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Assessment of phytochemical, antioxidant and antibacterial properties of *ziziphus lotus* leaves extracts

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Abstract Herbal medicines are used to treat a variety of chronic and acute diseases with little to no harmful side effects. They have played a significant part in health systems throughout the world. The phytochemical profile, antioxidant activity, and antibacterial activity of different extracts of *Ziziphus lotus* leaves (ZLL) were the subject of this study. According to the analysis of the extraction yield, the aqueous extract had the highest yield, followed by methanol, ethanol, and last, hexane ($p<0.05$). Phytochemical analysis of ZLL extracts revealed the presence of several bioactive molecules such as phenolic compounds and alkaloids. Water, methanol 50%, methanol 80%, methanol, ethanol, and hexane are the 6 different solvents which were used in order to evaluate the phytochemical profile as well as the biological activities of ZLL, and whose aqueous extract showed the best results. The aqueous extract showed the highest total contents of phenols, flavonoids, and tannins (120,53± 0. 37 mg GAE/g DM, 60.15 ± 0.06 mg QE/g DM, and 09.41 ± 0.36 mg CE/g DM, respectively), while the hexane extract revealed the lowest contents (20.42 ± 0.50 mg GAE/g DM, 14.45± 0.36 mg QE/g DM, and 04.20 ± 0.17 mg CE/g DM, respectively). By using the DPPH, ABTS, and FRAP methods, ZLL extracts demonstrated considerable antioxidant capacities, with the values IC50=31.20 ±0.69, IC50=61.35±0.30 and IC0.5=23.89±0.80, respectively, and this for the aqueous extract. All of the ZLL extracts, showed antibacterial efficacy against the bacterial strains of *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.

Keywords: phytochemistry, polyphenols, biological activity, phytotherapy , bacteria

1. Introduction

Because of its accessibility and wealth of resources, humanity has always prioritized using the plant kingdom for its many species (Cantwell-Jones et al., 2022). Plants are producers that occupy the pyramidal base of trophic networks due to their autotrophy and are the main source of carbon for herbivores and other living organisms in higher trophic links (Safi et al., 2019). Currently, exploratory studies of the botanical world are becoming increasingly focused, taking into account the nutritional and pharmacopic qualities of several plant species. Traditional medicine has found medication in a range of plant species, corroborated by numerous ethnobotanical studies investigating the practices used by populations of multiple ethnicities and geographies (Süntar, 2020).

The phytochemical exploration of the composition of plants, especially those qualified for medicinal use, has shown through extensive studies that a significant arsenal of secondary metabolites enriches their biochemical composition and is involved in their various pharmacological, nutritional, and ecological qualities, represented mainly by phenolic compounds, terpenoids, and alkaloids (Singh et al., 2023).

The normal functioning of human cells requires mitochondrial oxidation of energy metabolites involving the reduction of oxygen, which generates free radicals capable of oxidizing biological molecules and consequently altering their functions, such as the phospholipids of membrane bilayers, proteins, and DNA, which contributes to oxidative stress at the origin of several cellular dysfunctions and pathologies at the body level, such as diabetes, neurodegenerative diseases, hepatotoxicity, cardiovascular diseases, and cancer (Nagakannan et al., 2020).

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Thus, the increase in the rates of bacterial resistance to antibiotics and the widespread health problems linked to free radicals have motivated further research on great botanical gardens worldwide to explore in depth the different species of plants capable of being exploited for their antioxidant and pharmacological properties (AlSheikh et al., 2020).

Due to the diversity of edaphic and microclimatic factors, Morocco is considered a vast collection of plant species with important medicinal, aromatic, and taxonomic properties distributed throughout its territory (El Massoudi et al., 2019).

The jujube tree, *Ziziphus lotus*, is a shrub with dense thorny branches that can colonize vast regions of the world characterized by its arid climate, including Moroccan soil (Dahlia et al., 2020). *Ziziphus lotus* is known in Morocco by the name Sedra; it produces a small, reddish, red, and ripe fruit that is marketed for consumption in the fresh state and is known among local populations by the name Nbeg (Zarroug et al., 2021). The leaves of the shrub have been known for several years in the Arab-Muslim world, being cited in the Koran and being used in a therapeutic and preventive practice of a spiritual nature called Roquia (Elkhalloufi et al., 2021). The fruits of *Ziziphus lotus*, which have been used for a long time in traditional medicine, particularly in Morocco, are consumed to treat stomach and intestinal ailments as well as certain forms of anemia (Fadili et al., 2017) or are even utilized as a decoction or infusion before being given to patients with kidney stones (Khouchlaa et al., 2017). Thus, several recent studies have explored the phytochemical profile and evaluated the biological activities of *Zizyphus lotus* (Rached et al., 2019), as well as the use of its biomass for environmental applications (El Yakoubi et al., 2023 a; El Yakoubi et al., 2023b).

