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Phytochemical screening, phenolic compounds, antioxidant, and antibacterial activities of *Zizyphus lotus* from Beni Mellal region, Morocco

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ABSTRACT. The study thoroughly investigated the phytochemical composition, antioxidant, and antibacterial properties of *Zizyphus lotus* extracts from leaves, seeds, pulps, and root barks. Analysis revealed a diverse range of phytochemicals, with polyphenols, flavonoids, mucilage, gallic tannins, and catechic tannins being predominant. Highest total phenolic content was observed in leaves, followed by root barks and seeds, while root barks had the highest total anthocyanin content. Antioxidant assays (DPPH, ABTS, and FRAP) demonstrated robust activity in all extracts, notably in root bark (IC50 = 5.97 µg mL^{-1}) and leaf (IC50 = 9.68 µg mL^{-1}) extracts, surpassing the potency of BHT (IC50 = 11.30 µg mL^{-1}). Moreover, extracts exhibited significant antibacterial activity against *Escherichia coli*, Methicillin-Sensitive *Staphylococcus aureus* (MSSA), and Methicillin-resistant *Staphylococcus aureus* (MRSA) at concentrations of 1024 and 2048 µg/mL, with leaves and root barks showing the strongest effects. *Staphylococcus epidermidis* displayed the highest resistance. Such findings not only deepen our understanding of *Zizyphus lotus* but also pave the way for leveraging its pharmacological benefits in pharmaceuticals, nutraceuticals, and functional foods.

Keywords: antioxidant activity; antibacterial activity; anthocyanin; phenols; phytochemical screening; *Zizyphus lotus*.

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Introduction

Zizyphus lotus (L.) Lam. (*Z. lotus*), commonly known as lotus jujube or wild jujube, is a plant species belonging to the family Rhamnaceae. It is native to regions of North Africa, the Mediterranean, and parts of Asia (Benammar et al., 2010), where it thrives in arid and semi-arid climates (Khouchlaa et al., 2018). *Z. lotus* is a small deciduous tree or shrub characterized by its thorny branches, small yellow flowers, and round, red to black fruit, which resembles a small date (Dahlia et al., 2019). The fruits are appreciated for both unprocessed consumptions and for preparing bread, wine, and conserves (Regehr & El Brahli, 1995). Furthermore, several parts of *Z. lotus* have been used in traditional and ancestral medicine for the treatment of several pathologies including liver complaints, obesity, urinary troubles, diabetes, skin infections, fever, diarrhea, insomnia, inflammation, and peptic ulcers (Abdoul-Azize et al., 2013; Cadi et al., 2022). Many of these potential health benefits may have attributed to phenolic compounds, especially flavonoids.

Phenolic compounds represent one of the biggest groups of secondary metabolites, gaining increasing attention in the field of science. Although they are not nutrients, their dietary intake has several health-promoting effects (Gini & Jeya Jothi, 2018). Many researchers have made efforts to illustrate the effectiveness of *Z. lotus* as antioxidant agents (Bakhtaoui et al., 2014; Benammar et al., 2014; Hammi et al., 2015) and with the attempt to characterize the antioxidant compounds like alkaloids (Ghedira et al., 1995; Le Crouéour et al., 2002) and saponins (Maciuk et al., 2004; Abdoul-Azize, 2016). Recently researchers started to investigate the composition of phenolic compounds in this shrub species (Maciuk et al., 2003; Marmouzi et al., 2019; Rached et al., 2019; Dhibi et al., 2022; Yahia et al., 2020; Bencheikh et al., 2021).

The health benefits of phenolic compounds in *Z. lotus* may arise from their antioxidant and radical scavenging properties. Study has shown that the flavonoid fraction of *Z. lotus* root barks possesses significant and dose-dependent anti-inflammatory effects, suggesting potential relief for conditions like arthritis, inflammatory bowel disease, and asthma (Borgi et al., 2008). Furthermore, enriched phenolic compounds from *Z. lotus* pulp extract have been found to modulate cell signaling and exert immunosuppressive effects in

human T-cells (Abdoul-Azize et al., 2013). Moreover, both etheric and methanolic rich-phenolic extracts of *Z. lotus* fruits have demonstrated potent bactericidal effects, inhibiting bacterial growth (Rsaissi et al., 2013). The high antioxidant properties of *Z. lotus* have also been attributed to the presence of phenolic compounds (Benammar et al., 2010; Benammar et al., 2014; Bakhtaoui et al., 2014).

Unfortunately, there is currently no systematic information available on the phytochemical composition, antioxidant activity, and antibacterial activities of enriched-phenolic extracts of different parts of *Z. lotus* (leaves, root bark, seeds, and pulp) grown under the conditions of the Beni Mellal region in Morocco. Therefore, this study seeks to contribute to the existing knowledge base on Moroccan wild jujube. The primary objective of this research is to conduct a phytochemical screening of *Z. lotus* and perform a quantitative analysis of total phenolic compounds using the Folin-Ciocalteu method, alongside the assessment of total anthocyanins. Moreover, the antioxidant and antibacterial activities of phenolic-rich extracts from *Z. lotus* are being examined in vitro. Through this investigation, we aim to delineate the precise bioactive properties of this shrub species, potentially facilitating their utilization in industrial applications and incorporation into food formulations to promote human health.

Material and methods

Chemicals

Dichloromethane (⩾99% purity) was obtained from Sigma Chemicals Co. (Madrid, Spain). Dimethyl sulfoxide (DMSO) of cell culture grade was obtained from PanReac Applichem (Gatersleben, Germany). Acetone (⩾99% purity) was supplied by VWR. Mueller Hinton agar or broth was obtained from Liofilchem (Roseto degli Abruzzi, Italy). Brucella Broth was purchased from Fluka Analytical. 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2'-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid (ABTS), Ferric reducing antioxidant power (FRAP), and 3,5-di-tert-4-butylhydroxytoluene (BHT) (⩾99% purity) were obtained from Sigma Chemicals Co. (Madrid, Spain). Ascorbic acid (⩾99.5% purity) was supplied by Fluka Chemie (Madrid, Spain).

Preparation of *Zizyphus lotus* extracts

Various components of *Z. lotus* were gathered from the Beni Mellal regions of Morocco (32°20′21.998″ N; 6°21′38.999″ W) between September and October 2022. Prior to their utilization, the pulps, seeds, leaves, and root barks were manually separated. Subsequently, each component was shade-dried and ground into granules smaller than 2 mm before the extraction process.

