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Antimicrobial and radical scavenging activities of Moroccan *Ziziphus lotus* L. seeds

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ABSTRACT

Natural plant extracts contain a variety of phenolic contents, which are associated to various biological activities. In this study, we evaluated the antioxidant and antimicrobial activities of organic (ethanol and methanol) and aqueous extracts prepared from *Ziziphus lotus* L. seeds. The total polyphenol content of the extracts was determined using the Folin-Ciocalteu reagent, it was in the range of 50.67 ± 1.44 (Ethanol Extract), 39.32 ± 1.44 (Methanolic Extract) and 23.54 ± 0.44 (Aqueous Extract) mg gallic acid equivalent/g DW. The content in flavonoids was estimated at 69.19 ± 0.10 (Ethanol Extract), 53.13 ± 0.55 (Methanolic Extract) and 9.63 ± 0.88 (Aqueous Extract) mg equivalent quercetin/g DW. The condensed tannin assay revealed that the methanol extract was rich on tannin (9.12 ± 1.07 mg/g) relatively to the ethanol and aqueous extracts (4.97 ± 0.95 and 1.88 ± 0.47 mg/g respectively). The antioxidant activity was evaluated *in vitro* by DPPH and phosphomolybdate (total antioxidant capacity). The results reveal that the three extracts have a capacity to trap the DPPH radical with IC_{50} 1.33 ± 0.01 , 1.32 ± 0.09 and 3.11 ± 0.05 mg/ml for the methanol, ethanol and aqueous extract respectively. This antioxidant activity is confirmed by the phosphomolybdate test. The antimicrobial activity of the studied extracts was evaluated using the broth microdilution, on five microbial strains: *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis* and *Candida tropicalis*. Results revealed an activity on the four bacterial strains tested. While the fungal strain (*Candida tropicalis*) showed an amount of resistance to the tested extracts. All this results could justify the use of *Ziziphus lotus* in the treatment of various infections in traditional environments.

Keywords: *Ziziphus lotus*, Phenolic compounds, Antioxidant activity, Antimicrobial activity.

INTRODUCTION

The jujube (*Ziziphus lotus* L.), commonly known in Morocco as "Sedra", is an aromatic and medicinal plant widely used in traditional medicine to treat diarrhea, ulcers, fevers, and used as a sedative [1]. In addition, *Ziziphus lotus* fruits are described as emollients and included in the treatment of throat and bronchial irritation lung [2, 3]. The ziziphus, is abundantly present in north Africa (Libya, Algeria and morocco) and in southern Europe especially Spain, Sicily, Greece and Cyprus [4]. Several studies have suggested that the phytochemical content and anti-oxidant effect of plants contribute to show protective effect against chronic and degenerative diseases [5, 6]. Recently, there has been a renewed interest in the therapeutic potential of medicinal plants as antioxidants in the reduction of these free radical-induced tissue lesions [7]. In fact, free radicals play a role in the pathogenesis of chronic degenerative diseases, including cancer, autoimmune diseases, inflammatory diseases, cardiovascular diseases, neurodegenerative diseases and aging in general [8, 9, 5]. Because of these facts, synthetic and natural compounds with potential antioxidant activity received an increased attention in biological research, medicine and pharmacy [5, 6].

Multidrug-resistant microorganisms are difficult to treat [10]. Common multidrug-resistant pathogens, such as *Staphylococcus aureus* and *Pseudomonas aeruginosa*, demonstrate a high resistance to the majority of drugs [11]. The development of MDR (Multi Drugs resistant) bacteria has led many authors to invest in the research for new natural molecules with antimicrobial activity. These sought molecules must possess various chemical properties and useful against these pathogenic microbes [12].

Current research on the different pharmacological activities of *Ziziphus lotus* L. and its derivatives are of great importance for modern medicine. However, this species is often ignored or even forgotten. It is becoming increasingly degraded because of the impact of ever-increasing human factors (overgrazing, grubbing up by farmers to change the purpose of the land, etc.).

This looting is likely to jeopardize the survival of the species. Due to lack of pharmacological uses information of *Z. lotus* seeds, and in the aim of enhancing the potential that culminate this plant, we conducted this study to highlight its antimicrobial and antioxidant activities. Thus, the objective of this study is to evaluate the antioxidant, antimicrobial activities and determine the concentration of some secondary metabolites of *Z. lotus* seed extracts.

MATERIAL AND METHODS

Plant material

Plant used consists of *Ziziphus lotus* seeds, collected in August from Fez (Zouagha-Moulay Yaâcoub) in Morocco. The climatic and geolocalisation data are summarized in table 1.

Table 1: Climatic and geolocalisation data

| Climate Data | Station |
|-------------------|------------|
| | FEZ |
| Latitude | 34°02'13 N |
| Longitude | 4°59'59 W |
| Altitude | 403 |
| Rainfall mm/year | 375 |
| Bioclimatic stage | Semi-Arid |

N: North; W: west

The seeds were crushed with a mortar and sieved to obtain a fine and homogeneous powder; which will be used for extraction.