This study aimed to study, on the one hand, the phytochemical composition related to total phenolic compounds, total flavonoids and total tannins in several types of *Ziziphus lotus* leaf (ZLL) extracts and, on the other hand, to evaluate the quality of these extracts for their antioxidant capacities and antibacterial effects.

2. Materials and Methods

2.1. Leaf sampling

In September 2021, *Ziziphus lotus* shrubs were taxonomically identified due to differences in their morphological traits, which are expressed by differences in the vegetative apparatus of the plant and, more precisely, its fruits. Thus, the leaves were collected, returned to the laboratory to be washed with distilled water before being sun-dried, reduced to powder, packed in plastic bags and kept at a temperature of 20°C.

2.2. Phytochemical analysis of the ZLL extracts

Using hexane, ethanol, water, and methanol, 4 solutions containing 200 ml each were prepared. Then, 20 g of *Ziziphus lotus* leaf (ZLL) powder was added to each of the four solutions. For the aqueous extract, the mixture was boiled for 5 hours. For extraction with organic solvents, a Soxhlet extractor was used for a period of 5 hours before the crude extract was filtered and concentrated. Then, each extract was placed in a bottle and kept at 20°C.

2.3. Extraction yield

The extraction yield was estimated by applying the following formula (1):

$$Y(\%) = \frac{m_1}{m_2} \times 100 \quad (1)$$

where m_1 = the mass (g) of the ZLL extract and m_2 = the mass (g) of the raw material (ZLL powder).

2.4. Qualitative analysis of ZLL extracts

The ZLL extracts were subjected to qualitative analysis of phenolic compounds (flavonoids and tannins), terpenoids, alkaloids, amino acids, proteins, and reducing sugars.

2.4.1. Flavonoïdes

Four milliliters of diluted ammonia and a few drops of sulfuric acid were added to 50 mL of each of the four extracts. Yellowing of the reaction mixture indicates the presence of flavonoids.

2.4.2. Tanins

For each extract, a few drops of ferric chloride ($FeCl_3$) were added to a volume of 50 mL. Thus, the production of a precipitate with a color ranging from blue to black indicates the presence of tannins in a solution.

2.4.3. Terpenoids (Salkowski test)

For 4 mL of each extract, 2 mL of chloroform ($CHCl_3$) and 3 mL of concentrated sulfuric acid (H_2SO_4) were carefully added. The presence of terpenoids was revealed by the formation of a brownish-red ring.

2.4.4. Alkaloids

The presence of alkaloids was determined by the Wagner test. Thus, by adding a few drops of Wagner's reagent to 4 mL of each extract, the solution formed a red–brown precipitate if it contained alkaloids.

2.4.5. Amino acids

The ninhydrin test makes it possible to determine the presence of free amino acids in solution. By adding 2 to 3 drops of the reagent to 4 mL of extract, the solution turned purple if the amino acids were present.

2.4.7. Protein

By adding Biuret's reagent, the solution changes color to a mauve-violet, which indicates the presence of peptide bonds that are typical of proteins and peptides. Thus, two drops of copper sulfate ($CuSO_4$) and 1 mL of a base such as potassium hydroxide (KOH) were added to 4 mL of the extract.

2.4.8. Reducing sugars

A few drops of Fehling's solutions A and B were added to 4 mL of extract. The presence of reducing sugars is revealed by the presence of a red–orange precipitate.

2.5. Quantitative analysis of ZLL extracts

2.5.1. Total phenolic content (TPC)

The total phenolic content was estimated using the Folin-Ciocalteu method (Bajčan et al. 2013). To 3 mL of a solution diluted with Folin-Ciocalteu with distilled water (1:10), a volume of 1 mL of the extract was added, followed by the addition of 2 mL of 10% Na_2CO_3. The reaction mixture was incubated in the dark for 3 hours at room temperature, after which the absorbance was measured at 760 nm using a UV–Vis spectrophotometer against a blank sample. The gallic acid equivalent (mg GAE/g DM) was used to quantify the total quantity of phenolic substances. The standard curve for this measurement was generated under the same conditions as above utilizing a range of concentrations (0-200 mg/l).

2.5.2. Total flavonoid content (TFC)

Using quercetin as a standard, the total flavonoid content was measured using conventional techniques with a few modifications (Pandey et al., 2020). After the preparation of the following standard solutions (50, 100, 150, 200, 250, and 300 µg/mL), 1 mL of each standard solution and 1 mL of each extract were prepared, and 0.5 mL of 8% $AlCl_3$ and 3 mL of NaOH (1 M) were added before the final volume was completed with distilled water to make 10 mL of each test tube. Using a UV–vis spectrophotometer, the absorbance was determined at 510 nm. Quercetin equivalents (QEs) were utilized to express the total flavonoid content.

