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Original Research

Environmental and Biological Evaluation of *Ziziphus spina-christi* Aqueous Extracts: Phytochemical, Antioxidant, and Cytotoxic Perspectives

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Abstract

Ziziphus spina-christi, a medicinal plant widely used in traditional medicine, is known for its rich phytochemical composition and diverse therapeutic properties. This study investigates the phytochemical profile, antioxidant activity, and cytotoxic effects of the aqueous extract of *Z. spina-christi*. Phytochemical analysis revealed high concentrations of phenolics (105.48±0.42 GAE/g), tannins (99.02±0.45 GAE/g), flavonoids (32.39±0.15 QE/g), and flavonols (24.10±0.21 QE/g), indicative of its bioactive potential. Antioxidant assays demonstrated significant free radical scavenging activity, with IC_{50} values of 15.11±2.31 µg/mL (DPPH) and 18.30±0.11 µg/mL (ABTS). Cytotoxicity testing against Caco-2 cells showed moderate inhibitory effects with an IC_{50} of 209.245±8.32 µg/mL, suggesting potential anticancer activity. UV-vis and zeta potential analyses supported the stability and presence of phenolic compounds, while the IR spectrum highlighted hydroxyl and carbonyl functional groups. A comparative discussion with previous studies revealed variations in bioactivities based on dosage, duration, and extraction methods, emphasizing the dual nature of the plant as both a therapeutic agent and a potential toxicant at higher doses. These findings reinforce the promise of *Z. spina-christi* as a source of natural antioxidants and cytotoxic agents while underscoring the need for rigorous safety assessments for its clinical use.

Keywords: *Ziziphus spina-christi*, aqueous extract, phytochemical composition, antioxidant activity, cytotoxic effects, phenolics, flavonoids, Caco-2 cells

Introduction

Z. spina-christi, commonly referred to as Sidr, is a member of the Rhamnaceae family, native to the Middle East, North Africa, and parts of Asia [1]. Renowned for its resilience in arid environments, this species has been an integral part of traditional medicine, with various plant parts – including leaves, fruits, seeds, and bark – being utilized for their purported therapeutic properties [2]. The plant has been reported to exhibit a wide range of pharmacological activities, including antimicrobial, anti-inflammatory, antioxidant,

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and anticancer effects [3]. Despite its long-standing traditional use, the scientific exploration of its bioactive potential, particularly in aqueous extracts, remains an area of active investigation.

The phytochemical composition of *Z. spina-christi* is a rich repository of secondary metabolites [4]. Studies have identified the presence of flavonoids, alkaloids, tannins, saponins, and phenolic acids, many of which are known to exhibit significant biological activities [5]. The aqueous extraction of these compounds, which aligns with traditional preparation methods, offers a pragmatic and potentially effective way to harness their medicinal properties [6]. However, the specific profile and bioactivity of the aqueous extract need detailed elucidation to validate and optimize its therapeutic applications.

Oxidative stress, implicated in a host of chronic diseases, arises from an imbalance between the production of reactive oxygen species (ROS) and the body's ability to neutralize them [7]. Antioxidants from natural sources, such as plant-derived phenolics and flavonoids, are pivotal in mitigating oxidative damage [8]. *Z. spina-christi*, with its abundance of these compounds, has been shown to possess robust antioxidant potential [9-11]. Nevertheless, the contribution of its aqueous extracts to antioxidant activity remains underexplored and warrants detailed investigation.

Cytotoxic activity is another promising aspect of *Z. spina-christi* bioactivity. Initial studies have indicated the plant's potential to inhibit the growth of cancer cells [12], an effect attributed to the presence of bioactive molecules like betulinic acid and other triterpenoids [13]. However, most research has been conducted on organic solvent extracts [14], leaving a significant knowledge gap regarding the cytotoxic effects of aqueous extracts.

This study aims to bridge these gaps by evaluating the phytochemical composition, antioxidant activity, and cytotoxic effects of aqueous extracts from *Z. spina-christi*. By focusing on aqueous extracts, the research aligns with traditional medicinal practices while advancing the scientific understanding of the plant's therapeutic potential. The findings are expected to contribute to the growing body of evidence supporting the medicinal use of *Z. spina-christi* and to pave the way for its application in the development of novel therapeutic agents.

Materials and Methods

All animal procedures and treatments were conducted according to ethics committee guidelines at the Zoology Dept., College of Science, King Saud University, Riyadh, Saudi Arabia.

Botanical Material and Extraction Methodology

Leaves of *Z. spina-christi* were collected in the Dammam region (26.43°N, 50.1°E). A taxonomist from the Department of Botany at King Saud University authenticated the botanical identity of the leaves. Twenty grams of *Z. spina-christi* leaves were soaked in distilled water (200 mL) at 30ºC with shaking at 150 rpm for 24 h. Five grams of *Z. spina-christi* were processed using a Soxhlet apparatus. The suspensions were filtered using Whatman filter paper, and the residue was re-extracted and re-filtered as described above. The two filtrates were combined and evaporated in a rotary evaporator at 40ºC, as in our previous studies [15, 16].

Chemicals

The accompanying reagents and standards were procured from Sigma-Aldrich (St. Louis, MO, USA): DPPH, ABTS, linoleic acid, pyrogallol, gallic acid, Folin–Ciocalteu reagent, resorcinol, methyl gallate, catechin, 2-hydroxycinnamic acid, ellagic acid, quercetin, and cinnamic acid. All other compounds employed were of analytical grade.

Gas Chromatography – Mass Spectrometry Analysis (GC-MS)

The autosampler on the GC-MS QP2010 Ultra system, which is integrated with the Shimadzu GC-2010 Plus, was used to inject a 2 µL sample. Chemicals were located using programs that have their own database (NIST). A 100 m Rt-2560 capillary column (Restek) was used for separation, which had an internal diameter of 0.25 mm and a film thickness of 0.2 µm. With an input temperature of 250ºC and a split ratio of 50:1, helium was utilized as the carrier gas at a flow rate of 1 mL/min. During its 81 min of operation, the oven temperature increased from 50ºC to 250ºC. The MS detection parameters were as follows: an acquisition scan ranging from 40 to 500 g/mol, a scan speed of 1.56, a solvent delay of 9 min, and a source temperature of 230ºC [17].

IR Spectrum

A small amount of the dried extract was combined with potassium bromide (KBr) powder in a 1:100 ratio and finely ground to ensure a uniform consistency. The mixture was subsequently pressed into a thin, transparent pellet with the aid of a hydraulic press. The pellet, which held the sample, was positioned in the sample holder of the FTIR spectrometer, and the spectrum was recorded across the range of 4000-400 cm^{-1} with a resolution of 4 cm^{-1} (PerkinElmer Paragon 500, USA). The spectrum obtained was examined to pinpoint particular absorption bands linked to various functional groups found in the fenugreek seed extract [17].