Extraction

Samples were first subjected to Soxhlet extraction with dichloromethane for 8 hours to remove the lipophilic components. Subsequently, the solid residues (2 g) from the dichloromethane extraction were used to extract phenolic compounds by suspending them in a methanol/water (v/v) mixture. After 24 hours of constant stirring at room temperature, the suspension was filtered, and then the methanol and acetic acid were removed by low-pressure evaporation and water by freeze-drying. The dried extracts were weighed, and the extraction yield was determined as the percentage of dry biomass material (w/w, %). The phenolic-enriched extracts were kept at room temperature protected from light until analysis. Two extracts were prepared for each morphological part of *Z. lotus*.

Phytochemical screening

The phytochemical screening of various parts of *Z. lotus* extracts aimed to determine the presence of specific chemical families. This screening included solubility tests, color reactions using characteristic reagents, and precipitation assays. These analyses were conducted on the hydromethanolic extracts prepared beforehand. The presence of alkaloids was confirmed using Mayer's, Dragendorff (created an orange-red precipitate) and Wagner's reagent (formed a brown-colored precipitate) (Iqbal et al., 2015). Catechic and gallic tannins were detected using ferric chloride (Karumi et al., 2004), while terpenes and sterols were identified through the Liebermann (Békro et al., 2008). Saponins were determined based on their ability to form foam (Banso & Adeyemo, 2010), mucilage was detected by adding absolute ethanol (Karumi et al., 2004), and coumarins were revealed by the addition of NaOH (Békro et al., 2008). Polyphenolic substances were identified using $FeCl_3$ (Yeo et al., 2011), and the presence of flavonoids was confirmed through a reaction with cyanidine (N'Guessan et al., 2009).

Total phenolic content

The total phenolic content (TPC) of the extracts was determined using the Folin-Ciocalteu assay, following the procedures outlined by (Dewanto et al., 2002), with slight modifications. The dried extracts were first dissolved in methanol/water (1:1), to obtain stock solutions with concentrations ranging from 0.5 to 2 mg mL^{-1}. Briefly, aliquots of 0.125 mL of each extract solution were mixed with 0.625 mL of Folin Ciocalteu's reagent, previously diluted with water (1:5, v/v). The absorbance was then measured against a blank at 760 nm, using a UV/Vis V-530 spectrophotometer (Jasco, Tokyo, Japan) after 6 min of adding 1.25 mL of 7% sodium carbonate aqueous solution and 1 mL of water. The TPC was expressed as milligrams of gallic acid equivalent per g of dry weight material (mg GAE kg^{-1} dw). The analyses were carried out in triplicate for each extract and the average value from the two extracts was calculated for each morphological part.

Total anthocyanins

The total anthocyanin content (TAC) was estimated using the pH-differential method, as following the protocol described by (Lee et al., 2005). The dried extracts were first dissolved in an appropriate solvent to obtain stock solutions at a concentration of 50 mg mL^{-1}. These solution were then combined in a ration of 1:10 or 1:5 (v:v) with potassium chloride pH 1.0 (0.025 M), and sodium acetate pH 4.5 (0.4 M) buffers in separate vessels. The absorbance was measured at 510 and 700 nm, with wells containing buffer without the sample solution used as blanks. The results were expressed as milligrams of cyanidin-3-glucoside equivalents (cyn-3-glcEq) per gram of extract. The absorbance (A) was calculated according to the following formula:

$$A = (A_{510nm} - A_{700nm})_{pH\ 1.0} - (A_{510nm} - A_{700nm})_{pH\ 4.5} \quad (1)$$

Antioxidant activity

The antioxidant activity of phenolic-rich extracts from *Z. lotus* was assessed using the DPPH and ABTS free radical scavenging assays, along with the FRAP assay. Ascorbic acid and BHT were used as reference antioxidants in the DPPH assay.

DPPH scavenging effect assay

The free radical scavenging activity of phenolic rich-extract of different parts of *Z. lotus* was assessed following the procedure described by (Tepe et al., 2005) with some modification. The extract concentration ranged between 3 and 100 µg mL^{-1}. The absorbance of DPPH free radical was measured at 517 nm using a UV/Vis V-530 spectrophotometer. Ascorbic acid (AA: 1.6-3.8 µg mL^{-1}) and 3,5-di-tert-4-butylhydroxytoluene (BHT: 2-20 µg mL^{-1}) were used as reference compounds. The capacity to scavenge the DPPH was calculated using the following equation:

$$\%\ \text{scavenging effect} = [\frac{(A_{DPPH} - A_{DPPH})}{A_{DPPH}}] \times 100 \quad (2)$$

where A_{DPPH} is the control absorbance and A_S is the sample absorbance.

ABTS assay scavenging assay

ABTS scavenging assay was conducted according to the method of Re et al. (1999). The extract concentration ranged between 20 and 100 µg mL^{-1}. The absorbance was measured at 734 nm using a UV/Vis V-530 spectrophotometer. The Percent inhibition was calculated using the formula:

$$ABTS^{\cdot+}\ \text{scavenging effect}\ (\%) = [\frac{(AB - AA)}{AB}] \times 100 \quad (3)$$

where AB represents the absorbance of the ABTS radical and methanol, and AA represents the absorbance of the ABTS radical and the sample extract/standard. Trolox was used as a standard substance.

Ferric reducing antioxidant power assay (FRAP)

The ferric reducing antioxidant power assay was conducted following the protocol previously described by Thaipong et al. (2006). The antioxidant capacity of the samples was measured spectro-photometrically at 593 nm using a UV/Vis V-530 spectrophotometer. An analytical curve with different concentrations of Trolox (linearity: 50–750 µM; $R^2 = 0.9997$) was constructed to quantify the ferric reducing antioxidant power of the phenolic-rich extracts. The potential antioxidant activity was expressed as Trolox equivalent antioxidant capacity in µmol Trolox × g^{-1} of the extract.

Antibacterial activity

Bacterial strains

Bacterial cultures grown on Mueller-Hinton Agar (MHA) and then plates incubated overnight at 37ºC. These bacterial strains were stored at −80°C in Brucella Broth supplemented with 20% (v/v) glycerol and 5% of DMSO until use.

