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Assessment of Phytochemical, Antioxidant and Antibacterial Properties of *Ziziphus lotus* Fruit Extracts

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ABSTRACT

One of the main sources of novel chemicals with possible medical use is medicinal plants. Multiple diseases have been treated with them in traditional medicine. The purpose of this study was to explore the phytochemical characteristics, antioxidant effects, and antibacterial activities of several extracts of *Ziziphus lotus* fruits (ZLF). Phytochemical analysis of ZLF 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 ZLF, and whose aqueous extract showed the best results. The aqueous extract had the highest yield, followed by methanol, ethanol, and lastly hexane ($p < 0.05$). The aqueous extract showed the highest total contents of phenols, flavonoids, and tannins (77.13 ± 0.11 mg GAE /g DM, 33.36 ± 0.51 mg QE/g DM, and 03.72 ± 0.16 mg CE/g DM respectively), while the Hexane extract revealed the lowest contents (12.36 ± 0.26 mg GAE/g DM, 06.20 ± 0.23 mg QE/g DM, and 01.20 ± 0.10 mg CE/g DM respectively). By using the DPPH, ABTS, and FRAP methods, and for the aqueous extract, ZLF extracts demonstrated considerable antioxidant capacities, with the values IC50 = 37 ± 0.27, IC50 = 67 ± 0.18 and IC0.5 = 31 ± 0.22 respectively. All of the ZLF extracts, with the exception of the hexanic extract on *Staphylococcus aureus*, showed antibacterial efficacy against the bacterial strains of *Listeria monocytogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The results obtained reveal that ZLF exhibit significant biochemical composition and considerable biological activities encouraging its nutritional and therapeutic use.

Keywords: *Zizyphus lotus*, phytochemical compounds, antioxidant, antibacterial.

INTRODUCTION

The Mediterranean climate of Morocco has favored the floristic diversity of its ecosystems (Chebli et al., 2021), and has contributed to the country's wealth in terms of aromatic and medicinal plants, which makes the country one of the notorious floristic gardens of North Africa and the whole world (Redouan et al., 2020). Plants forever occupied an important place in the life of man on earth (Süntar, 2020). They are used as a source of nutrients (Sá et al., 2020), therapeutic substances (Anand et al., 2019), fuel for heating (Ozgen et al., 2021), and also as a barrier and shelter against the hostility of nature (Sutrisno et al., 2020).

The normal functioning of human cells requires oxidation of energetic metabolites involving the mitochondrial reduction of oxygen, which allows the generation of reactive oxygen species

ROS (Cheng et al., 2019), which although they are involved in certain cellular signaling processes, they can oxidize molecules biological of great importance such as proteins, lipids and DNA (Kapoor et al., 2019), which could threaten following their overproduction the cellular and tissue integrity of the organism by such oxidative stress, and could therefore contribute amply in the processes of aging and cancerization (Liao et al., 2019).

Following the considerable increase in cases of bacterial resistance to antibiotics on the one hand (Micoli et al., 2021), as well as the side effects arising from the administration of some of them on the other hand (Yadav et al., 2023), and taking into account the ability of plant constituents to reducing and combating oxidative stress, attention has been directed towards exploring the natural products expressed by several types of plants through their different types of biomass (Ros and Carrascosa, 2020). Several studies have demonstrated the anti-inflammatory, antibacterial, antiviral, antifungal, anticancer effects (Dahibhate et al., 2018), as well as multiple nutritional virtues linked to the ingredients forming the phytochemical arsenal of plants. Phytoactive molecules involved in antioxidative and bactericidal processes are secondary plant metabolites such as phenolic compounds, terpenoids, and alkaloids (Kumar et al., 2022).

Phenolic compounds are recognized for their potential to offer antioxidant activity linked to the scavenging of free radicals which expresses an antimutagenic role preventing cancers (Maya-Cano et al., as well as a neuroprotective (Shabani et al., 2020), and cardioprotective role by reducing cellular damage and lowering cholesterolemia (Zeb, 2021). Alkaloids, are organic compounds mostly of plant origin that are composed of nitrogenous heterocycles (Lee and Sperry, 2022). Originally produced by plants to defend themselves against herbivores and plant pathogens, alkaloids are now used both for their medicinal qualities and their pesticidal effects (Singh et al., 2021).