Phytochemical Analysis: Determination of Total Phenol

Through the utilization of the Folin-Ciocalteu colorimetric method, we were able to ascertain the total amount of Lipo-phenolic Eucam that was present overall. The test was conducted by transferring 100 µL of either the gallic acid standard or the Lipo-Eucam ethanol solution into two Eppendorf tubes with a capacity of 1 mL each. Following that, distilled water was used as a blank, and 200 µL of a 10% Folin-Ciocalteu reagent was introduced into the mixture. After a brief vortexing, the tubes were allowed to incubate in darkness for a duration of 2 h. Following this, 800 µL of a solution containing 0.7 mM sodium carbonate was added. A measurement of the absorbance was taken at 765 nm. Application of a calibration curve was carried out using gallic acid concentrations ranging from 0 to 0.1 mg/mL for the purpose of estimating the amount of lipophenolic eucam that was present in the sample [18].

Assay of Total Flavonoid Content

Lipo-Eucam's flavonoids were measured using a colorimetric aluminum chloride assay. The process comprised filling 2 mL Eppendorf tubes with 30 µL *Z. spina-christi* extract or quercetin standard and 160 µL methanol. Next, 30 µL of a freshly prepared 10% aluminum chloride solution in methanol was added and forcefully mixed. Then, 30 µL of 1 M sodium acetate solution and 850 µL of distilled water were added. Post-vortexing absorbance was measured at 415 nm after 30 min at room temperature. Flavonoid concentration was measured in mg QE/g DW of Lipo-Eucam [19].

Calculation of Total Tannin Concentration

Following the extraction of 0.1 mL of plant material, 1.5 mL of Milli-Q water, and 1 mL of diluted Folin-Ciocalteu reagent were amalgamated to determine the total tannin content (TTC). Subsequently, 0.8 mL of a sodium bicarbonate ($NaHCO_3$) solution with a pH of 7.5 was administered. The ingredients were thoroughly combined and allowed to incubate at 45ºC for 30 min. After 20 min of incubation, it was placed at ambient temperature in the absence of light. The content of tannins was measured in milligrams of tannic acid equivalents per gram of dry weight (mg TAE/g DW), calculated by assessing absorbance at 700 nm [20].

Assessment of Antioxidant Characteristics: DPPH Free Radical Scavenging Assay

The plant sample fraction was incubated in a water bath at 50ºC for 20 min with 2.5 mL of phosphate buffer and potassium ferrocyanide. Trichloroacetic acid (TCA) was added after centrifugation. A spectrophotometer recorded absorbance at 700 nm. Ascorbic acid was used as the positive control [21]. In the phosphomolybdate test, the plant sample's antioxidant capacity was measured using [22, 23]. In a microcentrifuge tube, 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate were mixed to make 4 mL of the phosphomolybdate reagent. In a Falcon tube, 4 mL of phosphomolybdate reagent and 1 mL of plant crude extract were mixed. For the blank, 4 mL of the reagent was added to 1 mL of ethanol. All tubes were wrapped in aluminum foil and rested for 10 min. The tubes were placed in a 95ºC water bath for 90 min. A spectrophotometer recorded absorbance at 695 nm after the tubes cooled. In the DPPH assay, the samples' DPPH radical scavenging activity was assessed using the method described in [23]. A 0.2 mM DPPH solution was prepared in methanol. The solution was left at room temperature. Equal volumes of plant extract and DPPH solution were mixed in a Falcon tube. Test samples were incubated for 30 min. Absorbance was measured at 517 nm. Methanol was used as the blank, while ascorbic acid served as the positive control. The IC_{50} (extract concentration inhibiting 50% of DPPH radicals) was calculated by plotting the inhibition percentage versus the extract concentration. A standard calibration curve plotting percentage inhibition against positive control concentration was used to quantify radical scavenging activity [23].

ABTS Radicals Scavenging Assay

This test quantifies color loss resulting from the interaction of an antioxidant with ABTS+ radical cations, leading to their conversion into ABTS and subsequent decolorization. The approach proposed by Ree et al. delineates the assessment of antioxidant activity by this procedure. ABTS radical cations are produced by combining potassium persulfate (2.45 mM) with a water stock solution of ABTS (7 mM). The operational solution is formulated by combining equal volumes of both stock solutions and incubating for 16 h at 25ºC in the absence of light, followed by dilution with methanol to obtain an absorbance of 0.70±0.2 units at 734 nm using spectrophotometry. Each experiment employed fresh solvent with Trolox as the antioxidant standard; its calibration curve encompassed values from 0 to 500 µM. In test tubes, diluted samples (1 mL) were combined with an equal amount of ABTS+ radical-cation solution; absorbance was recorded after 7 min at 734 nm to determine TEAC levels reported as Trolox equivalents (in µM) [24].

In Vitro Cytotoxicity Assessment

Establishment of Cell Line

This work utilized the human epithelial cell line Caco-2 as an in vitro model. Cancer cells were acquired from the American Type Culture Collection (ATCC, Manassas, WV, USA). The cells were cultivated in a flask with complete medium (Invitrogen, Carlsbad,

CA, USA) including 10% fetal bovine serum (FBS) and antibiotics (100 µg/mL streptomycin + 100 U/mL penicillin) at 37ºC and 5% CO_2. The culture medium was substituted every 2 to 3 days. Upon achieving 90% confluence, the cells were divided into sub-cultures. Normal cells were not used in this study.

MTT Assay

The main goal was to assess the bioactivity of the raw botanical extract by inhibiting proliferation in the Caco-2 cell line. Further experiments were conducted on the most potent crude sample, including hexane, dichloromethane, dichloromethane-ethyl acetate, ethyl acetate-methanol, methanol, and acetic acid. The study assessed the cytotoxicity of fractions against Caco-2 cell lines using MTT assays at 595 nm with a microplate reader (SunRise, TECAN, Inc., USA) and derived IC50 values. The growth inhibition experiment was performed in 96-well plates using the technique described in [25]. Two plates were used for MTT. Each plant crude extract (50 µL) was serially diluted in 100 µL of DMEM on the dilution plate row by row. The range was 6.3 ng/mL to 1.111 mg/mL. Methanol was used as the vehicle control, and all treatments were repeated and labeled. In the culture plate, 5×10^4 cells/mL of the cell lines were planted in 96-well plates (120 µL) and treated with 60 µL of the diluted treatments from the dilution plates. Twenty microliters of MTT salt were added after 4 days of incubation in a CO_2 incubator. After a 2-hour incubation, MTT salt formed formazan crystals. A vacuum aspirator was used to gently aspirate the solution to preserve the formazan crystals. Formazan was dissolved in isopropanol and incubated for another 10 min. After 10 min on a shaker, optical densities were measured at 595 nm using a plate reader. Results were shown as a percentage compared to the control value, derived by determining the IC_{50} concentration, which inhibited cell growth by 50% using Origin 8 software. Each chemical was tested in triplicate.