2.5.3. Total tanins content (TTC)

By applying some modifications to the method used by Ghazouani et al. (2016), the content of total tannins in each type of ZLL extract was estimated. Thus, 700 µl of solution containing vanillin (1% in 7 M H_2SO_4) was added to 300 µl of each sample. Finally, after incubation for 20 min at 25°C, the absorbance was measured at 500 nm. The results are presented in mg of catechin equivalent per gram of dry matter (mg CE/g DW).

2.6. Antioxidant activity

2.6.1. DPPH assay

The 1,1-diphenyl-2-picryl-hydrazyl (DPPH) method, with particular modifications, was applied to assess the free radical scavenging capacity of the leaf extracts (Kebede et al., 2021). Thus, a 0.1 mM ethanolic solution of DPPH was prepared, and a volume of 1 ml of this solution was added to 3 ml of each extract and kept at room temperature for 30 min. Then, the absorbance at 517 nm was read against blank samples.

Low absorbance values reveal the range of concentrations that induce powerful antiradical activity. The concentration of leaf extract that can reduce the amount of the radical species by half, known as the IC50, can be calculated using the graphical representation of the results of the antiradical activity of the extracts against DPPH. Each measurement was performed in triplicate, and ascorbic acid was used to generate the standard calibration curve.

The % DPPH trapped (DPPH) was calculated by applying the following formula (2):

$$\% \text{ DPPH} = \frac{A_C - A_S}{A_C} \times 100 \quad (2)$$

where A_C is the absorbance of the control and A_S represents the absorbance of the sample.

2.6.2. FRAP assay

The reducing power of different fruit extracts was evaluated by measuring their capacity to reduce the ferricyanide complex to the ferrous form by applying ferric ion reducing antioxidant power (FRAP) (Suwanwong and Boonpangrak 2021). First, 1 ml of the methanolic extract of ZLL (20 to 200 µg/mL) was added to 2.5 ml of 0.2 M sodium phosphate buffer (pH= 6.6) and 2.5 ml of a 1% (w/v) potassium ferricyanide ($K_3Fe(CN)_6$) solution. The reaction mixture was vortexed using a vortex and incubated for 30 min at 40°C. Second, a volume of 2.5 ml of 10% (w/v) trichloroacetic acid was added to the reaction mixture before centrifugation. Then, 0.5 ml of ferric chloride at 0.1% (w/v), as well as a volume of 2.5 ml of deionized water, was added to a volume of 2.5 mL of the supernatant characterizing each sample. The absorbance at 700 nm was measured after 30 minutes. An increase in the absorbance of the reaction mixture suggested a stronger reducing power.

To illustrate the results, the equation of the line showing how the absorbance varies with concentration was used to determine the sample concentration that corresponds to an absorbance of 0.5 (IC of 0.5).

2.6.3. ABTS assay

As described by Yun-Hyeok Choi et al. (2020), with slight adaptations, the antiradical activity of the different extracts of ZLL was also evaluated by the ABTS method. Thus, by mixing equal quantities of 7 mM ABTS reagent and 2.4 mM potassium persulfate ($K_2S_2O_8$), the ABTS solution was prepared and then left to stand in the dark for 15 h at room temperature. Second, the ABTS solution was diluted in ethanol to an absorbance of 0.700 at 734 nm. Finally, 0.5 mL of each ZLL extract was added to 1 mL of the previously prepared ABTS solution, and after approximately 15 minutes, the reaction mixture was sufficiently vortexed, after which the absorbance at 734 nm was read. The antioxidant activity of all the extracts was measured using ascorbic acid as the reference and assessed by the half maximal inhibitory concentration (IC50).

2.7. Antibacterial activity

Four bacterial strains, *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, were used to evaluate the antibacterial activity of different ZLL extracts by applying the diffusion method in agar wells on Mueller-Hinton agar. On nutrient agar, the 4 bacterial strains were activated for 24 hours while maintaining the temperature at 37°C. Then, in a saline solution, a bacterial suspension was prepared, with a turbidity of 0.5 McFarland corresponding to 1.5 × 108 CFU mL^{-1}, which was used for the inoculation of the Petri dishes by surface spreading, each containing 6 wells piercing the agar and each measuring 4 mm in diameter. Each well received 50 µL of a particular extract. After incubation of the plates for 24 h at 37°C, the diameters of the inhibition zones were measured to comparatively evaluate the effectiveness of the ZLL extracts against the strains tested. A solution of DMSO was used as a negative control, and the antibiotic gentamicin (20 µg/well) was used as a positive control.

2.8. Statistical analysis

The means of triplicate analyses were estimated, and the data are presented as the means ± SDs. One-way ANOVA was utilized to compare the means. When $P < 0.05$, a difference was considered to be statistically significant. The data were analyzed with IBM SPSS Statistics 25.0. OriginPro 9.0 was used to construct graphs showing the antioxidant potential of the various extracts.