Terpenoids, known as terpenes, are deeply embedded in the phytochemical arsenal of plants (Zhang et al., 2022). They allow plants to defend themselves against pathogens and herbivores (Kopaczyk et al., 2020). In addition, terpenoids contribute to the characteristic aromas of plants, which makes them important ingredients in the food industry and perfumery (Nagegowda and Gupta, 2020).Plants of the *Ziziphus* genus belong to the Rhamnaceae family, and are widespread in arid and semi-arid regions of many parts of the world including Morocco (Cadi et al., 2020a). These are two species that have been listed on Moroccan soils, *Ziziphus lotus* and *Ziziphus spina-christi*, although the genus has around a hundred species (El Maaiden et al., 2020). The *Ziziphus lotus* is a thorny shrub, known in Morocco by the name of Sedra, whose small rounded fruits are called jujubes, known among people by the name of Nbeg, insinuating from foliage supported by a very dense network of branches. While the fruits are generally edible fresh, the leaves are used in spiritual rituals known as Roquia, or even used with the branches as fuel for winter heating.

Literature reveals that extracts of *Ziziphus lotus* biomass were able to express several virtues such as: antihyperlipidemic (Bencheikh et al., 2021a), antiurolithiatic (Chakit et al., 2022), antimicrobial (Dahlia et al., 2020b), antidiabetic (Mahmoud et al., 2022), and anticancer effects (Sakna et al., 2022). *Ziziphus lotus* has been highlighted not only for its nutritional and pharmacognesic qualities, but also for the exploitation of its biomass for environmental purposes both for its leaves (Yakoubi et al., 2023) and for its fruits (El Yakoubi et al., 2023).

This study aims to study, on the one hand, the phytochemical composition relating to total phenolic compounds, total flavonoids and total tannins in in several types of *Ziziphus lotus* fruit (ZLF) extracts, and on the other hand to evaluate the quality of these extracts for their antioxidant capacities and their antibacterial effects. This study also aims to compare the results obtained relating to the ZLF characteristic of the study region (Al Hoceima) with other results relating to the same plant but characteristic of other regions.

MATERIALS AND METHODS

Fruit sampling

In September 2021, shrubs of *Ziziphus lotus* are taxonomically identified thanks to the different morphological traits expressed by the different vegetative apparatus of the plant and more precisely its fruits. Thus, the ripe fruits are collected, then brought back to the laboratory to be washed with distilled water before being reduced to powder then packed in plastic bags and kept at a temperature of 20 °C.

Phytochemical analysis of ZLF extracts

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

Extraction yield

The extraction yield was estimated by applying the following formula:

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

where: m_1 – mass (g) of ZLF extract, and m_2 – mass (g) of the raw material (ZLF powder).

Qualitative analysis of ZLF extracts

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

Flavonoïdes

4 mL 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.

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.

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 is revealed by the formation of a brownish-red colored ring.

Alkaloids

The presence of alkaloids is carried out by the Wagner test. Thus, by adding a few drops of Wagner's reagent to 4 mL of each extract, the solution gives a red-brown precipitate if it contains alkaloids.

Amino acids

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

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, to 4 mL of the extract are added two drops of copper sulphates ($CuSO_4$) and 1 mL of a base such as potassium hydroxide (KOH).

Reducing sugars

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

Quantitative analysis of ZLF extracts

Total phenolic content

The amount of 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 is added, followed by the addition of 2 mL of 10% Na_2CO_3. The reaction mixture is incubated in the dark for 3 hours and at room temperature, then the absorbance was measured at 760 nm using a UV-Vis spectrophotometer against a blank sample. Gallic acid equivalent (mg GAE/g DM) were employed to quantify the total quantity of phenolic substances. The standard curve for this measurement was generated under the identical conditions as above utilizing a range of concentrations (0–200mg/l).

Total flavonoid content

Using quercetin as a standard, the total flavonoid content was measured using conventional techniques with a few modifications (Pandey et al. 2020). In test tubes, and after the preparation of the following standard solutions 50, 100, 150, 200, 250, 300 µg/mL, a volume of 1 mL of

each standard solution as well as a volume of 1 mL for each extract are prepared and to which are added 0.5 mL of 8% $AlCl_3$, 3 mL of NaOH (1M), before the final volume is completed with distilled water to make 10 mL for each test tube. Using a UV-vis spectrophotometer, the absorbance was determined at 510 nm. Quercetin equivalents (QE) was utilized to express the results of the total flavonoids.

