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Original Article

LC-MS Analysis and Biological Activities of *Ziziphus spina-christi* L. (Christ's Thorn, Jujube) Leaves from Tunisia Oasis

Meriem Elaloui[1*], Badiaa Essghaier[2], Hanene Ghazghazi[1], Rim Driouich Chaouachi[3], Salima Bahri[1], Soumaya Hamdi[4], Rania Ben Nasr[5], Jouda Ben Jemaa[4], Youssef Ammari[1] and Abdelwahed Laamouri[1]

[1]Laboratory of Forest Ecology, National Institute of Researches on Rural Engineering, Water and Forests (INRGREF), University of Carthage, Tunis, Tunisia; [2]Laboratory Mycologies Pathologies and Biomarkers, Faculty of Sciences of Tunis, University of Tunis El Manar, Tunis, Tunisia; [3]Laboratory of Biotechnology and Bio-Geo Resources Valorization (LR11ES31) Higher Institute for Biotechnology, University of Manouba, Tunis, Tunisia; [4]Laboratory of Biotechnology Applied to Agriculture, University of Carthage, Tunis, Tunisia; [5]Faculty of Sciences of Bizerte, University of Carthage, Tunis, Tunisia

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Abstract

Background and objectives: Natural products from plants and their biological potentialities have increased environmental concerns. This study aimed to evaluate the phenolic composition, along with the antioxidant, antimicrobial, and insecticidal activities of *Ziziphus spina-christi* leaf extracts using LC-MS.

Methods: The plant materials were obtained from three Tunisian localities (Metlaoui, Degueche, and Tozeur).

Results: The leaf extracts contained phenols (8.157 mg GAE/DW), flavonoids (4.42 mg Quercetin Eq/gDW), and tannins (1.62 mg Cat Eq. g^{-1}). LC-MS analysis identified the rutin compound at a rate between 1.3 ± 0.005 µg/mL (Dgueche) and 2 ± 0.005 µg/mL (Metlaoui) while 3 ± 0.005 µg/mL in the extracts from the Tozeur. The extracts from the Tozeur had the highest antioxidant activity with a concentration for 50% inhibition of 0.125 mg/mL and the highest antibacterial activity against *Streptococcus agalactiae* specie with a superior diameter of zone inhibition of about 27.5 mm and against *Staphylococcus aureus* specie with a diameter of 16 mm. In contrast, the extracts from the Deguech displayed stronger activity against *Escherchia coli* specie with a diameter of 13.5 mm. There were only two Tozeur extracts that exhibited activity against *Candida albicans* and *Candida Sake* species. Furthermore, treatment with methanolic extracts caused 66.60% mortality in *Tribolium castaneum*.

Conclusions: This study showed that *Ziziphus spina-christi* extracts contained several components that had potent antioxidant and antibacterial activities against some types of bacteria. Hence, these extracts or their components may be recommended as an eco-friendly alternative for synthetic insecticides and used as natural antioxidants in phototherapy.

Keywords: Biological activities; leaves; phenolic compounds; Tunisia; *Ziziphus spina-christi*.

Abbreviations: CFU, colony forming units; CT, condensed tannin; DPPH, 2, 2-diphenyl-1-picrylhydrazyl; IC_{50}, concentration for 50% inhibition; MIC, minimum inhibitory concentration; TPC, total phenolic; TFC, total flavonoids.

*Correspondence to: Meriem Elaloui, Laboratory of Forest Ecology, National Institute of Researches on Rural Engineering, Water and Forests (INRGREF), University of Carthage, Tunis 2080, Tunisia. ORCID: https://orcid.org/0000-0002-8896-3877. Tel: +216 52 276 063, Fax: +216 71 717 951, E-mail: maryoumaa2000@yahoo.fr

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Introduction

The therapeutic applications of medicinal plants grown in Tunisian have increased; thus, a better understanding of their biological activities has become necessary.[1] Medical plants have become crucial therapeutic agents and valuable raw active compounds have been used for manufacturing many traditional and modern treatments.[2] Indeed, a majority of natural antioxidants are derived from plant materials and have been known to benefit human health because they have antidiabetic, antiproliferative, and antimicrobial activities.[3] These antioxidants have been given particular focus because

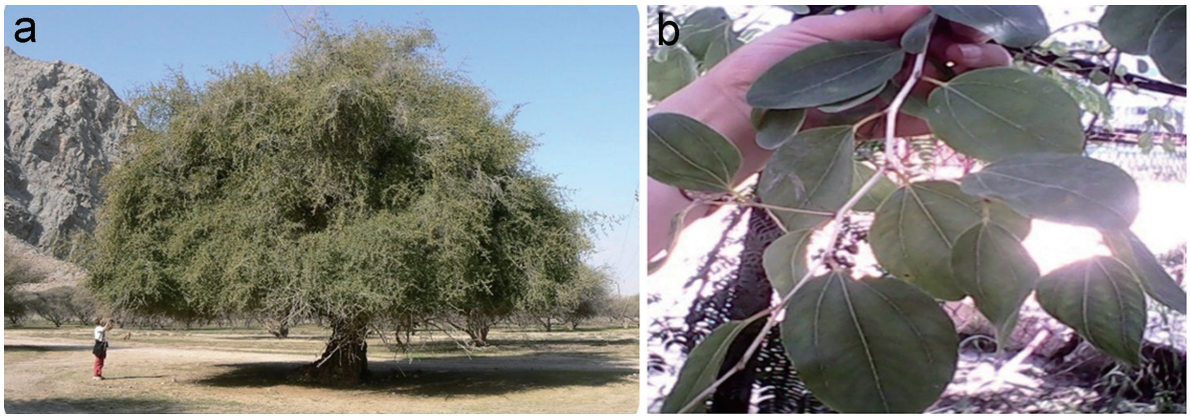

Fig. 1. *Z. spina-christi* tree (a) and leaves (b) in Tunisian oasis.