3. Results

3.1. Phytochemical screening of the ZLL extracts

The aqueous, methanolic and ethanolic extracts of ZLL contained flavonoids, tannins, alkaloids, proteins and reducing sugars. The hexanic extract was especially sensitive to terpenoids. Table 1 presents the results obtained.

3.2. Extraction solvent yield

Among the four solvents used in the extraction process, water showed the best extraction efficiency (p<0.05), with a value of 40.62 ±0.48%, followed by methanol, ethanol, and hexane, with values of 39.36±0.19, 27.71±0.28, and 08.41±0.16%, respectively, as shown in Table 2.

Table 1 Qualitative phytochemical exploration of the ZLL extracts.

Extract	Flavonoids	Tannins	Terpenoids	Alkaloids	Amino acids	Proteins	Reducing sugars
Water	++	++	-	++	+	±	++
Methanol	++	++	-	++	+	±	++
Ethanol	+	+	-	+	+	±	+
Hexane	+	-	+	-	±	-	-

++: abundant substance; +: presence of substance; -: absence of substance; ±: trace.

Table 2 ZLL extract yields.

		Yields %
Extract	Water	40.62 ±0.48 [a]
	Methanol	39.36±0.19 [b]
	Ethanol	27.71±0.28 [c]
	Hexane	08.41±0.16 [d]

The results are presented as the mean ± SD (n = 3). Significant differences at p < 0.05 are indicated by values in the same column with different letters.

3.3. Quantitative analysis of ZLL extracts

3.3.1. Total phenolic content (TPC)

The total phenolic content of the ZLL extracts ranged from 20.42 ± 0.50 to 120.53± 0. 37 mg GAE/g DM for the hexanic extract and the aqueous extract, respectively, as shown in Table 3. Thus, compared with the other solvents, the aqueous extract had the highest TPC (p<0.05) in the following order: water > 50% methanol > 80% methanol > methanol > ethanol > hexane.

Table 3 Total phenolic content, total flavonoid content and total tannin content in ZLL solvent fractions.

		TPC (mg GAE/g DM)	TFC (mg QE/g DM)	TTC (mg CE/g DW)
Extract	Water	120,53± 0. 37 [a]	60.15 ± 0.06 [a]	09.41 ± 0.36 [a]
	Methanol 50%	101,70± 0. 81 [b]	59.80± 0.30 [a]	08.11± 0.09 [b]
	Methanol 80%	81,68± 0.31 [c]	49.40 ± 0.34 [b]	07.47± 0.19 [c]
	Methanol	54.35 ± 0.25 [d]	40.57 ± 0. 30 [c]	05.34 ± 0.02 [d]
	Ethanol	32.05 ± 0.06 [e]	20.83 ± 0.60 [d]	05.01 ± 0.01 [d]
	Hexane	20.42 ± 0.50 [f]	14.45± 0.36 [e]	04.20 ± 0.17 [e]

GAE: gallic acid equivalent; DW: dry matter. ; QE: quercetin equivalent; CE: catechin equivalent. The results are presented as the mean ± SD (n = 3). Significant differences at p < 0.05 are indicated by values in the same column with different letters.

3.3.2. Total flavonoid and total tannin contents

The distribution of tannins and flavonoids in the ZLL extracts was generally similar to that of phenolic compounds, although there was no significant difference in the TFC between the aqueous extract and the 50% methanolic extract or between pure methanol and ethanol for the TTC content. The aqueous extract had the highest levels of flavonoids and tannins, with values of 60.15 ± 0.06 (mg QE/g DM) and 09.41 ± 0.36 (mg CE/g DW), respectively, as indicated in Table 3.

3.4. Antioxidant activity

3.4.1. DPPH assay

The different ZLL extracts, as well as ascorbic acid, showed DPPH free radical scavenging activity, which increased with increasing extract concentration, as shown in Figure 1. Furthermore, the aqueous extract demonstrated the greatest inhibitory effect, while the hexanic extract demonstrated the lowest inhibitory effect. For the IC50 estimates, a significant difference in the inhibitory potential against the DPPH free radical was recorded between all the extracts (P < 0.05), with the lowest value representing the highest inhibitory power against DPPH presented by the aqueous extract, while the highest value reflecting low inhibitory power was recorded by the hexanic extract, with values of 31.20 ±0.69 and 65.18±0.22 µg/mL, respectively, as mentioned in Table 4.