Total tanins content (TTC)

By applying some modifications to the method used by Ghazouani et al. (Ghazouani, Abderrabba, and Bouajila 2016), the content of total tannins in each type of ZLF extract was estimated. Thus, 700 µl of vanillin (4-hydroxy-3-methoxybenzaldehyde), prepared in 1% dissolved in 7 M H_2SO_4, is added to a volume of 300 µl of each sample. Finally, At 500 nm, absorbance was measured after incubating the ZLF sample for 20 min at 25 °C. In mg of catechin equivalent per gram of ZLF dry matter (mg CE/g DW), the results were reported.

Antioxidant activity

DPPH assay

1,1-diphenyl-2-picryl-hydrazil (DPPH) method, with particular modifications, was applied to assess the free radical scavenging capacity of fruit extracts (Kebede et al., 2021). Thus, a concentration of 0.1 mM of an ethanolic solution of DPPH was prepared, and a volume of 1 ml of this solution was added to a volume of 3 ml of each extract and, for 30 minutes, the ZLF extract was allowed to stand at the ambient temperature. Then the absorbance was read at 517 nm against blank samples.

Low absorbance values reveal the range of concentrations that induce powerful antiradical activity. The concentration of fruit extract that can reduce the amount of the radical species by half and is 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 adopted to generate the standard calibration curve.

The % DPPH trapped was calculated by applying the this formula:

$$\%\,\mathrm{DPPH} = \frac{A_C - A_S}{A_C} \times 100 \qquad (2)$$

where: A_s is the absorbance of the ZLF sample, while A_c represents the absorbance of the control.

FRAP assay

The ability to reduce ferricyanide complex to ferrous form in different fruit extracts was evaluated, and this by applying the method of ferric ion reducing antioxidant power (FRAP) (Suwanwong and Boonpangrak, 2021). Firstly, a volume of 1 ml of the methanolic extract of ZLF (20 to 200 µg/mL) was added to a volume of 2.5 ml of 0.2 M sodium phosphate buffer (pH = 6.6) and a volume of 2.5 ml of a 1% (w/v) potassium ferricyanide ($K_3Fe(CN)_6$) solution. The reaction mixture, vortexed using a vortex, was left for 30 min in incubation at a temperature of 40 °C. In a second time, a volume of 2.5 ml of 10% (w/v) trichloroacetic acid were added to the reaction mixture, before it is carried out in a centrifugation. 0.5 ml of ferric chloride at 0.1% (w/v), as well as a volume of 2.5 ml of deionized water were added to a volume of 2.5 mL of the supernatant characterizing each sample. The absorbance at 700 nm was estimated after 30 minutes. The reaction mixture's increased absorbance suggested a stronger reducing power. In order to illustrate the results, the equation of the line showing how the absorbance varies with concentration, is used to determine the sample concentration that corresponds to absorbance 0.5 (IC 0.5).

ABTS assay

As it was described by Yun-Hyeok Choi et al. (2020), with slight adaptations, the antiradical activity of the different extracts of ZLF 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 is prepared (kept for 15 hours in the dark and at ambient temperature). Secondly, the ABTS solution is diluted in ethanol to have an absorbance of 0.700 at 734 nm. Finally, to 1 mL of the previously prepared ABTS solution, a volume of 0.5 mL of each ZLF extract is added, and the reaction mixture, after about 15 minutes and being sufficiently vortexed, it was subjected to a reading of the absorbance at 734 nm. The antioxidant activity of all the extracts was measured using ascorbic acid as the reference, and assessed by the half maximal inhibitory concentration (IC50).

Antibacterial activity

4 bacterial strains: *Listeria monocytogenes*, *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, were used to evaluate

the antibacterial activity of different ZLF 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, and in a saline solution, a bacterial suspension was prepared, with a turbidity of 0.5 McFarland corresponding to 1.5×10^8 CFU mL^{-1}, and 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 a volume of 50 µL of a particular extract. The widths of the inhibitory zones were measured after the plates had been incubated for 24 h at 37 °C to comparatively evaluate the effectiveness of the ZLF extracts against the strains tested. A solution of DMSO was used as a negative control and the antibiotic Gentamicin (20 µg/wells) as a positive control.