of their ability to reduce cholesterol levels and ischemic damage.[4]

Among the different *Ziziphus* plants in Tunisia, *Ziziphus spina-christi* (*Z. spina-christi*), known as 'sidra', is habitually grown in Tunisia's oases, due to its greater pivoting root growth. This green tree belongs to the Rhamnaceae family and can continue to grow for over 900 years.[5] *Z. spina-christi* leaves are considered to have antiinflammatory, hypoglycemic, hypotensive, antinociceptive, and antitumoral effects.[6–10] A previous study identified that different *Ziziphus spina-christi* organs from Tunisian regions contain phenolic compounds[1] and the plant's roots are the most studied plant part.[11] However, there is no information on whether Z. *spina-christi* methanolic leaf extracts contain a primordial component, such as rutins. These components, known as vitamin P, are the most common flavonol glycol-sides and have potential to be developed as new medicines. This flavonol glyco-sides have antioxidant, cytoprotective, vasoprotective, anti-inflammatory, antibacterial, antiviral, antiulcerogenic, neuroprotective, cardioprotective, hepatoprotective, and nephroprotective propertiess.[12] The leaf extracts contain flavonoids, alkaloids, saponins, lipids, proteins, and free sugar.

In Tunisia, the biological activities of *Ziziphus spina-christi* leaves should be further studied for their anti-inflammatory, hypoglycemic, hypotensive, and antinociceptive effects.[13] The present study aimed to investigate the antioxidant, antimicrobial, and insecticidal activities of leaf extracts.

Materiels and methods

Plant materials

Z. spina-christi leaves (Fig. 1) were obtained from Metlaoui, Degueche, Tozeur, and Nefta of Tunisia in April 2018.

The *Z. spina-christi* trees were identified by a specialist at the National Institute of Researches on Rural Engineering, Water and Forests, and a voucher specimen them was deposited at the Herbarium of INRGREF (Tunisia). Leaves were collected and dried at room temperature for 15 days in a dry and airy environment. The dried leaves were ground using a mill equipped with a grid with a hole size of 1.00 mm in diameter. The generated powders were stored in plastic bags in the dark until chemical analysis. The leaf powder samples (1 g each) were macerated for 30 min in 10 ml of methanol (70%). The extracts were filtered through Whatman N°1 filter paper, pooled, and concentrated under a vacuum.

Chemical reagents

Folin-ciocalteu, phenol, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, catechin, sodium carbonates, hydrochloric acid, rutin standard, and all solvents were purchased from Sigma-Aldrich (St. Louis, MO, USA). All solvents and reagents used were of the highest purity.

Total phenol, flavonoid, and tannin contents

The total phenolic content in the leaf extracts was determined using the Folin-Ciocalteu method.[14] Briefly, 0.5 mL of a methanolic solution was mixed with 2.5 mL of Folin- Ciocalteu reagent and 2 mL of sodium carbonate (75 g/L) solution. After incubation for 30 min, the absorbance was measured at 765 nm using a Shimadzu 1600-UV spectrophotometer. The same procedure was repeated using gallic acid as a standard. Total phenols of each fraction, expressed in mg GAE/g dry weight (DW), were measured using the regression equation of a calibration curve $y = 0.0114x + 0.518$, $r^2 = 0.9932$. The flavonoid contents were measured by mixing 1 mL of $AlCl_3$ and 1 mL of plant extract, and incubated at room temperature for 40 min. Absorption was measured at 420 nm using a Shimadzu UV-1600 spectrophotometer. The results are expressed as mg quercetin equivalent per gram dray weight (mg QE/g DW). All experiments were carried out in triplicate. In contrast, the condensed tannins were tested calorimetrically as described by Earp et al.[15] The condensed tannins in the methanolic extracts are expressed as mg catechin equivalents per g of dry weight (mg CE. g^{-1} DW).

LC-ESI-MS analysis

Quantitative analysis of phenolic compounds was performed using the LC-MS 2020 system (Shimadzu, Tokyo, Japan) associated with an electro-spray ionization source (ESI). Briefly, practicals of 3 µm and pore size of 130 Å were eluted using a glass capillary C18 column (150×4.6 mm) (Phenomenex, Torrance, CA, USA). The mobile phase was set with a flow rate of 6 mL/min, and contained 0.5% formic acid in H_2O, v/v (solvent A) and acetonitrile (solvent B). The gradient elution was 90:10 (A: B, v/v) during 0–5 min, then it decreased to 86:14 (A: B, v/v) (during 5–7 min), and was held at 86:14 (A: B, v/v) from 7–17 min, before falling from 86:14 to 75:25 (A: B, v/v) during 17–19 min (linear gradient). Finally, the elution was held at 75:25 (A: B, v/v) and decreased to

10:90 (A: B, v/v) (linear gradient) during 19–24 min; 25–31 min held at 10:90; 31–32 min from 10:90 to 90:10; and 32–40 min held at 90:10 (reequilibration step). The injection volume was 20 µL at 40 °C temperature. High-purity (>99%) nitrogen was used as a nebulizer and an auxiliary gas. The spectra were monitored in selected ion monitoring (SIM) mode and processed using Shimadzu Lab Solutions LC-MS software. The mass spectrometer was operated in negative ion mode with a capillary voltage of −3.5 V, a dry gas flow rate of 12 L/min, a nebulizing gas flow rate of 1.5 L/min, a block source temperature of 400 °C, a dissolving line temperature of 250 °C, a 1.2 V voltage detector, and the full scan spectra from 50 to 2,000 m/z. Rutin was identified by comparing the retention time with those of the standard, and the content was calculated using the calibration curve by plotting the peak area against the concentration of the respective standard. The data were reported with convergence limits in triplicate.