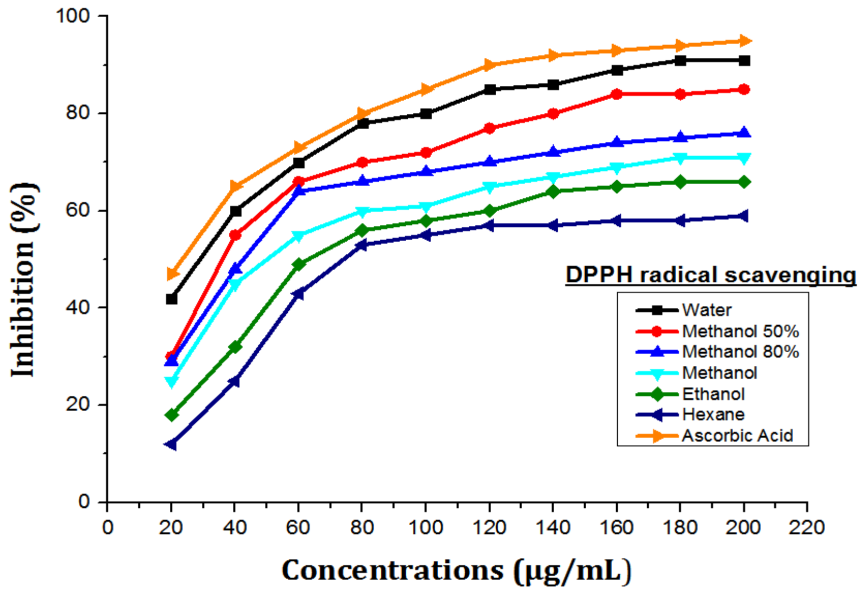

Figure 1 DPPH inhibition percentage according to the concentration of the different ZLL extracts.

Table 4 Antioxidant activity (DPPH, ABTS, and FRAP) of the different ZLL extracts.

Extract	Method		
	DPPH IC50 (µg/mL)	ABTS IC50 (µg/mL)	FRAP IC 0.5 (µg/mL)
Water	31.20 ±0.69 [a]	61.35±0.30 [a]	23.89±0.80 [a]
Methanol 50%	35.31±0.29 [b]	66.91±0.95 [b]	32.92±0.69 [b]
Methanol 80%	40.59±0.64 [c]	74.56±0.45 [c]	54.01±0.49 [c]
Methanol	47.97±0.62 [d]	77.23±0.08 [d]	70.89±0.17 [d]
Ethanol	56.65±0.28 [e]	84.41±0.53 [e]	80.73±0.24 [e]
Hexane	65.18±0.22 [f]	92.88±0.20 [f]	97.63 ±0.11 [f]
Ascorbic acid	27.35±0.40 [g]	56.08±0.05 [g]	10.14 ±0.14 [g]

The results are presented as the mean ± SD (n = 3). Significant differences at $p < 0.05$ are indicated by values in the same column with different letters.

3.4.2. ABTS assay

The ABTS method was used to assess the antiradical activity of the ZLL extracts, as was the method used for the DPPH test. Thus, the IC50 values of the aqueous extract, 50% methanolic extract, 80% methanolic extract, pure methanolic extract, ethanolic extract and hexane extract were 61.35±0.30, 66.91±0.95, 74.56±0.45, 77.23±0.08, 84.41±0.53, and 92.88±0.20, respectively, as shown in Table 4. Moreover, the free radical inhibition ability of ABTS increased with increasing concentrations of the extract, as illustrated in Figure 2.

3.4.3. FRAP assay

One potential indicator of antioxidant activity is the ability to reduce the ferric form of Fe^{3+} to the ferrous form of Fe^{2+}. This reductive capacity of the ZLL extracts was shown to be linearly correlated with the concentration, as was demonstrated for the antiradical activity, which is illustrated in Figure 3. Although all the extracts showed reductive activity, the aqueous extract demonstrated the highest reducing capacity ($p<0.05$). Thus, the IC 0.5 values ranged from 97.63 ±0.11 to 23.89±0.80 µg/mL for the hexane and aqueous extracts, respectively, as indicated in Table 4.

3.5. Antimicrobial activity

The results of the antibacterial activity were variable depending on the type of extract and the bacterial strain tested. Thus, the aqueous extract proved to be the most powerful of all the extracts used against all the bacterial strains used, as shown in Table 5. The highest activity ($p<0.05$) was recorded for the aqueous extract of ZLL against the bacterium *S. aureus,* with an inhibition zone diameter of 21.23 ± 0.29 mm, while the hexanic extract exhibited weak antibacterial activity, with an inhibition zone diameter of 03.05 ± 0.08 mm against *S. aureus*, as shown in Figure 4. The effects of the four extracts on each bacterial strain were statistically compared to those of gentamicin, which was used as a standard. Thus, although the antibacterial power of the aqueous extract was the highest among all the extract types, it nevertheless remained lower than that of gentamicin, and this effect was not observed for the four bacterial strains tested (p<0.05).