UV-vis Spectroscopy and Zeta Potential

UV-visible spectroscopy (Hitachi U2600) was utilized in order to assess the optical characteristics of the fenugreek extract. Additionally, dynamic light scattering (DLS) was utilized in order to determine the surface charge of the fenugreek extract in the culture medium.

Statistical Analysis

All analyses were performed in triplicate, and results are reported as mean±standard deviation (SD). Because the study evaluated a single combined extract rather than multiple experimental groups, inferential statistical tests (e.g., ANOVA, p-values) were not applied. Descriptive statistics were used to summarize biochemical and cytotoxicity measurements.

Results and Discussion

(GC-MS) Analysis

The GC-MS study of the aqueous extract of *Z. spina-christi* demonstrated a varied phytochemical profile, primarily consisting of fatty acids and their derivatives. Oleic acid was identified as the predominant chemical, constituting 58.03% of the total detectable components, with a retention time of 19.65 min. This monounsaturated fatty acid is recognized for its antioxidant and anti-inflammatory effects, considerably enhancing the biological activity of the extract. Linoleic acid, a polyunsaturated fatty acid with significant antioxidant properties, constituted 22.88% of the extract, making it the second predominant component. This molecule, with a retention time of 19.60 min, is essential for cellular health and the reduction of oxidative stress. The third principal constituent was n-Hexadecanoic acid, a saturated fatty acid referred to as palmitic acid, which comprised 11.71% of the extract and eluted at 17.88 min. This chemical is frequently linked to antibacterial and cytotoxic characteristics. Alongside these predominant components, lesser quantities of other fatty acids and derivatives were detected. Octadecanoic acid (stearic acid), with a retention time of 19.85 min and a peak area of 3.10%, is a saturated fatty acid recognized for its emollient and stabilizing characteristics. The research identified 9,12-Octadecadienoyl chloride (3.55%, retention period 22.61 min), a molecule associated with linoleic acid derivatives, potentially enhancing the extract's bioactivity. Finally, a minimal quantity of Diisobutyl phthalate was identified, constituting just 0.72% of the extract with a retention period of 16.94 min, perhaps indicating contamination during sample processing or analysis. The prevalence of oleic and linoleic acids in the extract highlights its potential for antioxidant and cytotoxic properties, consistent with the traditional therapeutic use of *Ziziphus spina-christi*. The varied composition of bioactive chemicals underscores the therapeutic adaptability of the extract, establishing a basis for further investigation of its pharmacological potential (Fig. 1 and Table 1).

FTIR Spectrum of Aqueous Z. *spina-christi* Extract

The infrared (IR) spectrum of the *Z. spina-christi* extract displays an intricate assortment of functional groups that elucidate its phytochemical makeup (Fig. 2). A significant broad absorption band at around 3400 cm^{-1} signifies the existence of hydroxyl groups, presumably derived from phenolic chemicals, alcohols, or residual water in the extract. This characteristic is indicative of bioactive chemicals linked to antioxidant action, hence reinforcing the plant's conventional therapeutic uses. The spectra exhibit distinct absorption bands at around 2920 cm^{-1} and 2850 cm^{-1}, signifying C-H stretching vibrations from aliphatic chains. The findings align

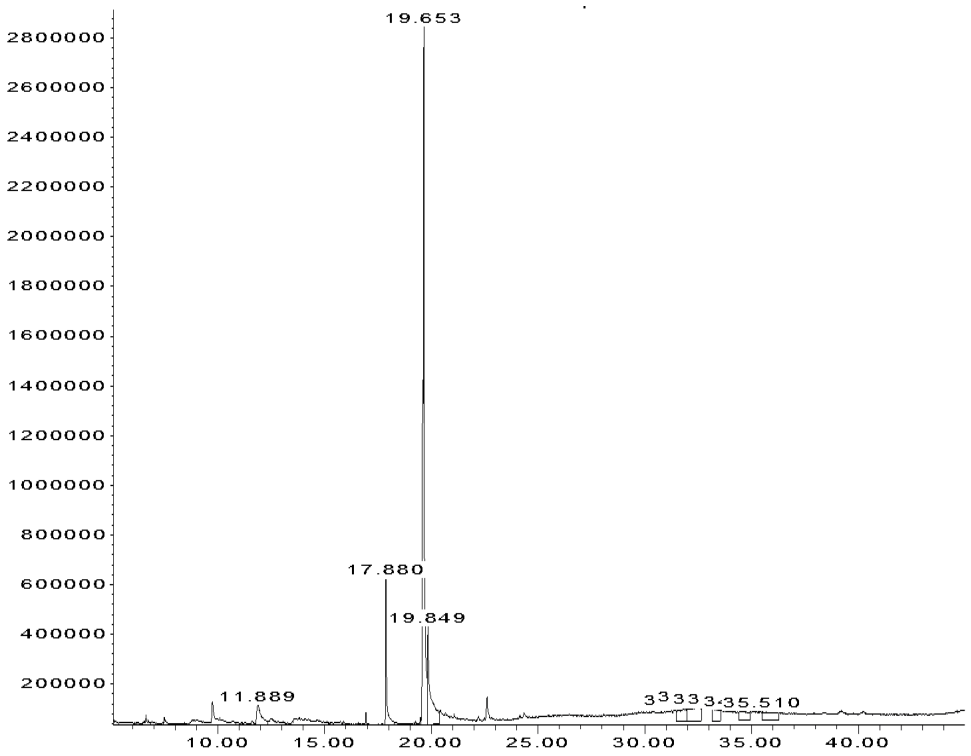
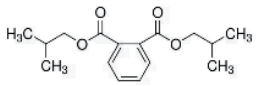
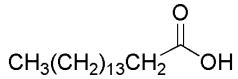
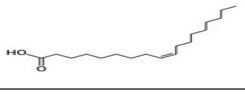
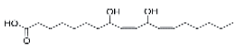


Fig. 1. GC-MS chromatogram of the aqueous extract of *Z. spina-christi.*

Table 1. GC-MS analysis of the aqueous extract of *Z. spina-christi*: identified chemical compounds, retention time, and peak area percentage.