Statistical analysis

Means of triplicate analysis were estimated and data was given as mean ± SD. One-way ANOVA was utilized to compare the means. When $P < 0.05$, a difference was deemed statistically significant. Utilizing IBM SPSS Statistics 25.0, the data was analyzed. OriginPro 9.0 is utilized to create the graphs that illustrate the antioxidant potential of the various extracts. It is noted that for all the results obtained, their representation in the different tables of the manuscript is proposed as mean ± SD ($n = 3$). Furthermore, significant differences at $p < 0.05$ are indicated by the values in same column with different letters.

RESULTS AND DISCUSSION

Phytochemical screening of ZLF extracts

Aqueous, methanolic and ethanolic extracts of ZLF revealed the presence of flavonoids, tannins, alkaloids, proteins and reducing sugars. The hexanic extract expressed a presence especially of terpenoids. Table 1 presents the results obtained.

Most of the therapeutic virtues of medicinal plants come 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 which are capable of neutralizing reactive species and preventing oxidative stress (Zihad et al., 2021). The results obtained by our study are in agreement with those obtained by Borgi et al. (2007), expressing a richness of ZLF extracts by the various secondary metabolites highlighted, as well as in agreement with the findings of earlier studies related to the qualitative analysis of phytochemicals extracts of Moroccan ZLF (Marmouzi et al., 2019).

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 39.20 ± 0.26%, followed in descending order by methanol, ethanol, and Hexane with 23.18 ± 0.32, 22.31 ± 0.33, and 04.24 ± 0.02% respectively, and as Table 2 illustrates.

Each extraction solvent is characterized by its relative polarity determining its capacity to retain certain molecules. Thus, the nature of the solvent used strongly affects the extraction yield (Xu et al., 2019). The relative polarity values of water, methanol, ethanol, and hexane which are 1.000, 0.762, 0.654, and 0.009 respectively could

Table 2. ZLF extract yields

Parameter		Yields %
Extract	Methanol	23.18 ± 0.32 [b]
	Ethanol	22.31 ± 0.33 [c]
	Water	39.20 ± 0.26 [a]
	Hexane	04.24 ± 0.02 [d]

Table 1. Qualitative phytochemical exploration of ZLF extracts

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

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

explain the variable yield obtained for the four ZLF extracts on the one hand, and on the other hand the high yield expressed by water while hexane showed low extraction yield. The high extraction efficiency of water compared to methanol was also reported by Letaief et al. (2021), by measuring a yield of 48% and 25.30% for the aqueous extract and the methanolic extract respectively, from a Tunisian ZLF.

Quantitative analysis of ZLF extracts

Total phenolic content

The ZLF extracts showed phenolic composition ranging from 12.36 ± 0.26 to 77.13 ± 0.11 (mg GAE/g DM) for the hexanic extract and the aqueous extract respectively, as shown in Table 3. Thus, the aqueous extract manifests the highest content in TPC compared to the other solvents ($p < 0.05$) and this in the following order: water > methanol 50% > methanol 80% > methanol > ethanol > hexane, although there is no significant difference between the pure methanol and 80% methanol.

Total flavonoid and total tannins contents – The distribution of tannin and flavonoid amounts in ZLF extracts is similar to that of phenolic compounds in general. Thus, it is the aqueous extract which showed the highest level of flavonoids and tannins with 33.36 ± 0.51 and 03.72 ± 0.16 (mg QE/g DM) respectively, as indicated in Table 3.

The obtained distribution of phytochemical compounds in the four types of extracts would depend on their degree of solubility in the solvent (Nagarajan et al., 2016). Previous investigations has also demonstrated that ZLF contain a range of phenolic constituents, alkaloids, terpenoids, amino acids and proteins (Cadi et al., 2020b). Although the aqueous extract also gave the highest values in relation to TPC, TFC, and TTC in the study of Touka Letaief et al. (Letaief et al., 2021), the values estimated by our work are significantly higher with the exception of the TPC content relating to the aqueous extract. The significant potential of water to sequester TPC, TFC, and TTC is also corroborated by the work of El Maaiden et al. (2019). Furthermore, this result disagrees with that reported by the work of Chaimae Rais et al. (2019) on ZLF, having found that TPC, TFC, TTC are widely distributed throughout the methanolic extract ZLF's composition, followed by the ethanolic extract then the aqueous extract. The quantities of TPC and TFC expressed by the methanolic extract, obtained during our work, remain significantly lower compared to those expressed by the methanolic extract in the work of Yassine Yahia et al. (Yahia et al., 2020), ranging from 167.30 ± 7.10 to 293.46 ± 17.20 (mg GAE/100 g DW) for TPC, and from 21.21 ± 2.69 to 46.51 ± 1.95 (mg QE/100 g DW) for TFC. The high content of TPC and TFC in the aqueous extract, followed by the methanolic then ethanolic extract is in agreement with the study by Ait Bouzid et al. (2022), having obtained average values of 18.16 ± 0.35 (mg GAE/g DM) and 15.49 ± 0.31 (mg QE/g DM) for TPC and TFC respectively, using water as solvent although their study showed that the acetone extract is more enriched in TPC and TFC compared to the aqueous extract. Several factors can explain the notable differences concerning both the extraction yield and the phytochemical screening of the different ZLF extracts in relation to the results obtained by previous work, such as climatic, edaphic, genetic factors, as well as the experimental conditions (Moghaddam and Farhadi, 2015).