MIC determination

The antibacterial activity of phenolic-rich extracts (root barks, leaves, pulps, and seeds) was evaluated using the Minimal Inhibitory Concentration (MIC) via the microbroth dilution method. The extracts were tested against bacterial strains including *Escherichia coli* (*E. coli*, ATCC 25922), Meticillin-Sensitive *Staphylococcus aureus* (MSSA), Methicillin-resistant *Staphylococcus aureus* (MRSA, ATCC 6538), and *Staphylococcus epidermis* (*S. epidermis*, clinical isolate). Briefly, bacterial strains in the exponential growth phase were suspended in Mueller-Hinton Broth (MHB) to reach a final inoculum concentration of $1x10^5$ CFU mL^{-1}, following the in Clinical and Laboratory Standards Institute guidelines (Clinical and Laboratory Standards Institute [CLSI] 2018).

In 96-well plates, serial dilutions of phenolic-rich extracts from *Z. lotus* were prepared, spanning a concentration range of 8 to 2048 µg mL^{-1}, using a stock solution of 50 mg mL^{-1} in DMSO or acetone. Additionally, the following controls were included: i) Solvent control: bacterial cultures with 4% (v/v) of DMSO or acetone; ii) Growth control: pure cultures (only bacterial inoculum); and iii) Sterility control: culture media. Each extract was tested in triplicate, and three independent experiments were conducted. The MIC values were determined after 24 hours of incubation at 37°C using the Resazurin assay, adapted from Sarker et al. (2007). The MIC value was defined as the minimum concentration of the tested sample at which the blue color of resazurin changed to pink and became fluorescent due to reduction to Resorufin by oxidoreductases within viable cells.

Statistical analysis

All data are presented as means ± SD. The Pearson correlation was performed to evaluate the correlation between the total phenolic content, total anthocyanin content, and antioxidant activities within the *Z. lotus* extracts under investigation, with significance levels set at 0.01 and 0.05.

Results and discussion

The phytochemical screening

The qualitative analysis of phytochemicals in the phenolic-enriched extracts from various parts of *Z. lotus* is detailed in Table 1. It was noted that all extracts contained phenols, flavonoids, gallic tannins, catechic tannins, and mucilage. Among these constituents, the leaf extract encompassed all phytochemicals except for terpenoids and coumarine, the latter being absent in all *Z. lotus* extracts except for terpenoids, which were solely present in the root barks. Alkaloids, phenolics, terpenenoids, and flavonoids are considered the most vital types of phytochemicals, as consistently reported across diverse investigations into various parts of *Z. lotus* (Cadi et al., 2022; Ghedira et al., 1995; Le Crouéour et al., 2002; Marmouzi et al., 2019; Rached et al., 2019; Yahia et al., 2020; Ghedira et al., 1993). These compounds are acknowledged for their significant role in the bioactive properties of medicinal plants, hence attributing to the observed antioxidant and antibacterial activities in the plant extract employed in this study.

Table 1. Phytochemical screening of *Zizyphus lotus* extracts.

Phytochemical groups		Alc	TG	TC	Tp	Mc	Coum	Pol	Sp	Flv
Roots bark		+	++	++	+	+	-	+	-	+
Leaves		+	+	+	-	++	-	++	+	++
Fruits	Seeds	-	+	+	-	+	-	+	-	+
	Pulp	-	+	+	-	++	-	+	++	+

Alc: Alkaloids, TG: gallic tannins, TC: catechic tannins, Tp: Terpenoids, Mc: Mucilage, Coum: Coumarine, Pol: Polyphenols, Sp: Saponins, Flv: Flavonoides. (-): absence of compound; (+): low compound content; (++): medium compound content.

Extraction yield, total phenolic and anthocyanins content

The Table 2 illustrates the yields of phenolic-enriched extraction from different parts of *Z. lotus*, including seeds, pulps, leaves, and root barks, along with their respective total phenolic compound content and total anthocyanin content. Notably, the pulp extract exhibits a significantly higher extraction yield (70.9%), followed by the extracts from leaves and root barks, whereas the seeds exhibit the lowest extraction yield at 5.9%. These findings contrast with those of Letaief et al. (2021), who reported that the aqueous extract of *Z. lotus* fruit had the highest yield, followed by leaves, then roots.

The total phenolic content varies across *Z. lotus* extracts, ranging from 64.4 mg g^{-1} in seeds to 192.6 mg g^{-1} in leaves, as outlined in Table 2. When compared to existing literature, the TPCs of pulp and seed extracts notably exceed those of the hydromethanolic fruit extract obtained from the Guercif region (4.8 mg GAE g^{-1} of dry matter) (Cadi et al., 2022). Furthermore, the TPC of the seed extract surpasses that reported for the aqueous extract from Fez (Zouagha-Moulay Yaâcoub) (23.54 mg g^{-1}) (Chaimae et al., 2019). However, it's important to note that several factors, including phenological stage, climatic conditions, extraction methods, and geographical location, may influence the content of phenolic compounds (Rached et al., 2019). To the best of our knowledge, the total phenolic content of the hydromethanolic extract from leaves and root barks of Morocco has not been previously studied.

In the evaluation of total anthocyanin content (Table 2), the root barks extract demonstrated the highest levels of these compounds (9.1 mg g-1 extract). Comparable total anthocyanin content was observed for pulp and leaf extracts. However, the seeds extract exhibited a lower content of these secondary metabolites (0.7 mg g-1 extract). As far as we know, this study marks the first exploration of the total anthocyanin content in the leaves, root barks, pulps, and seeds of *Z. lotus*. The only existing data pertains to the hydromethanolic fruit extract from the Guercif region, which we found to be less intriguing when compared to our results (Cadi et al., 2022).

Table 2. Extraction yield, total phenolic content and total anthocyanin content of methanol/water (v/v) extracts from *Zizyphus lotus*.

Z. lotus		Extraction yields (% w/w)	Total phenolic content mg GAE g^{-1} extract	Total anthocyanin content cyn-3-glcEq mg g^{-1} extract
Fruits	Pulp	70.9 ± 0.6[a]	51 ± 2.4[d]	2.6 ± 0.5[f]
	Seeds	5.9 ± 0.2[b]	64.4 ± 1.6[b]	0.7 ± 0.03[b]
Leaves		38.9 ± 1.5[b]	192.6 ± 4.9[b]	2.9 ± 0.4[b]
Root barks		27.6 ± 1.4[c]	83.1 ± 5.6[e]	9.1 ± 0.8[g]

Data are reported as mean (n = 3)±SD.[a-g] Different letters within the same column indicate significant differences between mean values (one-way ANOVA followed by Tukey-Kramer multiple comparisons test, $p < 0.05$).