$t_{R\ (min)}$	Proposed compound	MW	Peak area	Formula	Structural Formula
16.94	Diisobutyl phthalate	278	0.72	$C_{16}H_{22}O_4$	
17.88	n-Hexadecanoic acid	256	11.71	$C_{16}H_{32}O_2$	
19.60	Linoleic acid	280	22.88	$C_{18}H_{32}O_2$	
19.65	Oleic acid	282	58.03	$C_{18}H_{34}O_2$	
19.85	Octadecanoic acid	284	3.1	$C_{18}H_{36}O_2$	
22.61	9,12-Octadecadienoyl chloride	298	3.55	$C_{18}H_{31}ClO$	

with the fatty acid composition revealed in the GC-MS study, including oleic and linoleic acids, recognized for their medicinal and structural functions in bioactive formulations. A prominent peak at about 1650 cm^{-1} is indicative of the stretching vibrations of carbonyl (C=O) groups. This characteristic verifies the existence of carboxylic acids and esters, which are common in fatty acid derivatives and other carbonyl-containing phytochemicals identified in the extract. The absorption at 1450 cm^{-1} is associated with the bending vibrations of aliphatic C-H bonds, thus confirming the existence of both saturated and unsaturated fatty acid components. The fingerprint portion of the spectrum, extending below 1100 cm^{-1}, has unique peaks that signify C-O stretching vibrations. These signs indicate the presence of esters, ethers, or alcohols, which are frequently observed

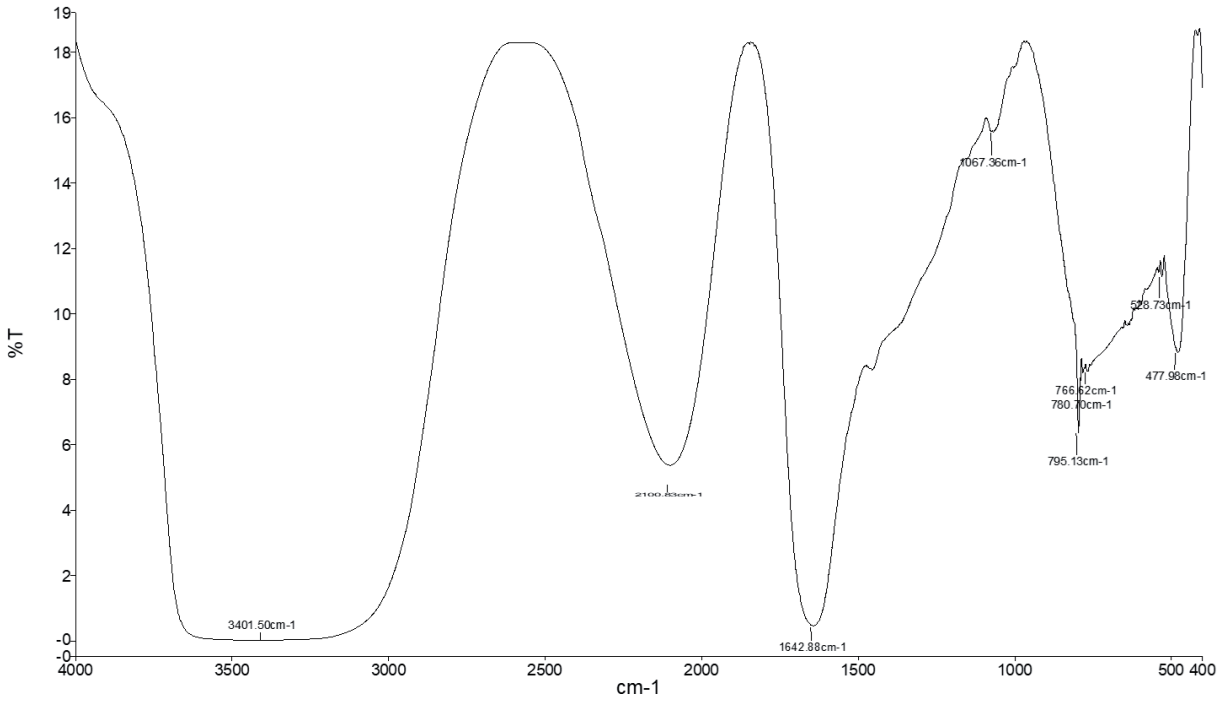


Fig. 2. The findings of the aqueous extract of *Z. spina-christi* samples' infrared spectroscopic study. Using a Nicolet 6700 FT-IR spectrometer and examining the data in the 400-4000 cm^{-1} range, the conclusions were reached. Fourier-transform infrared spectrometer, or FT-IR.

in plant-derived bioactive chemicals. Moreover, the area below 800 cm^{-1} has minor characteristics potentially linked to halogenated chemicals, including those identified in the GC-MS study, particularly 9,12-octadecadienoyl chloride. The IR spectrum reveals a diverse array of functional groups, including hydroxyl, carbonyl, and aliphatic chains, as well as potential halogenated compounds. The chemical complexity illustrates the extract's multifunctional characteristics and corresponds with its documented antioxidant and cytotoxic capabilities, enhancing the comprehension of its phytochemical attributes.

Table 2 presents quantitative data on the phytochemical composition of the seed extract of *Z. spina-christi*. The stated results pertain to the total phenolic content (TPC), tannins, flavonoids, and flavonols, each quantified by established tests and presented in appropriate units. The seed extract contains a total phenolic content of 105.48±0.42 GAE (gallic acid equivalents) per gram, indicating a substantial concentration of phenolic chemicals. These chemicals are renowned for their antioxidant qualities, which are essential to the plant's therapeutic functions. The extract possesses a tannin concentration of 99.02±0.45 GAE per gram. Tannins are polyphenolic chemicals that contribute to antioxidant effects and may exhibit additional antibacterial and anti-inflammatory characteristics, hence augmenting the extract's potential medicinal uses. The flavonoid content, quantified at 32.39±0.15 mg QE (quercetin equivalents) per gram, indicates a significant concentration of these beneficial chemicals. Flavonoids are recognized for their capacity to diminish oxidative stress, promote cardiovascular health, and have anticancer properties. The flavonol concentration is documented as 24.10±0.21 mg QE per gram. Flavonols, a subclass of flavonoids, are distinguished for their strong antioxidant and anti-inflammatory characteristics, which may enhance the overall bioactivity of the Z. *spina-christi* seed extract.

The results indicate that Z. *spina-christi* seeds are abundant in bioactive polyphenolic components, such as phenolics, tannins, flavonoids, and flavonols, which are essential to their medicinal potential. These findings corroborate the plant's historical application

Table 2. Total Phenolic Content (TPC), Total Flavonoid Content (TFC), Total Flavonol Content (TF-OL), and Total Tannin Content (TTC) in *Z. spina-christi* seed extract (expressed in mg GAE/g and mg QE/g).

Extract	Phenolics GAE/g	Tannin GAE/g	Flavonoid mg QE/g	Flavanol mg QE/g
Z. spina-christi	105.48±0.42	99.02±0.45	32.39±0.15	24.10±0.21

Note: Values represent the mean ± S.E. of three independent measurements.