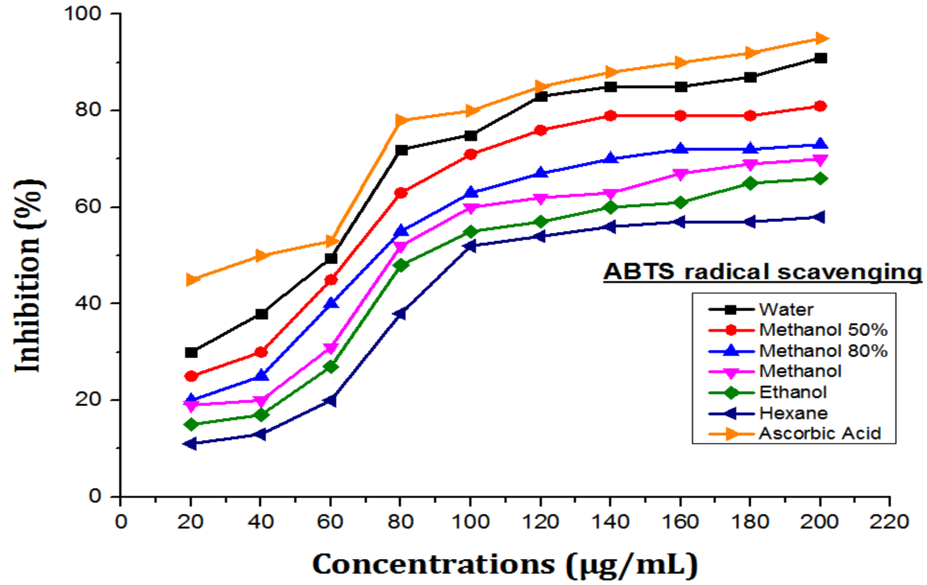

Figure 2 ABTS anti-radical activity as a function of the concentration of the ZLL extracts.

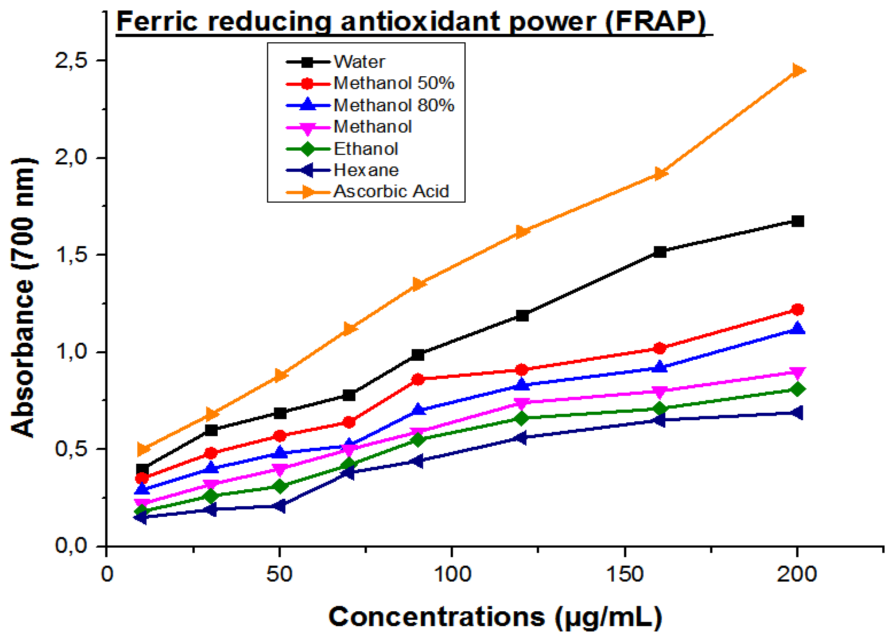

Figure 3 Ferric reducing antioxidant power (FRAP) of ZLL extracts at concentrations ranging from 20 to 200 µg/mL.

Table 5 Antimicrobial screening test of ZLL extracts (20 mg/ml) against several bacterial strains.

Strains	Inhibition zones (mm) for the different microbial strains using ZLL extracts (ø mm)				Gentamicin (20 µg/wells)
	Water	Methanol	Ethanol	Hexane	
S. a	21.23 ± 0.29 [a]	19.11 ± 0.11 [b]	13.68 ± 0.88 [c]	03.05 ± 0.08 [d]	24.48 ± 0.17 [A]
L. m	16.51 ± 0.36 [a]	14.28 ± 0.18 [b]	13.46 ± 0.77 [c]	11.57 ± 0.64 [d]	18.15 ±0.77 [A]
E. c	19.84 ± 0.05 [a]	17.83 ± 0.01 [b]	16.23 ± 0.12 [c]	13.74 ± 0.44 [d]	21.60 ± 0.14 [A]
P. a	18.15 ± 0.72 [a]	11.71 ± 0.85[b]	11.90 ± 0.03 [c]	06.08 ± 0.06 [d]	22.23 ± 0.28 [A]

The results are presented as the mean ± SD (n = 3). Significant differences at p < 0.05 are indicated by values in the same row with different letters. (S. a: *Staphylococcus aureus*; L. m: *Listeria monocytogenes*; E. c: *Escherichia coli*; P. a: *Pseudomonas aeruginosa*)