Biological activities

Antioxidant activity

The *in vitro* antioxidant activity of leaf extract samples was examined using the 2, 2-diphenyl-1-picrylhydrazyl radical, DPPH method.[9] The different concentrations of oil extracts (2.5 to 50.0 mg/mL) were prepared and incubated (V/V) at 25 °C for 30 min with standard or DPPH solution (2.36 mg in 100 mL of ethanol (70%)). The absorbance was measured at 734 nm. The concentration for 50% inhibition (IC_{50}) was estimated.[16] Tests were carried out in triplicate. The percent inhibition of the DPPH radicals by the samples was calculated according using the following formula:

$$\text{Scavenging effect (\%)} = \frac{A_0 - A_1}{A_0} \times 100,$$

where A_0 is the absorbance of the blank sample; and A_1 is the absorbance in the presence of the sample.

Antimicrobial activity

Agar diffusion method

The methanol extracts of *Z. spina-christi* leaves were dissolved in sterile water. Each extract solution was diluted to 50 µg/mL and sterilized by filtration through a 0.2 µm pore size filter. The antibacterial and antifungal activities were determined using the agar well diffusion method as described by Thakur *et al*.[17] The tested pathogens included *Streptococcus agalactiae*, *Staphylococcus aureus*, and *Escherichia coli*. Three *Candida* species – *Candida albicans*, *Candida sake*, and *Candida parapsilosis* – were included. The broth microdilution assay with sterile Mueller-Hinton media (BioRad, France) was used for bacterial strains and yeast malt extract agar (Bio-Rad, France) was used for antifungal tests. A fresh cell suspension (0.1 mL) adjusted to 10^7 CFU/mL for bacteria and 10^5 cells/mL for fungi was inoculated onto the surface of agar plates. The agar plates were punched with wells at a 6-mm diameter and the wells were filled with 30 µL of the extract dilution and culture medium. Negative control wells were filled with 30 µL of sterile water. The plates were allowed to stand for 40 min at 4 °C to permit extract diffusion and then incubated at 37 °C for 24 h for bacteria and at 48 h for yeast. Antibacterial and antifungal activities were evaluated by measuring the zones of inhibition (clear zone around the well) against the microorganisms. All tests were repeated three times.[18]

Determination of minimum inhibitory concentration (MIC)

The MIC of each extracted sample was determined using the microdilution broth method. MIC values were estimated visually by the absence of turbidity as previously reported by Khémiri *et al*.[19]

Insecticidal activity

Insecticidal assays of plant extracts were carried out at the National Agricultural Research Institute of Tunisia using *Tribolium castaneum* as a model insect. Experiments were accomplished in 25 ± 1 °C and 65 ± 5% relative humidity in the dark. Ten insects of mixed sex at 7–10 days were used for bioassay tests according to the method of Pascual-Villalobos and Robledo.[20]

Insects were treated with *Z. spina-christi* extracts (2 mg extract or control), transferred to Petri dishes, and kept at room culture at 25 ± 1 °C. The number of dead insects was observed and recorded every six hours. The percent of dead insects was calculated using the Abbott's[21] formula:

$$\text{Mortality (\%)} = \frac{Mt - Mc}{100 - Mc} \times 100$$

with Mt = % mortality in treated and Mc = % mortality in controls.

The median lethal concentrations (LC_{50} and LC_{95}) were calculated according to the Probit method.[22]

Statistical analysis

Results were statistically analyzed using STATISTICA (Statsoft, 1998). Each data presented was mean of three replicates (±standard deviation). Multiple mean comparisons were performed using the Student-Newman Keuls test with a significance level of $p < 0.05$.

Results

Phenolic content of Z. spina-christi leaf extracts

The contents of total phenolic (TPC), total flavonoids (TFC), and condensed tannin (CT) in *Z. spina-christi* leaf extracts from three Tunisian provenances are shown in Table 1.

Identification and quantification of phenolic compounds in Ziziphus spina-christi leaf extracts

LC-MS analyses identified that rutin was the major flavonoid compound present in *Z. spina-christi* leaf extracts (Fig. 2) and other phenolic compounds varied among these provenances. The rutin values varied from 1.3 ± 0.005 µg/mL to 3 ± 0.005 µg/mL. The leaf extracts from Tozeur provenance exhibited the highest rutin (Table 2). The contents were more important than those of *Ziziphus jujuba* leaf extracts (1.91 mg/g).