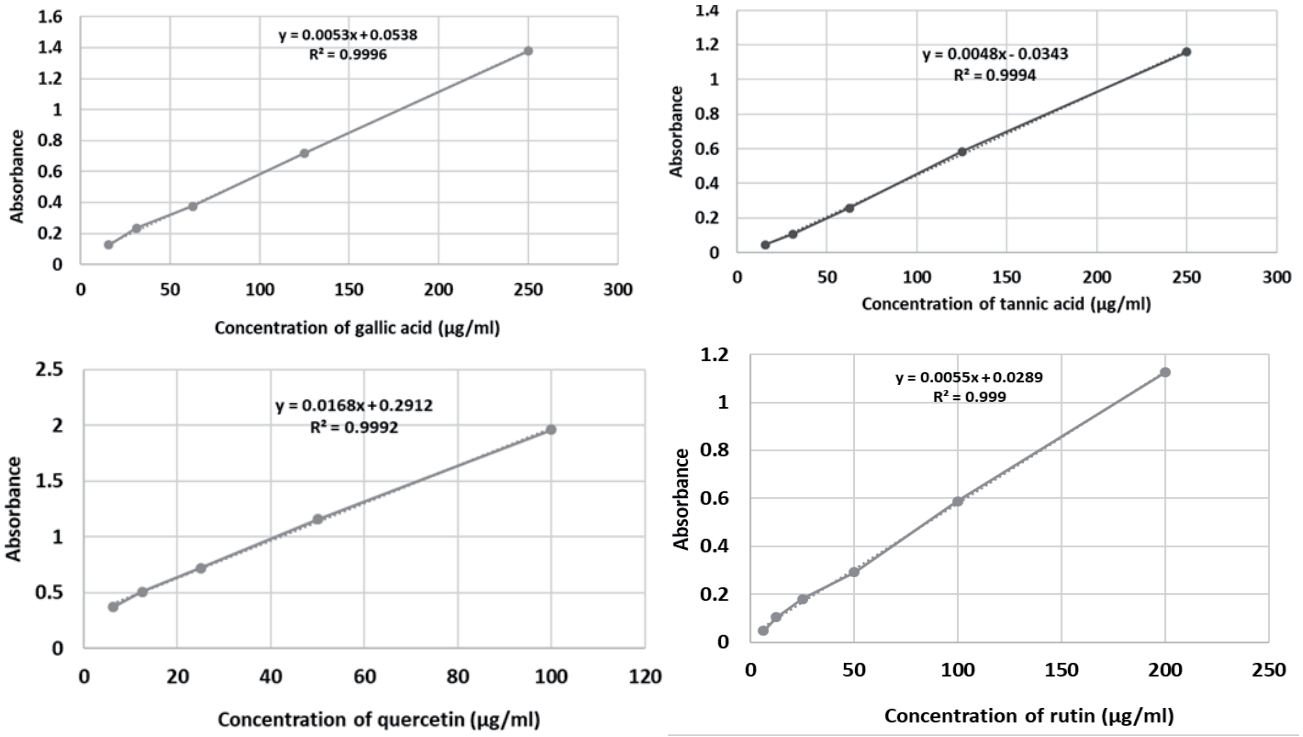


Fig. 3. Total Phenolic (TPC), Flavonoid (TFC), Flavonol (TF-OL), and Tannin (TTC) content in *Z. spina-christi* extract.

in addressing conditions associated with oxidative stress and inflammation, indicating the need for additional investigation as a natural reservoir of antioxidants and other bioactive substances (Fig. 3).

Antioxidant Activity

Table 3 presents the antioxidant activity of the aqueous extract of *Z. spina-christi*, evaluated using DPPH and ABTS radical scavenging tests. The findings demonstrate that the extract possesses considerable antioxidant capacity, with IC_{50} values denoting the quantity necessary to block 50% of free radicals in each experiment. The IC_{50} value for the DPPH test is 15.11±2.31 µg/mL. The low IC_{50} value indicates a robust free radical scavenging capacity, implying that the aqueous extract has active antioxidant components that can neutralize DPPH radicals. In the ABTS experiment, the IC_{50} value is 18.30±0.11 µg/mL, further validating the extract's significant antioxidant potency. The findings in both experiments highlight the reliability of the antioxidant activity across various radical scavenging methods.

Table 3. Inhibition percentage and IC_{50} value of the aqueous extract for DPPH scavenging activity.

Extracts	Antioxidant capacity inhibition%	
	IC_{50} DPPH (µg/mL)	IC_{50} ABTS(µg/mL)
Z. spina-christi	15.11±2.31	18.30±0.11

The results correspond with the elevated phenolic and flavonoid content identified in the extract, since these chemicals are recognized for their substantial contribution to antioxidant capabilities. The extract's capacity to efficiently scavenge free radicals highlights its potential as a natural antioxidant, which may aid in alleviating oxidative stress and avoiding associated illnesses. This study corroborates the conventional employment of *Z. spina-christi* in medicine and establishes a basis for its prospective therapeutic utilization in antioxidant-related treatments. For antioxidant assays (DPPH, ABTS), we used ascorbic acid and Trolox as positive controls.

MTT Assay

Fig. 4 depicts the cytotoxic impact of the aqueous extract of *Z. spina-christi* on Caco-2 cells, a colorectal cancer cell line, as assessed by cell viability (%) at various doses. The dose-response curve illustrates a progressive decline in cell viability with increasing extract concentration, resulting in a 50% reduction in viability at an IC_{50} value of 209.245±8.32 µg/mL. The findings demonstrate that the aqueous extract displays considerable cytotoxicity towards Caco-2 cells, indicating its potential as an anticancer drug. The sigmoidal shape of the curve illustrates the cumulative effect of rising extract concentrations on cell viability, where elevated concentrations impose a more pronounced inhibitory influence. The IC_{50} value indicates the dosage necessary for half-maximal suppression of cell proliferation, acting

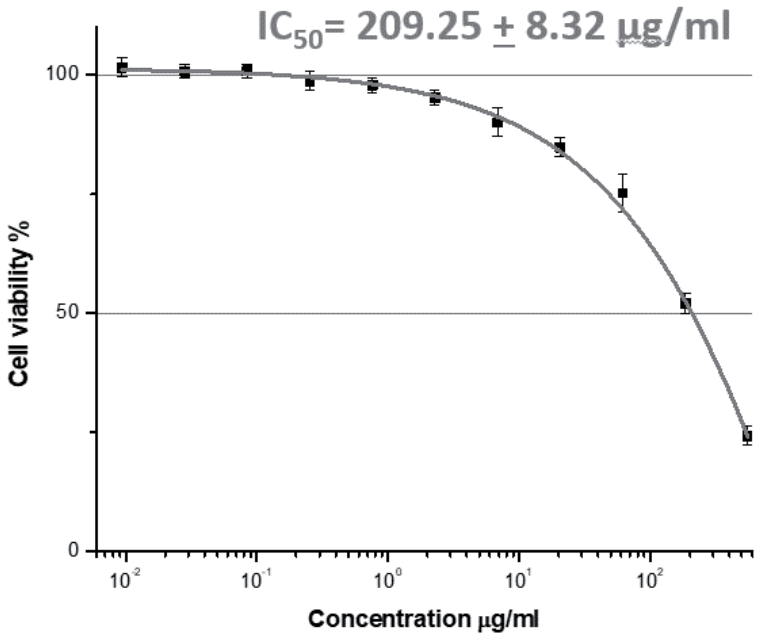


Fig. 4. The IC50 value represents the plant extract dose that inhibited cancer cell growth by 50% in Caco-2 cells (IC_{50} = 209.245 + 8.32 µg/mL).

as a quantitative assessment of the extract's cytotoxic efficacy.