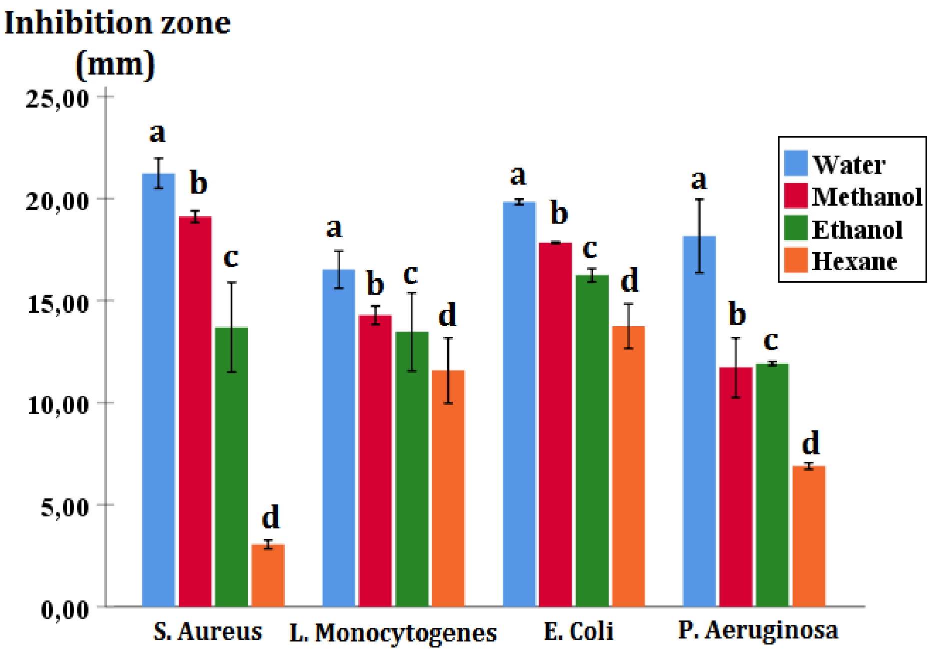

Figure 4 Antibacterial activity of the ZLL extracts.

4. Discussion

Most of the therapeutic benefits of medicinal plants stem from their biochemical arsenal in terms of secondary metabolites, particularly alkaloids and phenolic compounds (Prasathkumar, 2021). Phenolic acids, tannins and flavonoids are known to be the main metabolites responsible for the antioxidant properties of plants and are capable of neutralizing reactive species and preventing oxidative stress (Zihad et al., 2021). The results obtained by our study, which revealed the richness of ZLL extracts by the different secondary metabolites, are in agreement with the results revealed by previous studies related to the phytochemical screening of ZLL (Marmouzi et al., 2019). Each extraction solvent is characterized by its relative polarity, which determines its capacity to retain certain molecules. Thus, the nature of the solvent used strongly affects the extraction yield (Xu et al., 2019). The relative polarities of water, methanol, ethanol, and hexane, which are 1.000, 0.762, 0.654, and 0.009, respectively, could explain the variable yields obtained for the four ZLL extracts on the one hand, and on the other hand, the high yields obtained from water and hexane were low. The high extraction efficiency of water compared to methanol was also reported by Borgi Wahida et al. (2007), who obtained yields of 17.68, 15.28 and 2.37% for the aqueous extract, methanolic extract, and ethanolic extract, respectively, from a Tunisian ZLL. Furthermore, Bekkar et al. (2021) reported a higher extraction yield for the aqueous extract than for the methanolic extract. The distribution of phytochemical compounds in the four types of extracts depends on their degree of solubility in the solvent (Nagarajan et al., 2016). Previous investigations have also demonstrated that ZLL contains a range of phenolic constituents, alkaloids, terpenoids, amino acids and proteins (Cadi et al., 2020). In a study carried out by Touka Letaief et al. (Letaief et al. 2021), the methanolic extract had the highest TPC of 171.99 ± 1.14 (mg GAE/g DM), while the TPC of the aqueous extract and the ethanolic extract were 109.87 ± 2.07 and 41.70 ± 0.70 (mg GAE/g DM), respectively. In the same study, compared with the aqueous, methanolic, and ethanolic extracts, the ethanolic extract had the best TFC (28.54 ± 1.89 mg GAE/g DM), while a high quantity of TTC was provided by the aqueous extract (9.54 ± 0.26 mg GAE/g DM), as was the case in our study. In a phytochemical study on ZLL, Chaimae Rais et al. (2019) reported that TPC, TFC, and TTC are widely distributed in the ethanolic extract, followed by the methanolic extract and the aqueous extract. The quantities of TPC and TFC expressed by the methanolic extract obtained during our work were significantly lower than those expressed by the methanolic extract in the work of Yassine Yahia et al. (2020), ranging from 325.5 ± 10.13 to 949.87 ± 28.37 (mg GAE/100 g DW) for TPC and from 91.89 ± 5.23 to 92.22 ± 8.76 (mg QE/100 g DW) for TFC. Several factors can explain the notable differences concerning both the extraction yield and the phytochemical screening of the different ZLL extracts in relation to the results obtained by previous work, such as climatic, edaphic, genetic factors, and experimental conditions (Bruňáková et al., 2021; Moghaddam and Farhadi, 2015).