Antioxidant activities of *Zizyphus lotus* extracts

Phenolic compounds are recognized as the primary bioactive components in plants, possessing antioxidant properties to neutralize free radicals, engage in the restoration of other antioxidants, and shield cellular components from oxidative harm. In this investigation, we evaluated the free radical scavenging capabilities of *Z. lotus* phenolic-rich extract through DPPH and ABTS assays, and its ferric reducing abilities using the FRAP assay. The findings are outlined in Table 3. Additionally, we assessed the DPPH scavenging capacity of ascorbic acid and BHT for comparison. In our study, we observed distinct antioxidant activity among the four parts of *Z. lotus* phenolic-enriched extracts. This variation could be attributed to the unique profile of phenolic compounds in each fraction, particularly flavonoid derivatives.

Table 3. Antioxidant activities of phenolic-rich extract of different morphological parts of *Zizyphus lotus* by DPPH, ABTS, and FRAP assay.

Z. lotus extract		Antioxidant Assay		
		DPPH IC50 (µg mL^{-1})	ABTS IC50 (µg mL^{-1})	FRAP (µM Trolox equiv (TE) g^{-1} extract)
Fruits	Pulp	44.71 ± 1.75	97.20 ± 1.95	86 ± 1.2
	Seeds	75.45 ± 9.04	137.09 ± 1.07	51 ± 1.03
Leaves		9.68 ± 0.9	56.12 ± 0.47	245 ± 6.18
Root barks		5.97 ± 0.57	69.16 ± 0.53	601 ± 4.90
Ascorbic acid		2.44 ± 0.42	-	-
BHT		11.30 ± 2.17	-	-

Data are reported as mean (n = 3) ± SD.

DPPH scavenging effect of *Zizyphus lotus*

In the DPPH assay, the root barks and leaves phenolic-rich extracts of *Z. Lotus* exhibited stronger scavenging potential with IC50 values of 5.97 and 9.68 µg mL^{-1}, respectively (Table 2). The IC50 value of root

barks was higher than the other extracts and also showed good activity compared to the synthetic antioxidant BHT used as a positive control (IC50 = 11.30 µg mL^{-1}). Leaves also presented good activity compared to BHT, while both extracts exhibit weak activity against ascorbic acid (IC50=2.44 µg mL^{-1}). Moreover, the antioxidant activity of the leaves was found to be higher than that reported for the methanolic extract (IC50 = 700 µg mL^{-1}) in the Ihahen region (Aya & M'Hamed, 2020). Therefore, the antioxidant activity of root barks from the Moroccan region has not been previously studied. The only available results pertain to roots from the Oudhref-Gabes Region in the South of Tunisia. Our results show higher antioxidant activity compared to those reported in this study for both the methanolic extract (IC50 = 18.03 µg mL^{-1}) and the aqueous extract (IC50 = 16.46 µg mL^{-1}) (Letaief et al., 2021). Pulp extract exhibited moderate scavenging activity, showing higher activity than that reported for methanolic fruit extracts (IC50 = 5000 µg mL^{-1} and IC50 = 131 µg mL^{-1}) in the region of Ihahen (Aya & M'Hamed, 2020) and the Zaouiat Cheikh Area, Oued Zem City (Belmaghraoui et al., 2018), respectively, and higher than that reported for aqueous fruit extract (IC50 = 116 µg mL^{-1}) in Northeastern (Bencheikh et al., 2021). To our knowledge, the DPPH activity of the pulp extract has not been studied previously. However, seeds exhibited lower antioxidant activity among the studied parts, with a higher IC50 value (75.45 µg mL^{-1}). Our results were higher compared to those reported for both the methanolic extract (IC50 = 1300 µg mL^{-1}) and the aqueous extract (IC50 = 3110 µg mL^{-1}) in the Zouagha-Moulay Yaâcoub, Fez (Chaimae et al., 2019).

ABTS scavenging effect

The results of the ABTS radical scavenging activity are presented in Table 2, which showed similar trends to those observed in the DPPH radical scavenging activity. Leaves extract exhibited the highest capacity, followed by root barks with an IC50 value of 69.16 µg mL^{-1}. Consistent with the DPPH scavenging assay, the pulp and seeds phenolic-rich extracts of *Z. lotus* showed the weakest activity. The ABTS activity root barks has not been previously studied in Moroccan regions. While seeds and pulp followed the same order as observed in the studied *Z. lotus* aqueous extracts from Settat and Khouribga (El Maaiden et al., 2020). The value of the pulp was found to be lower than that reported for methanolic fruit extract (IC50 = 52.42 µg mL^{-1}) in the Zaouiat Cheikh Area, Oued Zem City (Belmaghraoui et al., 2018), while it is higher than the one reported for Tunisia in methanolic extract (IC50 = 173.93 µg mL^{-1}) and aqueous extract (IC50 = 342.25 µg mL^{-1}).

FRAP reducing power

The FRAP assessment provides clear information about the electron transfer potency of an antioxidant, offering a simple, rapid, and relatively inexpensive assay. Among the four parts, root barks exhibited the highest FRAP, while seeds showed the lowest, with concentrations ranging from 601 and 51 µM (TE) g^{-1} extract, respectively. These findings are consistent with the ABTS and DPPH radical scavenging activity results. Marmouzi et al. (2019) previously studied the FRAP activity of *Z. lotus* aqueous leaves and fruit extracts. They expressed the results as ascorbic acid equivalents per gram of extract and found that *Z. lotus* leaves extract exhibited the highest antioxidant capacities compared to the fruit, which is consistent with our results. However, seeds, pulp, and root barks have not been previously studied in this context.

Correlation between total anthocyanin content, total phenolic content, and antioxidant activities

To improve comprehension of the relationship between the antioxidant activity of *Z. lotus* and its phenolic compounds, we analyzed all prepared extracts. This analysis aimed to evaluate the correlation between TPC, TAC, and the results obtained from the DPPH, ABTS, and FRAP assays. In reference to Table 4, a statistically significant correlation was observed between the three different assays: DPPH, ABTS, and FRAP. This finding is consistent with previous studies (Dudonné et al., 2009; Floegel et al., 2011). Moreover, it was noted that a significant correlation ($r = 0.999$, $p < 0.01$) specifically existed between the ABTS and DPPH assays, suggesting a high degree of consistency between these two methods in assessing antioxidant activity.