The findings indicate that the bioactive components in the extract, including phenolics, flavonoids, and fatty acid derivatives found in previous tests, may have a role in its anticancer properties. This degree of cytotoxicity suggests potential for therapeutic uses; nevertheless, more research into the precise mechanisms of action and selectivity towards malignant vs. normal cells is necessary to confirm its viability for clinical usage. For cytotoxicity assessment (MTT assay), we included a vehicle control (methanol) to validate cell viability measurements.

UV-vis Measurements

The UV-vis spectra of *Z. spina-christi* extract display a prominent absorption peak at 325.26 nm, signifying the existence of certain chromophore chemicals within the extract. The absorption in the UV range is indicative of phenolic and flavonoid chemicals, often present in plant extracts and linked to their antioxidant properties. The prominence and vigor of the peak indicate a substantial concentration of these bioactive compounds. The progressive decrease in absorbance past 325 nm and the lack of significant peaks in the visible spectrum suggest that the main light-absorbing constituents of the extract are predominantly located in the UV range. This corresponds with the existence of conjugated systems, such as aromatic rings and double bonds, commonly observed in phenolics and flavonoids. The significant UV absorbance at 325.26 nm corroborates the phytochemical composition revealed in earlier investigations and substantiates the extract's potential for antioxidant and therapeutic uses. The findings align with the bioactivity ascribed to the extract, namely its capacity to engage with free radicals and avert oxidative damage, thus corroborating its conventional medical use (Fig. 5).

Surface Charge Analysis

The zeta potential distribution of the aqueous *Z. spina-christi* seed extract, shown in Fig. 6, has a peak centered at -23.3±5.87 mV. The negative zeta potential signifies that the particles in the extract are primarily negatively charged, possibly attributable to the presence of negatively charged functional groups, such as carboxyl and hydroxyl groups, on the surface of the bioactive molecules in the extract. The zeta potential indicates modest stability of the scattered particles in the aqueous medium. A zeta potential at -30 mV or below generally signifies favorable colloidal stability attributed to electrostatic repulsion; however, the measured zeta potential in this instance suggests that the particles may demonstrate some level of aggregation under specific conditions. This stability is essential for preserving the uniform distribution of bioactive chemicals and guaranteeing constant interaction with biological systems in therapeutic applications. The distribution peak is pronounced, signifying a generally homogeneous size and charge distribution among the particles in the extract. This consistency is crucial for repeatability in applications, especially in antioxidant and cytotoxic research, where consistent particle behavior can profoundly affect bioactivity results. The zeta potential measurement indicates

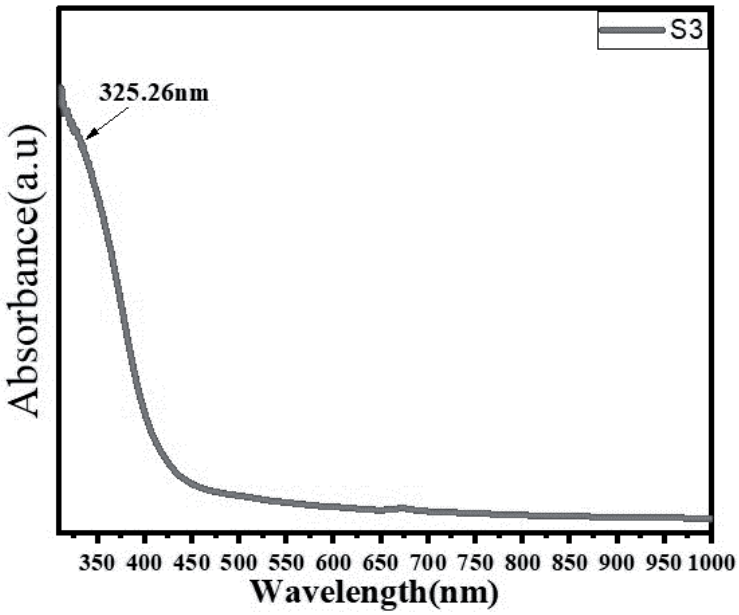


Fig. 5. UV spectra of *Z. spina-christi.*

the potential for persistent and effective biological interactions of the aqueous *Z. spina-christi* seed extract.

Hussein S. [6] provides a comprehensive analysis of the chemical composition and bioactivities of *Z. spina-christi*, offering a solid foundation for understanding the plant's medicinal properties. The study highlights the richness of Z. *spina-christi* in secondary metabolites, particularly phenolics, flavonoids, saponins, and tannins. These compounds are directly linked to the plant's antioxidant, antimicrobial, and anti-inflammatory effects. The results of our study align well with Hussein's findings, particularly in terms of the high phenolic and flavonoid content detected in the aqueous extracts. Hussein emphasizes the critical role of these compounds in neutralizing free radicals, which mirrors the significant antioxidant potential demonstrated in the DPPH and ABTS assays in our study. Furthermore, the cytotoxic effects observed here against Caco-2 cells are consistent with Hussein's documentation of the plant's anticancer properties, primarily attributed to bioactive compounds such as saponins and phenolics. Overall, Husseins S. [6] establishes a strong correlation between the chemical composition of Z. *spina-christi* and its bioactivities, reinforcing the findings of our study and supporting the plant's potential as a source of natural antioxidants and cytotoxic agents.

Naghmouchi S. et al. [7] examine the biochemical composition, antioxidant capacity, and allelopathic properties of *Z. spina-christi* from different provenances in Saudi Arabia. Their findings highlight the plant's abundance of phenolics, flavonoids, and tannins, which enhance its notable antioxidant activity, as assessed by

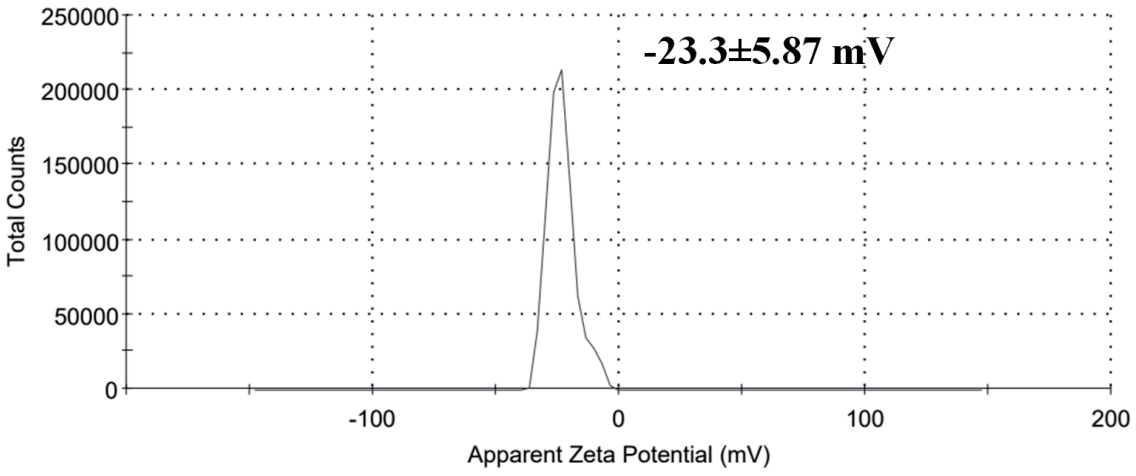


Fig. 6. Zeta potential distribution of the aqueous *Z. spina-christi* seed extract.