Antioxidant activity

DPPH assay

The different ZLF extracts, as well as ascorbic acid, show DPPH free radical scavenging activity, which increases with increasing the concentration

Table 3. Total phenolic content, total flavonoid content and total Tannins content, in ZLF solvent fractions

Parameter		TPC (mg GAE /g DM)	TFC (mg QE/ g DM)	TTC (mg CE / g DM)
Extract	Water	77.13 ± 0.11^{a}	33.36 ± 0.51^{a}	03.72 ± 0.16^{a}
	Methanol 50%	70.35 ± 0.29^{b}	30.43± 0.50^{b}	03.02 ± 0.11^{b}
	Methanol 80%	54.02 ± 0.06^{c}	28.53 ± 0.42^{c}	02.74 ± 0.07^{b}
	Methanol	53.84 ± 0.05^{c}	27.86 ± 0. 24^{c}	02.75 ± 0.11^{b}
	Ethanol	50.14 ± 0.76^{d}	20.53 ± 0.46^{d}	02.47 ± 0.19^{b}
	Hexane	12.36 ± 0.26 ^{e}	06.20± 0.23 ^{e}	01.20 ± 0.10 ^{c}

Note: GAE – gallic acid equivalent ; DW – dry matter. ; QE – quercetin equivalent ; CE – catechin equivalent.

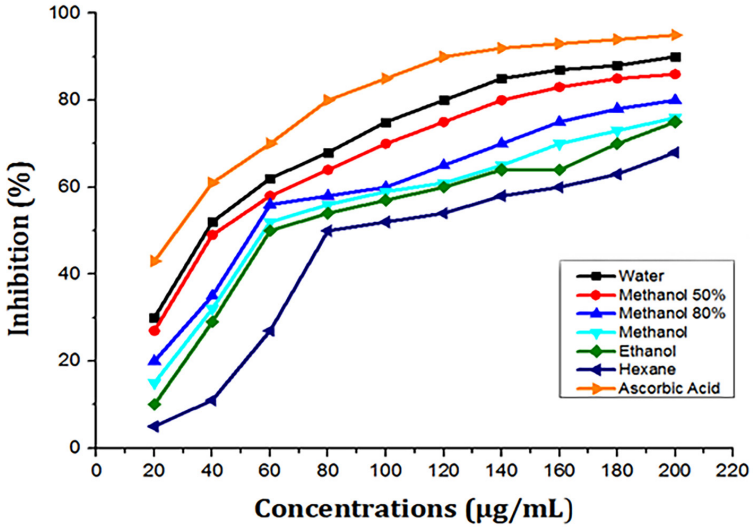

Figure 1. DPPH inhibition percentage according to the concentration of the different ZLF extracts

of the extract, as shown in Figure 1. Furthermore, it was the aqueous extract which demonstrated the highest inhibition power, while the hexanic extract demonstrated the lowest inhibition power. For the IC50 estimate, and with the exception of 80% methanol and pure methanol which did not show a significant difference ($p > 0.05$), the values of the other extracts are significantly different from each other ($p < 0.05$), with the lowest value expressing the highest inhibitory power with regard to DPPH is presented by the aqueous extract while the highest value reflecting a low inhibitory capacity is recorded by the hexane extract with 37 ± 0.27 and 85 ± 0.52 (µg/mL) respectively, as mentioned in Table 4.