Biological activities of Ziziphus spina-christi

Phenolic compounds from Tunisian *Z. spina-christi*, particularly from its roots have antibacterial, antifungal, and antioxidant properties.[23] The most identified phenolic compound was epicathechin, which can protect organisms against oxidative stress caused by re-

Table 1. Biochemical composition of *Z. spina-christi* leaves

Provenance	TPC (mg GAE/g DW)	TFC (mg QE/g DW)	CT (mg CE/g DW)
Metlaoui	6.08 ± 0.06[a]	2.22 ± 0.03[b]	0.925 ± 0.003[a]
Tozeur	8.16 ± 0.05[b]	4.42 ± 0.03[c]	0.82 ± 0.007[b]
Dgueche	8.04 ± 0.04[b]	1.96 ± 0.01[a]	1.62 ± 0.001[c]

Data are mean values of three measurements. The confidence intervals were calculated at the threshold of 5%. For each column, values with the same alphabetical letter indicate no significant difference at 5% by the Duncan test. [a]$p < 0.05$; [b]$p < 0.005$, [c]$p < 0.005$. DW, dry weight; GAE, gallic acid equivalent; QE, quercetin equivalent; CE, catechin equivalents.

active oxygen and nitrogen species.[11]

Antioxidant activity

The results indicated that the reduction ability of DPPH radical was determined by the decrease in absorbance induced by plant antioxidants. The scavenging effects of each extract sample on the DPPH radical are expressed as IC_{50} values in Table 3. The IC_{50} values were 0.125 mg/mL (Tozeur provenance) and 1 mg/mL (Dgueche provenance), respectively. The radical scavenging properties of natural products are frequently linked with their ability to shape stable radicals. Superior antioxidant activity was observed from leaf extracts from Tozeur and Metlaoui provenances.

The results indicated that all leaf extracts of *Z. spina-christi* from different Tunisian provenances had high antioxidant activity. Statistical analyses revealed that this composition varied significantly among the extracts from different provenances (Fig. 3). The leaf extracts from Tozeur provenance exhibited the highest values.

Antimicrobial activity

Overall, the leaf extracts from Tozeur provenance had superior antibacterial potential against the tested bacteria species (Fig. 4). The results indicated that the tested extracts were able to inhibit *Streptococcus agalactiae* with inihibitory zone varying between 19.5–27.5 mm and the highest activtiy was obtained by the extract from Tozeur provenance. The Gram-negative bacteria species belonging to *E. coli* were sensitive to Dgueche and Tozeur extracts with 13.5 and 11.5 mm inhibitory zones, respectively. Only extracts from the Tozeur provenance were able to inhibit the growth of *Staphylococcus aureus* with an inhibiotry zone diameter 16 mm (Fig. 5). Numerous reports have shown that antibacterial potential is related to the bacteria strain species tested, and the composition of the extract can be influenced by many factors such as geographical region, plant organs, season, extraction methods, and solvent.

The antifungal activities of three extracts were analyzed using three *Candida* species. The results indicated the highest antimicrobial potential of the extract from Tozeur provenance, which was the only extract that effectively inhibited the growth of two *Candida* clinical species – *Candida albicans* and *Candida sake* – with a high zone of inhibition of 11.5 mm and 21 mm, respectively. There was no extract tested to inhibit the growth of *Candida parapsilosis* specie. The extract from Dgueche provenance was effective against *Candida albicans* specie with a inhibitory zone diameter of 12 mm (Fig. 5).

The same MIC value of 25 µg/mL was obtained for antifungal activity as well as for antibacterial activity against *Streptococcus agalactiae* specie. The extract from Tozeur had superior CMI values of approxiamatley 6.25 µg/mL; the extract from Tozeur had potent antifungal activity (Table 4).

Insecticidal activity

The insecticidal activities of different doses (1 mL, 500 µL, and 200 µL) of three extracts against *Tribolium castaneum* (*T. castaneum*) were evaluated. Treatment with individual extracts from Degueche, Tozeur, and Metlaoui at 500 µL for 72 h caused *T. castaneum* mortality of 60.66%; 77.33%, and 100%, respectively (Fig. 6).

Discussion

Our data indicate that *Ziziphus spina-christi* leaf extracts from different provenances have varying biological activities. This variability may reflect the influence of geographical conditions (humidity, temperature, and altitude), soil-growth conditions, and origin and period of leaf harvest.[23] A previous study reported that the total phenolic content of *Z. spina-christi* leaf extracts differs significantly among the various extraction methods (ethanolic, aqueous extracts).[24] In addition, *Z. spina-christi* leaf extracts contain higher levels of phenolic compounds than that in *Z. jujuba* leaf extracts, with values of 3.97 and 6.04 mg GAE/g, respectively.[25]

Rutin was identified as the major flavonoid compound in *Z. spina-christi* leaf extracts. Our data are in disagreement with a previous report that identified luteolin and quercetin as the major compounds in *Z. spina-christi* leaf extracts.[9] These compositions differ among the specie. In fact, chlorogenic acid was identified as the major phenolic compound in *Z. lotus*, while caffeic and salviolinic acids were detected in *Z. mauritiana* samples.[26] Another study reported a correlation between total phenol, flavonoid, and condensed tannin contents and antioxidant activities.[27] Thus, our findings support the application of this kind of extract in pharmacology and cosmetic industries.

The results revealed that rutin was the richest compound in the leaf extracts from Tozeur. However, we did not detect quercetin in the extracts, but we did detect antifungal activity of these *Z. spina-christi* leaf extracts.[18] In addition, we detected strong antibacterial activities of these extracts against *Staphylococcus aureus* and *E. coli*, and the activities were stronger than a recent report.[28]

A previous study reported that *Ziziphus* leaf extracts contain alkaloids, saponins, tannins, glycosides, flavonoids, and terpenoids.[29] In our study, we provide evidence that the *Z. spina-christi* leaf extracts contain the major compound of rutin, which had potent antibacterial and antifungal activities because the amount of rutin in the extracts correlated with the antibacterial and antifungal activities. The efficiency of the specific phytochemical composition in the extracts was also confirmed by comparing the ethanolic with methanolic extracts.[30] More importantly, the MIC values from our studies ranged from 25–6.5 µg /mL, which were more efficient than that in a previous report.[29]