DPPH and ABTS tests. The findings of our investigation closely correspond with those presented by Naghmouchi and Alsubeie. The aqueous extract of *Z. spina-christi* exhibited elevated concentrations of phenolics (105.48 GAE/g) and flavonoids (32.39 QE/g), which corresponded with significant antioxidant activity. The IC_{50} values for DPPH (15.11 µg/mL) and ABTS (18.30 µg/mL) demonstrate significant free radical scavenging capabilities, corroborating the observations of Naghmouchi S. et al. [7], who emphasized the plant's efficacy in alleviating oxidative stress in various settings. Moreover, the allelopathic effects identified by Naghmouchi S. et al. [7] indicate that secondary metabolites, including phenolics and flavonoids, are crucial in ecological interactions, suggesting the extract's multifunctional bioactivity. These insights enhance the cytotoxic findings of our investigation, highlighting the varied therapeutic potential of *Z. spina-christi*. Collectively, these investigations validate the plant's worth as a natural source of bioactive chemicals possessing considerable antioxidant properties and prospective uses in health and agriculture.

The research conducted by Almustafa I. and Yehia S. [8] examines the bioactivities of Pestalotiopsis neglecta, an endophytic fungus extracted from *Z. spina-christi*. Their research emphasizes the strong antioxidant, cytotoxic, and DNA damage protective properties of bioactive chemicals generated by the fungus. While the study primarily concentrates on the fungal endophyte, it indirectly highlights the phytochemical abundance of *Z. spina-christi* as the host plant, which presumably affects the bioactive characteristics of its associated microorganisms. Our research illustrates the considerable antioxidant activity of the aqueous extract of Z. *spina-christi*, evidenced by low IC_{50} values for DPPH (15.11 µg/mL) and ABTS (18.30 µg/mL), comparable to the robust antioxidant potential documented for fungal extracts. The cytotoxic effects of Z. *spina-christi* extract on Caco-2 cells (IC_{50} = 209.245 µg/mL) correspond with the cytotoxic activity of fungal metabolites, indicating a common involvement of secondary metabolites including phenolics, flavonoids, and triterpenoids.

A study by Khaleel M. [26] analyzed the heavy metal composition in Z. *spina-christi* leaves grown in Jordan and reported concentrations of lead, copper, cadmium, nickel, and cobalt were below the permissible limits for medicinal plants. This suggests a favorable safety profile under normal environmental conditions, but also highlights the importance of considering geographic location due to potential environmental contamination, as shown in other studies. Furthermore, Wibowo G. [27] discuss the potential of Lemna minor in eradicating ionic dyes from contaminated water, further reinforcing the importance of plant-based detoxification systems. Although Z. *spina-christi* has not been extensively studied in this context, its ability to thrive in diverse environments suggests a potential mechanism for avoiding toxic element accumulation, reinforcing its safety as a medicinal plant.

The results of our study, which highlight the significant antioxidant and cytotoxic potential of *Z. spina-christi* aqueous extracts, align with existing research in terms of the plant's bioactivity but also raise important questions when compared to our previous studies [15], which explore its potential side effects on vital organs. Apparent contradictions in results can often be attributed to differences in experimental design, such as dosage, duration, and biological context. One of the most important factors influencing these results is the concentration of the extract used. Our study highlights strong antioxidant activity at low IC_{50} values for DPPH (15.11 µg/mL) and ABTS (18.30 µg/mL) assays, as well as moderate cytotoxicity against Caco-2 cancer cells (IC_{50} = 209.245 µg/mL). These low doses emphasize therapeutic efficacy. However, Ammari A. et al. [15] report side effects in the liver and kidneys of female rats, likely due to chronic exposure to higher concentrations. Prolonged high doses can overwhelm the body's natural defense mechanisms, leading to organ toxicity.

These findings highlight the dual nature of Z. *spina-christi* extracts: therapeutic at controlled doses but potentially toxic at high concentrations or with prolonged use. Future studies should perform dose-response and time-course studies to establish safe and effective therapeutic ranges, examine the metabolic fate of bioactive compounds to understand their in vivo behavior, and evaluate mechanisms of action through combined in vitro and in vivo studies to bridge experimental gaps.

Overall, our results reaffirm the therapeutic potential of Z. *spina-christi*, particularly its significant antioxidant and cytotoxic properties, consistent with existing literature. However, safety evaluations, such as those by Ammari A. et al. and Wibowo G. et al. [15, 27], provide a crucial reminder of the importance of assessing toxicological parameters. By understanding the interplay between dosage, duration, and biological systems, researchers can reconcile these findings and develop a framework for the safe and effective therapeutic use of *Z. spina-christi*. This balance is essential for maximizing its potential benefits while mitigating risks.

Conclusions

This study demonstrates the strong bioactivity of the aqueous extract of *Z. spina-christi*, attributed to its high phytochemical content and potent antioxidant capacity, along with moderate cytotoxic effects against Caco-2 cells. The abundance of phenolics and flavonoids underlies its effective free radical scavenging activity. While the extract shows promise as a natural source of bioactive and anticancer agents, variations in dosage and biological context highlight the need

for cautious interpretation due to potential toxicity at high or prolonged exposure. Overall, the findings support the traditional medicinal use of Z. *spina-christi* and emphasize the necessity for standardized extraction methods, dose-response evaluations, and further in vivo and clinical studies to ensure safety and efficacy.

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Conflict of Interest

The author of this article declares that there are no conflicts of interest.