ABTS assay

The ABTS method is used to assess the antiradical activity of ZLF extracts, same as the DPPH test. Thus, the IC50 values of aqueous extract, 50% methanolic extract, 80% methanolic extract, pure methanolic extract, ethanolic extract and hexane extract were respectively 67 ± 0.18, 77 ± 0.58, 84 ± 0.26, 87 ± 0.40, 94 ± 0.08, and 112 ± 0.55, as shown in Table 4. Moreover, the free radical inhibiting ability of ABTS increased with increasing concentration of the extract as illustrated in Figure 2.

FRAP assay

One of the potential indicators of antioxidant activity is the ability to reduce the ferric form Fe^{3+} to the ferrous form Fe^{2+}. This reductive capacity of the ZLF extracts was shown to be linearly correlated with the concentration, as was demonstrated for the antiradical activity, and which is illustrated in Figure 3. Although all the extracts showed reductive activity, it is the aqueous extract that demonstrated the highest reducing capacity ($p < 0.05$). Thus, the IC 0.5 values spread from 120 ± 0.60 to 31 ± 0.22 (µg/mL) for the hexane and aqueous extracts respectively as indicated in Table 4.

The antioxidant properties of plants relate to the antioxidant effect of certain of its metabolites acting according to the mechanism of hydrogen

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

Extract	Method		
	DPPH IC50 (µg/mL)	ABTS IC50 (µg /mL)	FRAP IC 0.5 (µg/mL)
Water	37.27 ± 0.24 [a]	67.18 ± 0.32 [a]	31.22 ± 0.29 [a]
Methanol 50%	43.31 ± 0.29 [b]	77.58 ± 0.43 [b]	52.19 ± 0.11 [b]
Methanol 80%	57.25 ± 0.11 [c]	84.26 ± 0.33 [c]	63.04 ± 0.06 [c]
Methanol	58.07 ± 0.05 [d]	87.40 ± 0.22 [d]	63.89 ± 1.01 [c]
Ethanol	58.62 ± 0.54 [d]	94.08 ± 0.12 [e]	70.27 ± 0.36 [d]
Hexane	85.52 ± 0.33 [e]	112.49 ± 0.55 [f]	120.60 ± 0.05 [e]
Ascorbic acid	27.25 ± 0.06 [f]	56.08 ± 0.05 [g]	10.14 ± 0.14 [f]

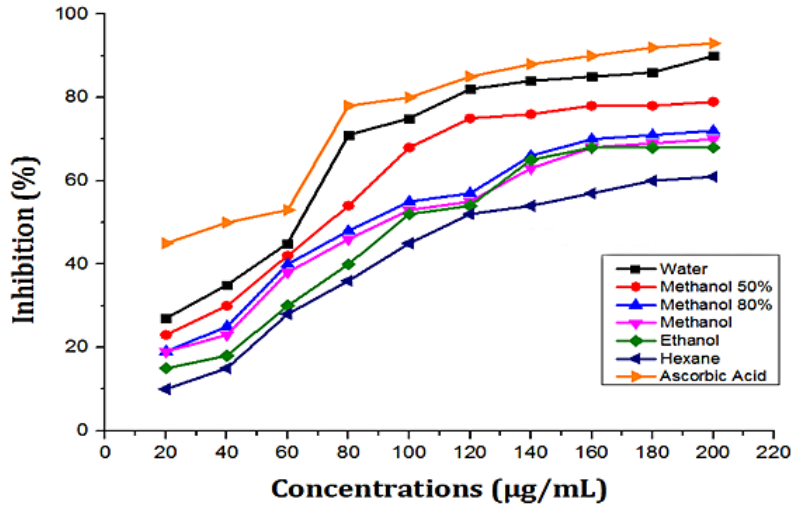

Figure 2. ABTS anti-radical activity as a function of the concentration of ZLF extracts