We also observed that the *Z. spina-christi* leaf also had high insecticidal activities, although a previous study reported that the n-hexane and ethyl acetate fraction in *Zizyphus oxyphylla* had moderate insecticidal activity against *Callosobruchus analis*.[31] To

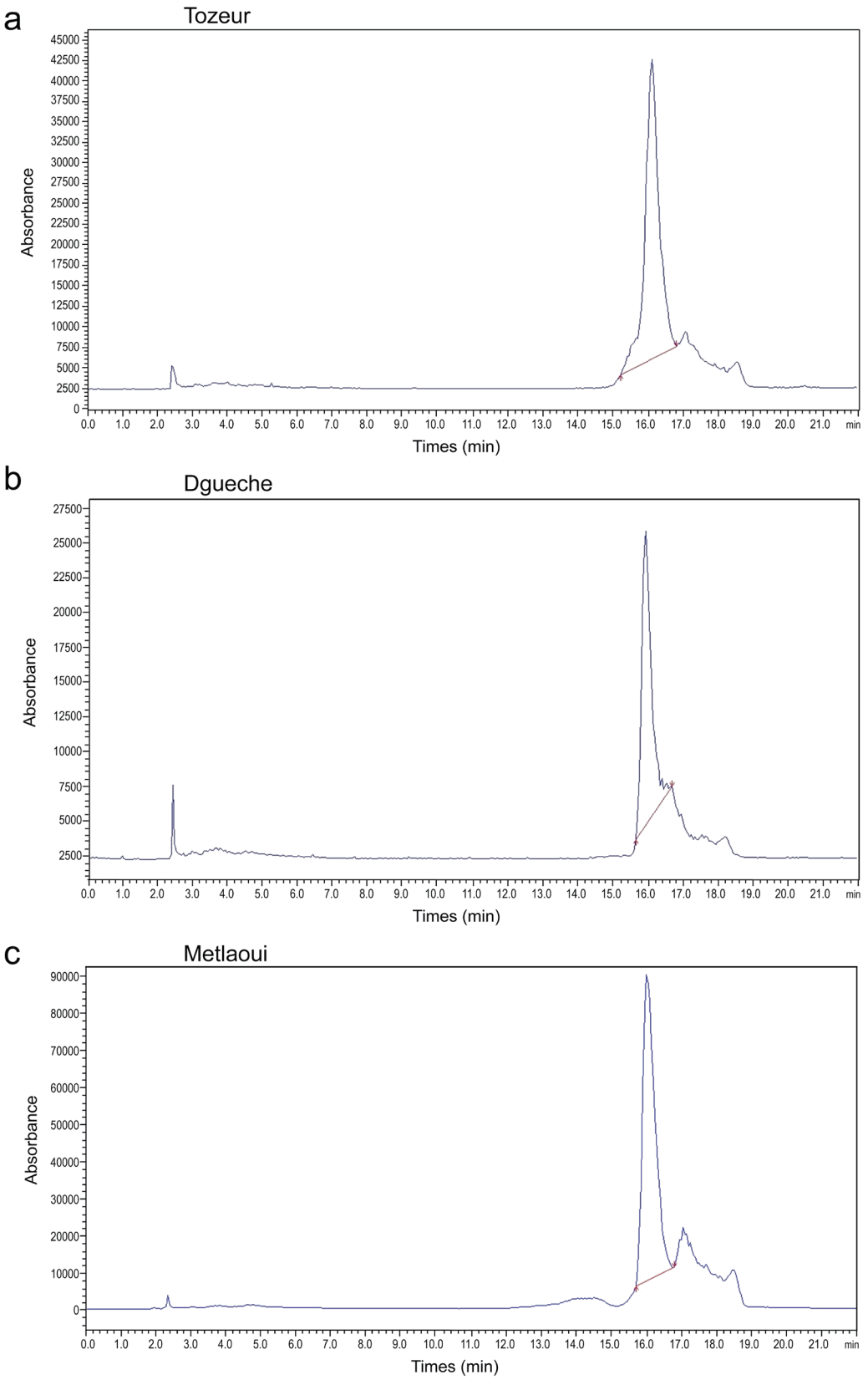

Fig. 2. Identification and quantification of phenolic compounds in *Z. spina-christi* leaf extracts using LC-ESI-MS analysis.

Table 2. Rutin amounts (µg/mL) in *Z. spina-christi* leaf extracts

Provenance	RT	Quantity (µg/mL)
Metlaoui	15.85 ± 0.03	2 ± 0.005
Tozeur	16.06 ± 0.05	3 ± 0.005
Dgueche	15.93 ± 0.004	1.3 ± 0.005

Data are mean values of three measurements. The confidence intervals were calculated at the threshold of 5%. For each column, values with the same alphabetical letter indicate no significant difference at 5% by the Duncan test.

