med. 2013;10(5):305-9.
34. Benmehdi H, Bounoua N, Amrouche A, Lahcene D, Maazouzi A. Phytochemical study, antioxidant and antimicrobial activities of *Euphorbia resinifera*. Int Res J Pharm. 2013;4(9):44-50.
35. Zarshenas MM, Ansari R, Dadbakhsh A, Mohammadi M. A Review of Herbal Remedies for Multiple Sclerosis-Like Disorders in Traditional Persian Medicine (TPM). Curr Drug Metab. 1 2018;19(5):392-407.