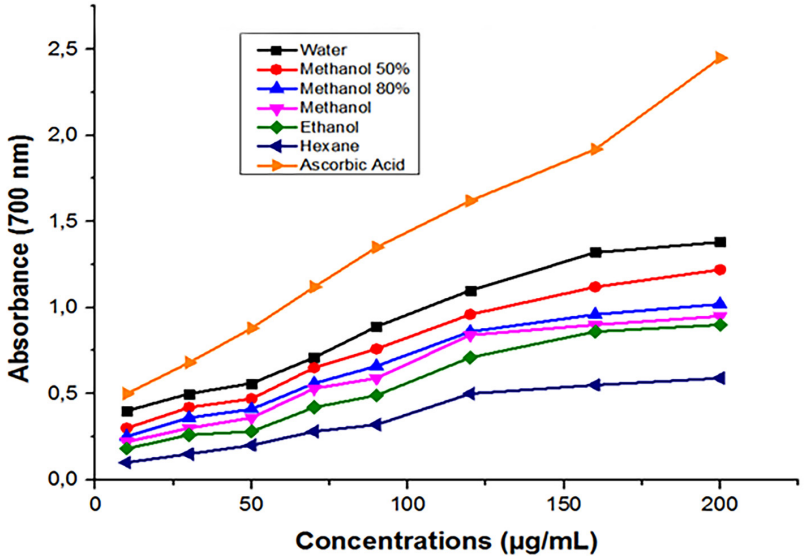

Figure 3. Ferric reducing antioxidant power (FRAP) of ZLF extracts for concentrations ranging from 20 to 200 µg/mL

atom transfer or according to the mechanism of electron transfer (Olszowy, 2019).The results obtained through our work show that the different types of extracts demonstrate antioxidant activity by the three methods used, and among all the extracts, the ZLF aqueous extract demonstrated the strongest performance. Using the DPPH method, and in a previous study carried out by Bouzid et al. (2022), it was the aqueous extract of ZLF which also demonstrated the highest inhibition power among all types of extracts used in all sampling regions. The value of IC 50 relating to the DPPH test obtained in our study is both lower compared to that obtained in a previous study in the northeastern Moroccan region concerning the aqueous extract, as well as that relating to the methanolic extract in the Moroccan region of Cheikh (Bencheikh et al., 2021). Although the results obtained by our study are in agreement with several previous studies, a disagreement was reported by the study of Letaief et al. (2021), having recorded a higher power of DPPH inhibition by the methanolic extract compared to that manifested by the aqueous extract. The evaluation of the antioxidant activity by the ABTS and FRAP tests showed that the different types of extracts evaluated by our study have a significant antioxidant capacity, and which was corroborated by previous studies on ZLF extracts (Bouzid et al., 2022). 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 having been manifested by the different ZLF extracts is largely linked to the quantity of TPC expressing a positive correlation with the radical inhibition power and the reducing capacity for each of the extracts tested by our study. This observation of TPC-antioxidant activity correlation is corroborated by previous studies including that of Letaief et al. (2021), having found a significant antioxidant activity relating to the methanolic extract having previously expressed the highest level of phenolic compounds among the extracts tested.

Antibacterial 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, and this against all the bacterial strains used, as shown in Table 5. The highest activity ($p < 0.05$) is recorded by the aqueous extract of ZLF against the bacteria S. aureus with an inhibition zone diameter of 20.23 mm, while the hexanic extract exhibited weak antibacterial activities by recording an inhibition zone diameter of 06.08 mm against *P. aeruginosa* and no activity against *S. aureus* (resistance to its effect), and as shown in Figure 4. The effect of the four extracts on each bacterial strain was statistically compared to gentamicin used as a standard. Thus, although the antibacterial power of the aqueous extract is the highest among all the extract types, it nevertheless remains lower compared to that of gentamicin, and this on the four bacterial strains tested ($p < 0.05$).

In order to survive by fighting against herbivores and phytopathogens, plants deploy a set of mechanisms including the phytochemical arsenal of secondary metabolites, like phenolic molecules, terpenoids, and alkaloids (Adedeji and Babalola, 2020). Our study revealed that ZLF extracts exhibit antibacterial activity against both gram negative bacteria (*E. coli* and *P. aeruginosa*) and gram positive bacteria (*S. aureus* and *L. monocytogenes*) and which was proven

Table 5. Antibacterial activity screening test of ZLF extracts (20 mg/ml) against some bacterial strains

Strains	Inhibition zones (mm) for the different microbial strains using ZLF extracts (ø mm)				Gentamicin (20 µg/wells)
	Water	Methanol	Ethanol	Hexane	
S. aureus	20.23 ± 0.29^{a}	17.28 ± 0.57^{b}	14.35 ± 0.34^{c}	00 ± 00^{d}	24.48 ± 0.17^{A}
L. monocytogenes	15.28 ± 0.26^{a}	13.45 ± 0.46^{b}	12.13 ± 0.19^{c}	09.04 ± 0.16^{d}	18.15 ±0.77^{A}
E. coli	18.64 ± 0.33^{a}	18.19 ± 0.15^{b}	15.23 ± 0.12^{c}	11.04 ± 0.16^{d}	21.60 ± 0.14^{A}
P. aeruginosa	17.49 ± 0.15^{a}	12.28 ± 0.21^{b}	10.84 ± 0.13^{c}	06.08 ± 0.06^{d}	22.23 ± 0.28^{A}