In many cases, a positive correlation has been observed between the total phenolic content and antioxidant activities (Kowalczyk et al., 2013; Kumar et al., 2014; Muflihah et al., 2021). This suggests that phenolic compound contribute significantly to the antioxidant capacity of a sample. Therefore, in current study, a weak correlation was found between the antioxidant parameters and the total phenolic content determined by the Folin-Ciocalteu assay (Table 2). These results are likely explained by that Folin assay measures the total phenolic content but does not differentiate between individual phenolic compounds. Different phenolic compounds have varying antioxidant capacities, and their composition

and concentration in the sample can influence the overall antioxidant activity. Further, the lack of a correlation could be also explained by variety of compounds in the plant extracts besides phenolics that can contribute to antioxidant activity. These include vitamins, minerals, pigments, and other phytochemicals (Sánchez-Rangel et al., 2013). The presence of these compounds may confound the relationship between the total phenolic content and antioxidant activity. However, these findings cannot be generalized. Other studies have indeed reported strong correlations for all three assays in *Z. lotus* fruit extract (Bouzid et al., 2022), as well as for the DPPH assay in methanol extracts from various parts of *Z. lotus* (leaves, fruits, and seeds) (Yahia et al., 2020).

Anthocyanins are a group of water-soluble pigments found in various fruits, vegetables, and flowers, renowned for their potent antioxidant properties. Numerous studies have confirmed the correlation between anthocyanins and antioxidant activities (Khoo et al., 2017; Kharadze et al., 2018; Kumari et al., 2022). According to Table 4, a negative correlation between anthocyanins and antioxidant activity, as assessed by DPPH and ABTS assays, was observed. This suggests that the presence of anthocyanin may not significantly contribute to the scavenging of free radicals in these assays. Conversely, a positive correlation between anthocyanins and antioxidant activity, as measured by the FRAP assay, was noted. This indicates that higher levels of anthocyanins are associated with increased antioxidant activity, as indicated by the reducing power measured by the FRAP assay.

Table 4. Linear correlation coefficients (r) between total anthocyanin content, total phenolic content and antioxidant assays (DPPH, ABTS, and FRAP) in *Z. lotus* extracts obtained by Pearson's analysis.

Parameters	DPPH	ABTS	FRAP	TPC	TAC
DPPH	1				
ABTS	0,999**	1			
FRAP	-0,916	-0,904	1		
TPC	-0,235	-0,264	0,094	1	
TAC	-0,949	-0,953*	0,754	0,182	1

**Significant correlation with $p < 0.01$;* significant correlation with $p < 0.05$

Antibacterial activity

Given the ongoing reports of bacterial resistance to antibiotics, the urgency for discovering potent and novel antibacterial agents becomes increasingly apparent (Chaturvedi & Dwivedi, 2017). Numerous studies have established a correlation between the inhibitory effect of plant extracts against bacterial pathogens and their phenolic composition (Baydar et al., 2004; Rodríguez Vaquero et al., 2007; Álvarez-Martínez et al., 2021). Thus, the antibacterial activity of diverse parts of *Z. lotus* phenolic-enriched extracts was assessed using the Resazurin assay against both Gram-negative and Gram-positive bacterial strains, as outlined in Table 5. The root bark extracts exhibited the most potent activity, with MIC values ranging from 1024 to 2048 µg mL^{-1}, and MRSA displayed heightened sensitivity to the root bark extract (MIC = 1024 µg mL^{-1}). The leaf extract also demonstrated antibacterial effects, with MIC values comparable to those of the root bark extract (MIC = 2048 µg mL^{-1}). However, the seed and pulp extracts failed to inhibit bacterial growth within the tested concentration range (8–2048 µg mL^{-1}), with *S. epidermidis* proving to be the most resistant strain (MIC > 2048 µg mL^{-1}). Generally, the antibacterial efficacy of plants is contingent upon their chemical composition and geographical origin (Al-mariri & Safi 2014).

Table 5. MIC values of *Z. lotus* extracts against *E. coli*, MSSA, MRSA, and *S. epidermidis*, determined through Resazurin assay.

Z. lotus extracts		MIC (µg mL^{-1})			
		E. coli	MSSA	*S. epidermidis*	MRSA
Root barks		2048	2048	>2048	1024
Leaves		2048	2048	>2048	2048
Fruits	Seeds	>2048	>2048	>2048	>2048
	Pulp	>2048	>2048	>2048	>2048

Both methanolic and aqueous seed extracts from Fez (Zouagha-Moulay Yaâcoub) City, exhibited respective MIC and IC50 values (MIC= 100 mg mL^{-1} and IC50= 200 mg mL^{-1}), against *E. coli* (Chaimae et al., 2019). Additionally, a study investigating the antibacterial activity of methanolic extract of *Z. lotus* fruits in Zaouiat Cheikh, Oued Zem City, using disc diffusion and micro-dilution methods, demonstrated notable

efficacy against *E. coli*, with a MIC range from 400 µg mL^{-1} (Belmaghraoui et al., 2018). Leaves, pulp, and root barks from Morocco have not yet been subjected to antibacterial investigations. Additionally, the specific antibacterial potential of pulps and root barks remains unexplored in the existing literature. The efficacy of the leaf extract against *E. coli* was found to be weaker compared to the methanolic leaf extract from Libya (MIC = 1000 µg mL^{-1}) (Naili et al., 2010) yet stronger than the reported value for methanolic leaf extract from Tunisia (MIC = 10 µg mL^{-1}) (Yahia et al., 2020). Notably, the substantial potential of *Z. lotus* root bark against MRSA, a highly resistant bacterial strain, underscores a promising avenue for the development of natural antimicrobial agents sourced from botanical origins. This investigation sheds light on a promising pathway for the development of natural antimicrobial agents derived from botanical sources.

Conclusion

The study provides valuable insights into the pharmacological properties of four different parts of *Z. lotus* (leaves, seeds, pulp, and root bark) from the Beni Mellal region in Morocco, significantly enriching the existing literature in this field. It delves into their phytochemical profile, encompassing total phenolic content, total anthocyanin content, and explores their biological activities, including antioxidant and antibacterial properties. The results of this study unveil that the four parts of *Z. lotus* are rich in bioactive compounds, mainly polyphenols, flavonoids, mucilage, gallic tannins, and catechic tannins. The phenolic enriched extract of *Z. lotus* may be explored for the search of flavonoids, flavonols, etc. owing to their highest total phenolic compounds content in the current study. Meanwhile, the highest TAC content was observed in the root bark extract. Additionally, the evaluation of antioxidant and antibacterial activities revealed that extracts from both leaves and root bark can serve as sources of natural antioxidants and antibacterial agents. Taken all together, these findings provide a scientific basis to promote the value-added utilization of *Z. lotus* as a safe source of promising antioxidant and antibacterial agents.