Table 3. Inhibitory concentrations of *Z. spina-christi* leaf extracts using the free radical scavenging activity (DPPH) method

Provenances	Metlaoui	Tozeur	Dgueche
DPPH radical-scavenging (mg/ml)	0.5 ± 0.00^{b}	0.125 ± 0.01^{a}	1 ± 0.02^{c}

Data are mean values of three measurements. The confidence intervals were calculated at the threshold of 5%. For each column, values with the same alphabetical letter indicate no significant difference at 5% by the Duncan test. $^{a}p < 0.05$; $^{b}p < 0.005$, $^{c}p < 0.005$

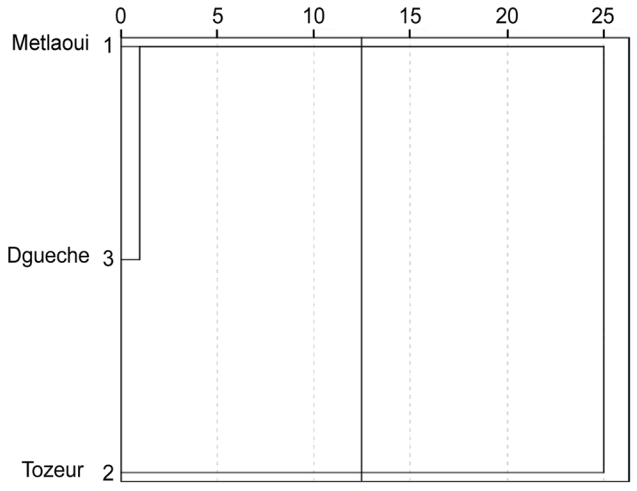

Fig. 3. Dendrogram to compare *Z. spina-christi* leaf extracts from three provenances.

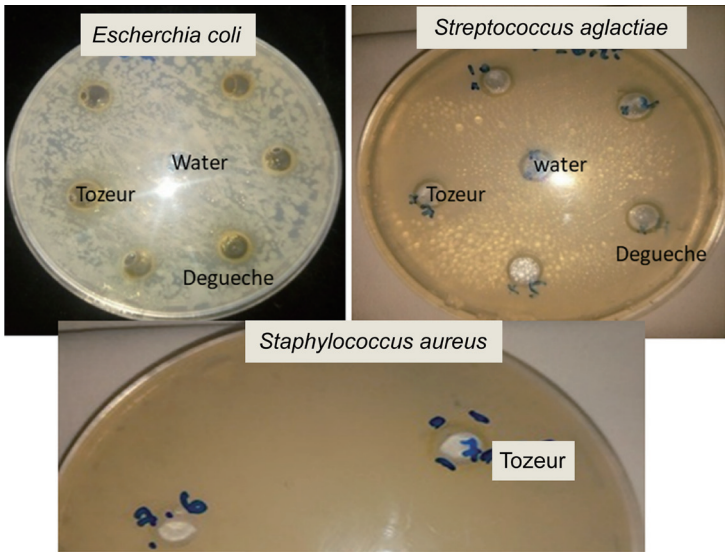

Fig. 4. Agar well diffusion method analysis of antibacterial activities of three *Z. spina-christi* extracts.

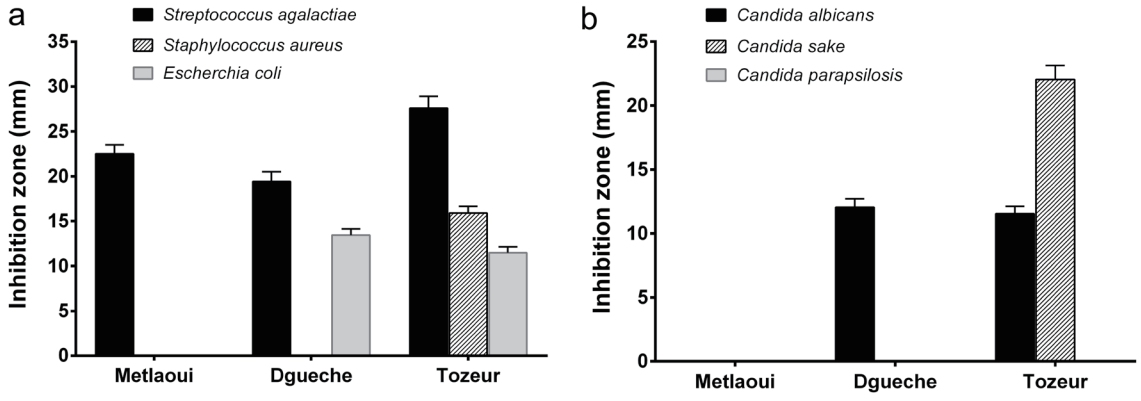

Fig. 5. Antibacterial (a) and antifungal (b) activities of methanolic extracts of three *Z. spina-christi* extracts analyzed using the agar well diffusion method. Values indicate the diameters of zone inhibition (mm).

Table 4. MIC comparison of various extracts against bacteria and *Candida* expressed in µg/mL

Extracts	*MIC (µg/mL)*				
	Candida albicans	***Candida sake***	***Streptococcus agalactiae***	***Eschcherchia coli***	***Staphylococcus aureus***
Tozeur	*25*	*25*	*25*	*6, 25*	*6, 25*
Dguech	*25*	–	*25*	*25*	*25*
Metlaoui	*25*	*25*	*25*	*25*	*25*