References

1. FARIDA T., ABDELLATIF S., ICHRAK E. Ichrak. Medicinal Plant Modulates Renal Toxicity and Oxidative Stress Induced by Heavy Metals in Rats. Diss. Université de Echahid Cheikh Larbi Tébessi–Tébessa-, PhD Thesis. Université de Echahid Cheikh Larbi Tébessi–Tébessa. **2024**.
2. Al-MOHAMED R., MAJAR A., FAHED K., DAGAR C., SILESHI W. Agroforestry for plant diversity and livelihood security in southwest asia. Agroforestry for sustainable intensification of agriculture in Asia and Africa. Singapore: Springer Nature Singapore, **2023**.
3. KENDRICHA A., RIAH T., ZGHEIDA I. Isolation and characterization of new strains of arbuscular mycorrhizal fungi and evaluation of their effect on secondary metabolites contents in wild jujube (*Ziziphus lotus* L.). Diss. Faculté des Sciences de la Nature et de la Vie, PhD Thesis. Faculté des Sciences de la Nature et de la Vie. **2021**.
4. GUEZZOUN N., SELMANE M., KHEZZANI B., ELKHALIFA A., ZEMMOULI N. *Ziziphus spina-christi* (L.) as a source of environmentally friendly biocides: phytochemical characteristics and insecticidal potential of leaves and stem bark extracts on Aphis fabae Scopoli (Aphididae). African Journal of Biological Sciences, **6** (13), 7509, **2024**.
5. ABU-ODEH M., TALIB H. Middle East medicinal plants in the treatment of diabetes: a review. Molecules, **26** (3), 742, **2021**.
6. HUSSEIN S. *Ziziphus spina-christi*: Analysis of bioactivities and chemical composition. Wild Fruits: Composition, Nutritional Value and Products. Cham: Springer International Publishing, **2019**.
7. NAGHMOUCHI S., ALSUBEIE M. Biochemical profile, antioxidant capacity and allelopathic effects from five *Ziziphyus spina-christi* (L.) provenances growing wild in Saudi Arabia. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, **48** (3), 1600, **2020**.
8. ALMUSTAFA I., YEHIA S. Antioxidant, cytotoxic, and DNA damage protection activities of endophytic fungus Pestalotiopsis neglecta isolated from *Ziziphus spina-christi* medicinal plant. Microorganisms, **11** (1), 117, **2023**.
9. BILEL H., ABDELZAHER M., MOUSTAFA M. Biochemical profile, antioxidant effect and antifungal activity of Saudi *Ziziphus spina-christi* L. for vaginal lotion formulation. Plant Science Today, **10**, 22, **2023**.
10. ELALOUI M., HAMDI H., GHAZGHAZI H., NASR B., BOUSLIH E., AMMARI, Y., LAAMOURI A. Characterization of epicathechin contents in the *Ziziphus spina-christi* L. root extracts using LC-MS analyses and their insecticidal potential. Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology. **155** (4), 685**, 2021**.
11. ARSLAN L., KAYA E. Investigation of Antimicrobial and Antioxidant Activities of *Paliurus spina-christi* Mill. in Kahramanmaras, Turkey. Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi, **24** (6), 1161, **2021**.
12. IMRAN S., YAMIN I., QAYYUM A., SOBIA A., SHAHZAD R., ALHARBI A., ANSARI J. Biological and phytochemical analysis of fractionated extracts of *Ziziphus nummularia* against pathogenic bacteria, oxidative stress and cytotoxicity. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, **52** (3), 13663, **2024**.
13. ELMAIDOMY H., El ZAWILY A., SALEM K., ALTEMANI H., ALGEHAINY A., ALTEMANI H., SHADY H. New cytotoxic dammarane type saponins from *Ziziphus spina-christi*. Scientific Reports, **13** (1), 20612, **2023**.
14. ADS N., RAJENDRASOZHAN S., HASSAN I., SHARAWY S., HUMAIDI R. Phytochemical, antimicrobial and cytotoxic evaluation of *Ziziphus spina-christi* (L.) stem bark. Biomedical Research, **28** (15), 6646, **2017**.
15. AMMARI A., ALHIMAIDI R., AMRAN A., RADY M. The possible side effects of Ziziphus spina-christi extract on the liver, kidneys of female rats. Indian Journal of Animal Research, **58** (7), 1221, **2024**.
16. ALHIMAIDI R., AMMARI A., AMRAN A., RADY M. Assessment of biochemical and histological effects of one of Ziziphus genus extract on ovarian function in female rats. Indian Journal of Animal Research, **58** (8), 1275, **2024**.
17. KANTHAL K., DEY A., SATYAVATHI K., BHOJARAJU R. GC-MS analysis of bio-active compounds in methanolic extract of *Lactuca runcinata* DC. Pharmacognosy Research, **6** (1), 58, **2014**.
18. MWAMATOPE B., TEMBO D., CHIKOWE I., KAMPIRA E., NYIRENDA C. Total phenolic contents and antioxidant activity of Senna singueana, Melia azedarach, Moringa oleifera and Lannea discolor herbal plants. Scientific African, **9**, e00481, **2020**.
19. DZOYEM P., MCGAW J., ELOFF N. In vitro antibacterial, antioxidant and cytotoxic activity of acetone leaf extracts of nine under-investigated Fabaceae tree species leads to potentially useful extracts in animal health and productivity. BMC Complementary and Alternative Medicine, **14** (1), 147, **2014**.
20. OJHA S., RAJ A., ROY A., ROY S. Extraction of total phenolics, flavonoids and tannins from *Paederia foetida* L. Leaves and their relation with antioxidant activity. Pharmacognosy Journal, **10** (3), 541, **2018**.
21. PADALIA H., CHANDA S. Synthesis of silver nanoparticles using *Ziziphus nummularia* leaf extract and evaluation of their antimicrobial, antioxidant, cytotoxic

and genotoxic potential (4-in-1 system). Artificial Cells, Nanomedicine, and Biotechnology, **49** (1), 354, **2021**.
22. MESHGINFAR N., SADEGHI A., HOSSEINIAN F., GHORBANI M., TSOPMO A. Production of antioxidant peptide fractions from a by-product of tomato processing: mass spectrometry identification of peptides and stability to gastrointestinal digestion. Journal of Food Science and Technology, **55** (9), 3498, **2018**.
23. FERNÁNDEZ-POYATOS P., ZENGIN G., SALAZAR-MENDÍAS C., RUIZ-MEDINA A., SINAN I., LLORENT-MARTÍNEZ J. Study on three Sarcocapnos species as potential sources of bioactive compounds: relation between phenolic content and bioactivity by multivariate Analysis Journal of Analytical Methods in Chemistry, **2020** (1), 8885169, **2020**.
24. KASEKE T., OPARA L., FAWOLE A. Effect of microwave pretreatment of seeds on the quality and antioxidant capacity of pomegranate seed oil. Foods, **9** (9), 1287, **2020**.
25. MOSMANN T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. Journal of Immunological Methods, **65** (1), 55, **1983**.
26. KHALEEL M. Studying the heavy metals composition and the impact of different common solvents on the extraction efficiency of phytochemical secondary metabolites from the leaves of *Ziziphus spina-christi* grown in Jordan. Pakistan Journal of Nutrition, **17** (8), 392, **2018**.
27. WIBOWO G., SYAHNUR T., Al-AZIZAH S., GINTHA A., LULULANGI G. Phytoremediation of high concentration of ionic dyes using aquatic plant (*Lemna minor*): A potential eco-friendly solution for wastewater treatment. Environmental Nanotechnology, Monitoring & Management, **20**, 100849, **2023**.