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References

Abdoul-Azize, S. (2016). Potential benefits of jujube (*Zizyphus lotus* L.) bioactive compounds for nutrition and health. *Journal of Nutrition and Metabolism*, *2016*(2867470), 1-13. https://doi.org/10.1155/2016/2867470

Abdoul-Azize, S., Bendahmane, M., Hichami, A., Dramane, G., Simonin, A. M., Benammar, C., Sadou, H., Akpona, S., El Boustani, E. S., & Khan, N. A. (2013). Effects of *Zizyphus lotus* L. (Desf.) polyphenols on Jurkat cell signaling and proliferation. *International Immunopharmacology*, *15*(2), 364-371. https://doi.org/10.1016/j.intimp.2012.11.013

Al-Mariri, A., & Safi, M. (2014). In vitro antibacterial activity of several plant extracts and oils against some gram-negative bacteria. *Iranian Journal of Medical Sciences*, *39*(1), 36-43.

Álvarez-Martínez, F. J., Barrajón-Catalán, E., Herranz-López, M., & Micol, V. (2021). Antibacterial plant compounds, extracts and essential oils: An updated review on their effects and putative mechanisms of action. *Phytomedicine*, *90*, 1-16. https://doi.org/10.1016/j.phymed.2021.153626

Aya, K., & M'Hamed, T. (2020). Chemical compounds, antioxidant activity, and in vitro and in silico litholytic effects of *Zizyphus lotus* extracts. *Journal of Basic and Clinical Physiology and Pharmacology*, *31*(3), 1-12. https://doi.org/10.1515/jbcpp-2019-0091

Bakhtaoui, F.-Z., Lakmichi, H., Megraud, F., Chait, A., & Gadhi, C.-E. A. (2014) Gastroprotective, anti-Helicobacter pylori and, antioxidant properties of Moroccan *Zizyphus lotus* L. *Journal of Applied Pharmaceutical Science*, *4*(10), 81-87. https://doi.org/10.7324/JAPS.2014.401015

Banso, A., & Adeyemo, S. (2010). Phytochemical screening and antimicrobial assessment of *Abutilon mauritianum, Bacopa monnifera* and *Datura stramonium*. *Biokemistri*, *18*(1), 39-44. https://doi.org/10.4314/biokem.v18i1.56390

Baydar, N. G., Özkan, G., & Sağdiç, O. (2004). Total phenolic contents and antibacterial activities of grape (*Vitis vinifera* L.) extracts. *Food Control*, *15*(5), 335-339. https://doi.org/10.1016/S0956-7135(03)00083-5

Békro, Y.-A., Békro, J. A. M. A., Boua, B. B., Trabi, F. H., & Éhilé, E. E. (2008). Étude ethnobotanique et screening phytochimique de *Caesalpinia benthamiana* (Baill.) Herend. et Zarucchi (Caesalpiniaceae). *Science & Nature*, *4*(2), 217-225. https://doi.org/10.4314/scinat.v4i2.42146

Belmaghraoui, W., El Madani, N., Manni, A., Harir, M., Filali-Maltouf, A., El Hajjaji, S., & El Fatni, O. K. (2018). Total phenolic and flavonoid content, antioxidant and antibacterial activity of *Ziziphus lotus* from Morocco. *Pharmacologyonline*, *3*, 176-183.

Benammar, C., Baghdad, C., Belarbi, M., Subramaniam, S., Hichami, A., & Khan, N. A. (2014). Antidiabetic and antioxidant activities of *Zizyphus lotus* L. aqueous extracts in wistar rats. *Journal of Nutritional Science*, *8*, 1-6. https://doi.org/10.4172/2155-9600.S8-004

Benammar, C., Hichami, A., Yessoufou, A., Simonin, A.-M., Belarbi, M., Allali, H., & Khan, N. A. (2010). *Zizyphus lotus* L. (Desf.) modulates antioxidant activity and human T-cell proliferation. *BMC Complementary and Alternative Medicine*, *10*(54), 1-9. https://doi.org/10.1186/1472-6882-10-54

Bencheikh, N., Bouhrim, M., Kharchoufa, L., Al Kamaly, O. M., Mechchate, H., Es-Safi, I., Dahmani, A., Ouahhoud, S., El Assri, S., Eto, B., Bnouham, M., Choukri, M., & Elachouri, M. (2021). The nephroprotective effect of *Zizyphus lotus* L. (Desf.) fruits in a gentamicin-induced acute kidney injury model in rats: A biochemical and histopathological investigation. *Molecules*, *26*(16), 1-16. https://doi.org/10.3390/molecules26164806

Bencheikh, N., Bouhrim, M., Merrouni, I. A., Boutahiri, S., Kharchoufa, L., Addi, M., Tungmunnithum, D., Hano, C., Eto, B., Legssyer, A., & Elachouri, M. (2021). Antihyperlipidemic and antioxidant activities of flavonoid-rich extract of *Ziziphus lotus* (L.) Lam. Fruits. *Applied Sciences*, *11*(17), 1-13. https://doi.org/10.3390/app11177788

Borgi, W., Recio, M. C., Ríos, J. L., & Chouchane, N. (2008). Anti-inflammatory and analgesic activities of flavonoid and saponin fractions from *Zizyphus lotus* (L.) Lam. *South African Journal of Botany*, *74*(2), 320-324. https://doi.org/10.1016/j.sajb.2008.01.009

Bouzid, H. A., Sakar, E. H., Bijla, L., Ibourki, M., Zeroual, A., Gagour, J., Koubachi, .J, Majourhat, K., & Gharby, S. (2022). Physical fruit traits, proximate composition, antioxidant activity, and profiling of fatty acids and minerals of wild jujube (*Ziziphus lotus* L. (Desf .)) fruits from eleven Moroccan origins. *Journal of Food Quality*, *2022*, 1-15. https://doi.org/10.1155/2022/9362366

Chaimae, R., Meryem, B., Chaimae, S., Bouchamma, El-O., Hamza, E., Laila, E., Lahsen, El-G., & Meryem, B. (2019). Antimicrobial and radical scavenging activities of Moroccan *Ziziphus lotus* L. seeds. *Journal of Phytopharmacology*, *8*(4), 155-160. https://doi.org/10.31254/phyto.2019.8402

Chaturvedi, D., & Dwivedi, P. K. (2017). Recent developments on the antidiabetic sesquiterpene lactones and their semisynthetic analogues. In *Discovery and development of antidiabetic agents from natural products* (pp. 185-207). Elsevier. https://doi.org/10.1016/B978-0-12-809450-1.00006-5

Clinical and Laboratory Standards Institute [CLSI]. (2018). *M07: Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically* (11th ed.). CLSI.