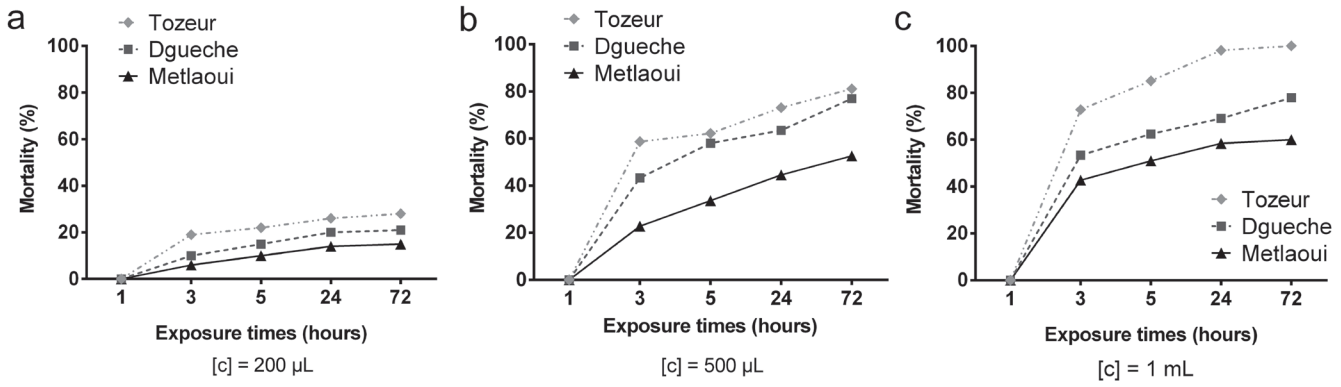

Fig. 6. The methanolic leaf extracts of *Z. spina-christi* displayed insecticidal activities that resulted in *Tribolium castaneum* mortality.

the best of our kownledge, our data study is the first report on the insecticidal activity of the *Z. spina-christi leaf* extracts, supporting the potential application of these extracts for industrial use.

Future directions

In this study, we sought to optimize our experiments in order toval-oriz e our *Z. spina-christi* extracts and demonstrate their potential use as synthetic insecticides or natural antioxidants in the cosmetics industry. We hope also to substantiate the therapeutic uses of the extracts and to enhance *Z. spina-christi* fruit trees growth in gardens and fields.

Conclusions

The present study highlights the importance of *Z. spina-christi* leaf extracts in medicinal and pharmaceutical industries. Due to their richness of phenolic contents, these extracts may be used as antioxidant, insecticidal, and allelopathic agents. *Z. spina-christi* leaves may be a good candidate for food and pharmaceutical supplement formulations, as well as industrial uses.

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None.

Conflict of interest

The authors have no conflicts of interest related to this publication.

Author contributions

Conceived of the presented idea (EM and AL), carried out the experiment (ME, BE, SH, JBJ, RBN), contributed to the interpretation, discussed the results and co-wrote the manuscript (ME, BE, contributed to English revision of the manuscript (ME, BE and RDC), contributed to statistical analysis (BS), contributed to the final manuscript (YA and AL)

Data sharing statement

No additional data are available.

References

[1] Elaloui M, Ghazghazi H, Ennajah A, Manaa S, Guezmir W, Karray NB, *et al*. Phenolic profile, antioxidant capacity of five *Ziziphus spina-christi* (L.) Willd provenances and their allelopathic effects on *Trigonella foenum-graecum* L. and *Lens culinaris* L. seeds. Nat Prod Res 2017; 31(10):1209–1213. doi:10.1080/14786419.2016.1226830, PMID:27618365.

[2] Makhawi AM, Mustafa MI, Uagoub HA. Phytochemical screening and antimicrobial activity of *Ziziphus spina-christi* stem barks. bioRxiv [Preprint] 2020:2020.02.24.963157. doi:10.1101/2020.02.24.963157.

[3] Prakash P, Kumari N, Gayathiri E, Selvam K, Ragunathan MG, Chandrasekaran M, *et al*. In vitro and in silico toxicological properties of natural antioxidant therapeutic agent *Azimatetracantha*. LAM. Antioxidants (Basel) 2021;10(8):1307. doi:10.3390/antiox10081307, PMID: 34439555.