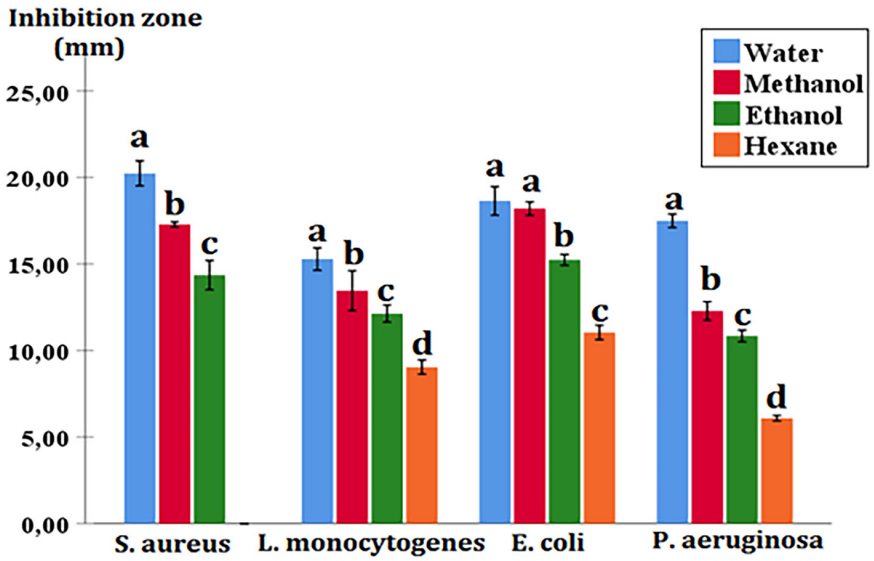

Figure 4. Antibacterial activity of ZLF extracts

by other previous studies (Hamada-Saoud et al., 2023). Thus, Rais et al. (2019), showed that the aqueous extract is more effective against *S. aureus*, compared to the methanolic and ethanolic extracts and this in agreement with the results of our study, although contrary to our result the effect against *E.coli* was more significant when applying methanolic and ethanolic extracts compared to the aqueous extract. The values of the diameter of the inhibition zone recorded by the aqueous extract of ZLF during our study were much higher compared to those obtained by Dahlia et al. (2020), recording 12.148 ± 2.12 mm against S. aureus, 13.648 ± 2.18 mm against *E. coli*, and 8.152 ± 2.04 mm against *P. aeroginosa*.

The data reported in the literature express a correlation between the biochemical composition of the plant in secondary metabolites and its antimicrobial activity (Bucekova et al., 2019), and which could explain in our study, the strong antibacterial potential of the aqueous extract having already shown the highest contents of phenolic compounds, and the weak effect of the hexane extract, or even the absence of effect as was the case against *S. aureus*, and whose phytochemical report was shown to be depleted in phenolic molecules.

CONCLUSIONS

The present study focused on the qualitative and quantitative evaluation of the phytochemical potential of ZLF, 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. ZLF expressed the presence of several secondary metabolites in its biochemical heritage (phenolic compounds, alkaloids, and terpenoids) as well as other biochemical compounds (amino acids, proteins and reducing sugars). Concerning the phytochemical composition (TPC, TFC, and TTC) on the one hand, and the antioxidant properties (DDPH, ABTS, and FRAP) on the other hand, it is the aqueous extract that showed the best values while the hexane extract was the least efficient. The ZLF extracts were tested for their antimicrobial potential against four bacterial strains which all showed sensitivity against the different extracts with the exception of the hexane extract which had no effect against S. aureus. Considering the results of this research, and others already presented in several corners of the world, jujubes of *Ziziphus lotus*, natives of the Al Hoceima (northeastern Morocco), are identified as being an estimable source of molecules of high nutritional importance and secondary metabolites offering a shield against oxidative stress and having pharmacological properties against several harmful germs, which will make this fruit a path to develop active ingredients with therapeutic effects or to make food products with high nutritional value.

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