Dahlia, F., Benito, C., & Boussaid, M. (2019). Genetic diversity of fruits in wild jujube (*Ziziphus lotus* L. desf.) natural populations from Algeria. *Agriculture & Forestry*, *65*(1), 165-183. https://doi.org/10.17707/AgricultForest.65.1.17

Dhibi, M., Amri, Z., Mnari, A., Hammami, S., & Hammami, M. (2022). Measurement: Food Comparative study of the phenolic profile and antioxidant activities of Moringa (*Moringa oleifera* Lam .) and Jujube (*Ziziphus lotus* Linn.) leaf extracts and their protective effects in frying stability of corn oil. *Measurement: Food*, *7*, 100045. https://doi.org/10.1016/j.meafoo.2022.100045

Dewanto, V., Wu, X., Adom, K. K., & Liu, R. H. (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *Journal of Agricultural and Food Chemistry*, *50*(10), 3010–3014. https://doi.org/10.1021/jf0115589.

Dudonné, S., Vitrac, X., Coutière, P., Woillez, M., & Mérillon, J.-M. (2009). Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial Interest Using DPPH, ABTS, FRAP, SOD, and ORAC assays. *Journal of Agricultural and Food Chemistry*, *57*(5), 1768-1774. https://doi.org/10.1021/jf803011r

El Cadi, H., El Bouzidi, H., Selama, G., El Cadi, A., Ramdan, B., El Majdoub, Y. O., Alibrando, F., Dugo, P., Mondello, L., Lanjri, A. F., Brigui, J., & Cacciola, F. (2022). Physico-chemical and phytochemical characterization of Moroccan wild jujube "*Zizyphus lotus* (L.)" fruit crude extract and fractions. *Molecules*, *25*(22), 1-27. https://doi.org/10.3390/molecules25225237

El Maaiden, E., El Kharrassi, Y., Lamaoui, M., Allai, L., Essamadi, A. K., Nasser, B., & Moustaid, K. (2020). Variation in minerals, polyphenolics and antioxidant activity of pulp, seed and almond of different *Ziziphus* species grown in Morocco. *Brazilian Journal of Food Technology*, *23*, 1-7. https://doi.org/10.1590/1981-6723.20619

Floegel, A., Kim, D.-O., Chung, S.-J., Koo, S. I., & Chun, O. K. (2011). Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. *Journal of Food Composition and Analysis*, *24*(7), 1043-1048. https://doi.org/10.1016/j.jfca.2011.01.008

Ghedira, K., Chemli, R., Caron, C., Nuzilard, J.-M., Zeches, M., & Le Men-Olivier, L. (1995). Four cyclopeptide alkaloids from *Zizyphus lotus*. *Phytochemistry*, *38*(3), 767-772. https://doi.org/10.1016/0031-9422(94)00669-K

Ghedira, K., Chemli, R., Richard, B., Nwllard, J.-M., Zeches, M., & Le Men-Olivier, L. (1993). Two Cyclopeptide alkaloids from *Zizyphus lotus*. *Phytochemistry*, *32*(6), 1591-1594. https://doi.org/10.1016/0031-9422(93)85186-U

Gini, T. G., & Jeya Jothi, G. (2018). Column chromatography and HPLC analysis of phenolic compounds in the fractions of *Salvinia molesta* Mitchell. *Egyptian Journal of Basic and Applied Sciences*, *5*(3), 197-203. https://doi.org/10.1016/J.EJBAS.2018.05.010

Hammi, K. M., Jdey, A., Abdelly, C., Majdoub, H., & Ksouri, R. (2015). Optimization of ultrasound-assisted extraction of antioxidant compounds from Tunisian *Zizyphus lotus* fruits using response surface methodology. *Food Chemistry*, *184*, 80-89. https://doi.org/10.1016/j.foodchem.2015.03.047

Iqbal, E., Abu, K., & Lim, L. B. L. (2015). Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of *Goniothalamus velutinus* (Airy Shaw) from Brunei Darussalam. *Journal of King Saud University Science*, *27*(3), 224-232. https://doi.org/10.1016/j.jksus.2015.02.003

Karumi, Y., Onyeyili, P. A., & Ogugbuaja, V. O. (2004). Identification of active principles of *M. balsamina* (balsam apple) leaf extract. *Journal of Medical Sciences*, *4*(3), 179-182. https://doi.org/10.3923/jms.2004.179.182

Kharadze, M., Japaridze, I., Kalandia, A., & Vanidze, M. (2018). Anthocyanins and antioxidant activity of red wines made from endemic grape varieties. *Annals of Agrarian Science*, *16*(2), 181-184. https://doi.org/10.1016/j.aasci.2018.04.006

Khoo, H. E., Azlan, A., Tang, S. T., & Lim, S. M. (2017). Anthocyanidins and anthocyanins: Colored pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food & Nutrition Research*, *61*(1), 1-17. https://doi.org/10.1080/16546628.2017.1361779

Khouchlaa, A., Talbaoui, A., El Yahyaoui-El Idrissi, A., Bouyahya, A., Lahsen, S. A., Kahouadji, A., & Tijane, M. (2018). Détermination des composés phénoliques et évaluation de l'activité litholytique *in vitro* sur la lithiase urinaire d'extrait de *Zizyphus lotus* L. d'origine Marocaine. *Phytothérapie*, 1-7. https://doi.org/10.1007/s10298-017-1106-3

Kowalczyk, D., Świeca, M., Cichocka, J., & Gawlik-Dziki, U. (2013). The phenolic content and antioxidant activity of the aqueous and hydroalcoholic extracts of hops and their pellets. *Journal of Institute of Brewing and Distilling*, *119*(3), 103-110. https://doi.org/10.1002/jib.73

Kumar, S., Sandhir, R., & Ojha, S. (2014). Evaluation of antioxidant activity and total phenol in different varieties of *Lantana camara* leaves. *BMC Research Notes*, *7*(560), 1-9. https://doi.org/10.1186/1756-0500-7-560

Kumari, P., Raju, D. V. S., Prasad, K. V., Saha, S., Panwar, S., Paul, S., Banyal, N., Bains, A., Chawla, P., Fogarasi, M., & Fogarasi, S. (2022). Characterization of anthocyanins and their antioxidant activities in Indian rose varieties (Rosa × hybrida) using HPLC. *Antioxidants*, *11*(10), 1-18. https://doi.org/10.3390/antiox11102032