[4] Kim JT, Chung IM, Kim MJ, Lee JS, Son BY, Bae HH, *et al*. Comparison of antioxidant activity assays in fresh purple waxy corn (*Zea mays* L.) during grain filling. Appl Biol Chem 2022;65:1. doi:10.1186/s13765-021-00671-w.
[5] Laamouri A. Contribution to the study of jujube trees in Tunisia: Identification, characterization, adaptation to water deficit and multiplication. National Agronomic Institute. [Dissertation]. Tunisia: Cartage University; 2009.
[6] Moustafa MF, Hesham AE, Quraishi MS, Alrumman SA. Variations in genetic and chemical constituents of *Ziziphus spina-christi* L. populations grown at various altitudinal zonation up to 2227 m height. J Genet Eng Biotechnol 2016;14(2):349–362. doi:10.1016/j.jgeb.2016.09.001, PMID:30647633.
[7] Bukar AM, Kyari MZ, Gwaski PA, Gudusu M, Kuburi FS, Abadam YI. Evaluation of phytochemical and potential antibacterial activity of *Ziziphus spina-christi* L. against some medically important pathogenic bactevria obtained from University of Maiduguri Teaching Hospital, Maiduguri, Borno State-Nigeria. J Pharmacol Phytochem 2015;3(5):98–101.
[8] Kadioglu O, Jacob S, Bohnert S, Naß J, Saeed ME, Khalid H, *et al*. Evaluating ancient Egyptian prescriptions today: Anti-inflammatory activity of *Ziziphus spina-christi*. Phytomedicine 2016;23(3):293–306. doi:10.1016/j.phymed.2016.01.004, PMID:26969383.
[9] Manar PA, Utama SEP, Hasan GM, Hidayat MS. The potential of Sarang Semut (*Myrmecodia* spp) as medicinal plants. IOP Conf Ser: Earth Environ Sci 2019;394:012032. doi:10.1088/1755-1315/394/1/012032.
[10] Basuny AM, Arafat MS, Soliman HM. Chemical analysis of olive and palm pollen: Antioxidant and antimicrobial activation properties. Wudpecker J Food Technol 2013;1:14–21.
[11] Elaloui M, Hamdi SH, Ghazghazi H, Ben Nasr RB, Boushih E, Ammari Y, *et al*. Characterization of epicathechin contents in the *Ziziphus spina-christi* L. root extracts using LC-MS analyses and their insecticidal potential. Plant Biosyst 2020;155(4):685–690. doi:10.1080/11263504.2020.1779837.
[12] Chen W, Wang S, Wu Y, Shen X, Xu S, Guo Z, *et al*. The physiologic activity and mechanism of quercetin-like natural plant flavonoids. Curr Pharm Biotechnol 2020;21(8):654–658. doi:10.2174/1389201021666200212093130, PMID:32048963.
[13] Dkhil MA, Kassab RB, Al-Quraishy S, Abdel-Daim MM, Zrieq R, Abdel Moneim AE. *Ziziphus spina-christi* (L.) leaf extract alleviates myocardial and renal dysfunction associated with sepsis in mice. Biomed Pharmacother 2018;102:64–75. doi:10.1016/j.biopha.2018.03.032, PMID:29549730.
[14] Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. Am J Enol Vitic 1965;16:144–158.
[15] Earp CF, Akingbala JO, Ring SH, Rooney LW. Evaluation of several methods to determine tannins in sorghums with varying kernel characteristics. Cereal Chem 1981;58:234–283.
[16] Fu R, Zhang Y, Guo Y, Liu F, Chen F. Determination of phenolic contents and antioxidant activities of extracts of Jatropha curcas L. seed shell, a by-product, a new source of natural antioxidant. Ind Crops Prod 2014;58:265–270. doi:10.1016/j.indcrop.2014.04.031.
[17] Thakur R, Srivastava M. A study on the impact of consumer risk perception and innovativeness on online shopping in India. Int Rev Retail 2015;43(2):148–166. doi:10.1108/IJRDM-06-2013-0128.
[18] Essghaier B, Dhieb C, Rebib H, Ayari S, Rezgui A, Boudabous A, *et al*. Antimicrobial Behavior of Intracellular Proteins from Two Moderately Halophilic Bacteria: Strain J31 of *Terribacillus halophilus* and Strain M3-23 of *Virgibacillus marismortui*. J Plant Pathol Microb 2014;5(1):214. doi:10.4172/2157-7471.1000214.
[19] Khemiri L, Larsson H, Kuja-Halkola R, D'Onofrio BM, Lichtenstein P, Jayaram-Lindström N, *et al*. Association of parental substance use disorder with offspring cognition: a population family-based study. Addiction 2020;115(2):326–336. doi:10.1111/add.14813, PMID:31503371.
[20] Pascual-Villalobos MJ, Robledo A. Anti-insect activity of plant extracts from the wild flora in southeastern Spain. Biochem Syst Ecol 1999;27(1):1–10. doi:10.1016/S0305-1978(98)00051-9.
[21] Abbott WS. A method of computing the effectiveness of an insecticide. J Economic Entomol 1925;18(2):265–267. doi:10.1093/jee/18.2.265a.
[22] Finney Jr EE. Random vibration techniques for non-destructive evaluation of peach firmness. Acad J Agric Res 1971;16(1):81–87. doi:10.1016/S0021-8634(71)80007-0.
[23] Dharajiy D, Shah M, Bajpai B. Biosorption of acid black 52, an azo dye from aqueous solution using pre-treated biomass of Aspergillus fumigatus A23. Poll Rese 2015;34(4):667–676.
[24] Khaleel SM, Jaran AS, Haddadin MS. Evaluation of total phenolic content and antioxidant activity of three leaf extracts of *Ziziphus spina-christi* (Sedr) grown in Jordan. J Adv Med Med Res 2016;14(6):1–8. doi:10.9734/BJMMR/2016/24935.
[25] Rigane G, Ben Younes S, Ghazghazi H, Ben Salem R. Investigation into the biological activities and chemical composition of *Calendula officinalis* L. growing in Tunisia. Int Food Res J 2013;20(6):3001–3007.
[26] Yahia Y, Benabderrahim MA, Tlili N, Bagues M, Nagaz K. Bioactive compounds, antioxidant and antimicrobial activities of extracts from different plant parts of two *Ziziphus Mill*. species. PLoS One 2020;15(5):e0232599. doi:10.1371/journal.pone.0232599, PMID:32428000.
[27] Badr SF, El-Sherif NA, Ii S, El-Deeb M, Al-Shinqiti FYM. Evaluation of genetic variation, antioxidant and antibacterial activities of two Sidr varieties in Medina. Pak J Pharm Sci 2020;33(1):61–69. PMID:32122832.
[28] Salih RRM, Gibreel HH, Ahmed DM, Hammad ZM, Ahmed AI. Antimicrobial effect of aqueous and ethanolic leaves extracts of *Ziziphus* species against Animal Bacterial Pathogens. Curr Trends Forest Res 2019;3(2):10–36. doi:10.29011/2638-0013.101036.
[29] Ads EN, Rajendrasozhan S, Hassan SI, Sharawy SMS, Humaidi JR. Phytochemical, antimicrobial and cytotoxic evaluation of *Ziziphus spina-christi* (L.) stem bark. Biomed Res 2017;28(15):56–64.
[30] Al-Mutairi MH, Ali S, Aly DS, Aldebasi Y. Antibacterial activity of Sider (*Ziziphus spina-christi*), leaves extract against selected pathogenic bacteria. Europ J pharma Med Research 2016;3(5):138–144.
[31] Nisar M, Kaleem WA, Qayum M, Hussain A, Zia-Ul-Haq M, Ali I, *et al*. Biological screening of *Zizyphus oxyphylla* edgew leaves. Pak J Bot 2010;42(6):4063–4069.