The antioxidant properties of plants are related to the antioxidant effects of certain metabolites, which act according to the mechanism of hydrogen atom transfer or the mechanism of electron transfer (Olszowy, 2019). The results obtained through our work show that the three different types of extracts demonstrate antioxidant activity, and among all the extracts, the ZLL aqueous extract demonstrated the strongest performance. Using the DPPH method, and in a previous study carried out by Meriem Elaloui et al. (2016), the ethanolic extract of ZLL also demonstrated the greatest inhibitory effect among all types of extracts used in all sampling regions, with an IC50 value of 0.03 µg/mL. The IC50 value of the aqueous extract, as determined by the DPPH test, was lower in our study than in a previous study carried out with ZLL aqueous extract in the Oudhref-Gabès region of southern Tunisia (Bencheikh et al., 2021). Touka Letaief et al. (2021) reported that the methanolic extract demonstrated the highest antiradical activity against DPPH with an IC50 = 33.66 ± 0.11 mg/L, followed by the aqueous extract and the ethanolic extract with 64.80 ± 0.36 and 375.50 ± 1.50 mg/L, respectively. The evaluation of the antioxidant activity by the ABTS and FRAP tests showed that the different types of extracts evaluated in our study have a significant antioxidant capacity, which was corroborated by previous studies on ZLL extracts (Bencheikh et al. 2021). The antioxidant capacities of plant extracts are due to the free radical scavenging activities of several types of phytochemical compounds, particularly phenolic compounds (Adebayo et al., 2019). Thus, the antioxidant power of the different ZLL extracts is largely linked to the quantity of TPC, which is positively correlated with the radical inhibition power and the reducing capacity of each of the extracts tested in our study. This correlation between TPC and antioxidant activity is corroborated by previous studies, including that of Touka Letaief et al. (2021), who reported significant antioxidant activity related to the methanolic extract having previously expressed the highest level of phenolic compounds among the extracts tested.

To survive by fighting against herbivores and phytopathogens, plants deploy a set of mechanisms, including the phytochemical arsenal of secondary metabolites, such as phenolic molecules, terpenoids, and alkaloids (Adedeji and Babalola, 2020). Our study revealed that ZLL extracts exhibit antibacterial activity against both gram-negative bacteria (*E. coli* and *P. aeruginosa*) and gram-positive bacteria (*S. aureus* and *L. monocytogenes*), which was proven by other previous studies (Hamada-Saoud et al. 2023). Thus, Bekkar et al.(2021) showed that the aqueous extract is more effective against *S. aureus* and against *E. coli* than the methanolic extract, which is in agreement with the results of our study, although the values of the diameter of the inhibition zone obtained by their study were lower than those relating to our study, with 14.03 ±0.06 and 11.1 ±0.1 mm for the aqueous and methanolic extracts against *S. aureus,* respectively, and against *E. coli*, with 17.03 ±0.08 and 16.1 ±0.1 mm for the aqueous and methanolic extracts, respectively. The data reported in the literature indicate a correlation between the biochemical composition of the plant secondary metabolites and its antimicrobial activity (Vaou et al., 2021; Bucekova et al., 2019), which could be explained by the strong antibacterial potential of the aqueous extract, which has already shown the highest content of phenolic compounds, and the weak effect of the hexane extract, whose phytochemical report was shown to be depleted of phenolic molecules.

5. Conclusions

The present study focused on the qualitative and quantitative evaluation of the phytochemical potential of ZLL, as well as the evaluation of the antioxidant and antibacterial properties of the extracts in vitro, by testing the use of several types of solvents. Thus, ZLL contains several secondary metabolites (phenolic compounds, alkaloids, and terpenoids) as well as other biochemical compounds (amino acids, proteins and reducing sugars). On the one hand, the phytochemical composition (TPC, TFC, and TTC) and the antioxidant properties (DDPH, ABTS, and FRAP) were the best, while the hexane extract was the least efficient. The antimicrobial potential of the ZLL extracts was tested against four bacterial strains, which all showed sensitivity to the different extracts. Considering the results of this research, and others already presented in several corners of the world, the leaves of *ziziphus lotus* are identified as being an estimable source of molecules of high biological and physiological importance, as well as a source of secondary metabolites that shield against oxidative stress and have pharmacological properties against several harmful germs.

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Ethical considerations

Not applicable.

Conflict of interest

The authors declare that we have no conflicts of interest.

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