Le Crouéour, G., Thépenier, P., Richard, B., Petermann, C., Ghédira, K., & Zèches-Hanrot., M. (2002) Lotusine G: A new cyclopeptide alkaloid from *Zizyphus lotus*. *Fitoterapia*, *73*(1), 63-68. https://doi.org/10.1016/S0367-326X(01)00363-X

Lee, J., Durst, R. W., & Wrolstad, R. E. (2005). Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: Collaborative study. *Journal of AOAC International*, *88*(5), 1269-1278. https://doi.org/10.5555/jaoi.2005.88.5.1269

Letaief, T., Garzoli, S., Masci, V. L., Mejri, J., Abderrabba, M., Tiezzi, A., & Ovidi, E. (2021). Chemical composition and biological activities of tunisian *Ziziphus lotus* extracts: Evaluation of drying effect, solvent extraction, and extracted plant parts. *Plants*, *10*(12), 1-15. https://doi.org/10.3390/plants10122651

Maciuk, A., Ghedira, K., Thepenier, P., Lavaud, C., & Zeches-Hanrot, M. (2003). A new flavonol glycoside from leaves of *Zizyphus lotus*. *Pharmazie*, *58*(2), 158-159. https://doi.org/10.1002/chin.200323205

Maciuk, A., Lavaud, C., Thépenier, P., Jacquier, M. J., Ghédira, K., & Zèches-Hanrot, M. (2004). Four new dammarane saponins from *Zizyphus lotus*. *Journal of Natural Products*, *67*(10), 1639-1643. https://doi.org/10.1021/np0499362

Marmouzi, I., Kharbach, M., El Jemli, M., Bouyahya, A., Cherrah, Y., Bouklouze, A., Heyden, Y. V., & El Abbes Faouzi, M. (2019). Antidiabetic, dermatoprotective, antioxidant and chemical functionalities in *Zizyphus lotus* leaves and fruits. *Industrial Crops & Products*, *132*, 134-139. https://doi.org/10.1016/j.indcrop.2019.02.007

Muflihah, Y. M., Gollavelli, G., & Ling, Y.-C. (2021). Correlation study of antioxidant activity with phenolic and flavonoid compounds in 12 indonesian indigenous herbs. *Antioxidants*, *10*(10), 1-15. https://doi.org/10.3390/antiox10101530

N'Guessan, K, Kadja, B., ZIrihi, G. N, Traoré, D, & Aké-Assi, L. (2009). Screening phytochimique de quelques plantes médicinales ivoiriennes utilisées en pays Krobou (Agboville, Côte-d'Ivoire). *Science of Nature*, *6*(1), 1-15. https://doi.org/10.4314/scinat.v6i1.48575

Naili, M. B., Alghazeer, R. O., Saleh, N. A, & Al-Najjar, A. Y. (2010). Evaluation of antibacterial and antioxidant activities of *Artemisia campestris* (Astraceae) and *Ziziphus lotus* (Rhamnacea). *Arabian Journal of Chemistry*, *3*(2), 79-84. https://doi.org/10.1016/j.arabjc.2010.02.002

Rached, W., Barros, L., Ziani, B. E. C., Bennaceur, M., Calhelha, R. C., Heleno, S. A., Alves, M. J., Marouf, A., & Ferreira, I. C. F. R. (2019). HPLC-DAD-ESI-MS/MS screening of phytochemical compounds and the bioactive properties of different plant parts of *Zizyphus lotus* (L.) Desf. *Food and Function*, *10*(9), 5898-5909. https://doi.org/10.1039/c9fo01423c

Re, R., Pellegrini, N, Proteggente. A, Pannala. A, Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved abts radical cation decolorization assay. *Free Radical Biology & Medicine*, *26*(9-10), 1231-1237. https://doi.org/10.1016/S0891-5849(98)00315-3

Regehr, D. L., & El Brahli, A. (1995). Wild Jujube (*Ziziphus lotus*) control in Morocco. *Weed Technology*, *9*(2), 326-330.

Rodríguez Vaquero, M. J., Alberto, M. R., & Manca de Nadra, M. C. (2007). Antibacterial effect of phenolic compounds from different wines. *Food Control*, *18*(2), 93-101. https://doi.org/10.1016/j.foodcont.2005.08.010

Rsaissi, N., El Kamili, Bencharki, B., Hillali, L., & Bouhache, M. (2013) Antimicrobial activity of fruits extracts of the wild jujube "*Ziziphus lotus*" (L.) Desf. *International Journal of Engineering Research*, *4*(9), 1521-1528.

Sarker, S. D., Nahar, L., & Kumarasamy, Y. (2007). Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the in vitro antibacterial screening of phytochemicals. *Methods*, *42*(4), 321-324. https://doi.org/10.1016/j.ymeth.2007.01.006

Sánchez-Rangel, J. C., Benavides, J., Heredia, J. B., Cisneros-Zevallos, L., & Jacobo-Velázquez, D. A. (2013). The Folin-Ciocalteu assay revisited: Improvement of its specificity for total phenolic content determination. *Analytical Methods*, *5*(21), 5990-5999. https://doi.org/10.1039/c3ay41125g

Tepe, B, Daferera, D, Sokmen, A, Sokmen, M, & Polissiou, M. (2005). Antimicrobial and antioxidant activities of the essential oil and various extracts of *Salvia tomentosa* Miller (Laiaceae). *Food Chemistry*, *90*(3), 333-340. https://doi.org/10.1016/j.foodchem.2003.09.013

Thaipong, K, Boonprakob, U, Crosby, K, Cisneros-Zevallos, L, & Byrne, D. H. (2006). Comparison of ABTS , DPPH , FRAP , and ORAC assays for estimating antioxidant activity from guava fruit extracts. *Journal of Food Composition and Analysis*, *19*(6-7), 669-675. https://doi.org/10.1016/j.jfca.2006.01.003

Yahia, Y., Benabderrahim, M. A., Tlili, N., Bagues, M., & Nagaz, K. (2020). Bioactive compounds, antioxidant and antimicrobial activities of extracts from different plant parts of two *Ziziphus* Mill. species. *PLoS ONE*, *15*(5), 1-16. https://doi.org/10.1371/journal.pone.0232599

Yeo, D., Dinica, R., Yapi, H. F., Furdui, B., Praisler, M., Djaman, A. J., & N'Guessan, J. D. (2011). Évaluation de l'activité anti-inflammatoire et screening phytochimique des feuilles de *Annona s enegalensis*. *Thérapie*, *66*(1), 73-80. https://doi.org/10.2515/therapie/2010076