Preparation of extracts

Seeds extracts were obtained using three solvents (methanol, ethanol and distilled water). The aqueous extract was prepared by Soxhlet apparatus according to plant: solvent ratio of 1/10 (w/v) for 4 hours.

Ten grams of the seed powder were subjected to 6 h of extraction using maceration, according to the plant: solvent ratio of 1/10 (w/v). The solvent is removed under reduced pressure at 40 °C and then stored at 4 °C.

Phytochemical screening of the plant extract

Total polyphenols contents

The total phenolic contents were performed according to the method of Singleton and Rossi (1985) [13] which consists of mixing 1 ml of extract with 0.5 ml of Folin-Ciocalteu reagent (1:1 distilled water) and 2.5ml of sodium carbonate solution (20%). The mixture was vortexed, then incubated for 2 h in the obscurity. The absorbance was determined at 725 nm by an UV-visible spectrophotometer. The calibration curve was performed with gallic acid and the final concentrations were expressed in µg of gallic acid equivalent (GAE)/mg of extract.

Total flavonoids

Total flavonoids content was performed according to the aluminum trichloride's method described by [14]. Indeed, 0.3 ml of NaNO₂ 5% was mixed with 1 ml of extract. After 5 min, 0.3 ml of AlCl₃ 10% was added. Once 6 minutes have elapsed, 2 ml of NaOH 1M were added and the total volume was made up to 10 ml with distilled water. The absorbance was measured at 510 nm. Total flavonoids content was calculated from the calibration curve established with quercetin standard, and were expressed as mg of quercetin equivalent (mg QE)/g of extract.

Determination of condensed tannins

Condensed tannins were performed according to Ribereau-Gayon and Stonestreet (1966) [15]. Briefly, 1ml of extract diluted 2 times were mixed with 1.5ml of HCl and 0.5 ml of distilled water. The mixture was heated for 30 min in a water bath at 100°C. The optical density was measured at 550 nm. The difference in optical density between the hydrolysed tube and the control (tube at room temperature) corresponds to the amount of tannins present in the extract. The results are expressed by following relation:

$$\text{Tannins (mg/ml)} = (\text{OD}_{\text{hydrolysis}} - \text{OD}_{\text{Control}}) \times 19,33 \text{ Antimicrobial activity}$$

Bacterial strains

The antimicrobial activity was performed on two Gram positive bacteria: *Staphylococcus aureus* (ATCC29213); *Enterococcus faecalis* (ATCC51299), and two Gram negative bacteria: *Pseudomonas aeruginosa* (ATCC27853) and *Escherichia coli* (ATCC 25922) and one yeast *Candida tropicalis* H195A (KP674873.1).

Antimicrobial assays

From a 24 h-old pure agar culture, 1 to 2 colonies were transferred in sterile saline for each strain. Then, the solutions were well-vortexed and their turbidity were adjusted to 0.5 McFarland.

The minimal inhibitory concentration (MIC), is defined as the lowest concentration of the extract that inhibits the microbial growth. For its determination, the broth microdilution was performed according to [16]. The microplates were incubated for bacteria at 37 °C for 24 h and at 30 °C for 48 h for *C. tropicalis*. After incubation, 10 µl of resazurine dye are added to reveal the microbial growth.

To determine the MBC/MFC, 3 µl of the negative wells were deposited on the surface of LB/YPG agar plate and incubated at 37 °C for 24 h for bacteria and 30 °C for 48 h for *C. tropicalis*. After incubation, the MBC or MFC has been recorded as the lowest concentration yielding a negative visible growth. To highlight the nature of the antimicrobial effect of the extracts tested the MBC/MIC ratio was calculated (Levison, 2004). [17]

Antioxidant activity

DPPH radical scavenging assay

The free radical DPPH was used to evaluate the antioxidant capacity of the extracts. For the extracts, a serial dilution was prepared from a stock solution of 4 mg/ml prepared in methanol. Then, 1 ml of each dilution was added to 1 ml of DPPH (0.004 %). The mixture was left in the dark for 30 minutes, and the discoloration against the negative control containing only the DPPH solution was measured at 517 nm. The percentage of the antioxidant activity was calculated according to the following equation:

$$\text{Antioxidant activity (\%)} = \frac{\text{Abs DPPH} - \text{Abs final}}{\text{Abs DPPH}} \times 100$$

The antioxidant capacity of the various extracts studied was interpreted from their IC₅₀, that scavenges 50 % of the initial DPPH [18]. The value of IC₅₀ is expressed in µg/ml. The results obtained are expressed in µg equivalent BHT/ ml.

Total antioxidant capacity

The phosphomolybdenum method was also used to evaluate the antioxidant activity of the extracts [19]. Indeed, 200 µl of the extract

were mixed with 3 ml of reagent solution (sulfuric acid, sodium phosphate and ammonium molybdate). The screwed tubes were incubated at 95 °C for 90 minutes. Then, the absorbance was measured at 695 nm. The total antioxidant capacity is expressed in milligram equivalent of vitamin C (mg Evitamin C), from a calibration curve, carried out by vitamin C standard.

Method of statistical analysis

The data obtained were the subject of a statistical analysis (averaging, analysis of variance ANOVA, SE) to search the variability between the different factors studied. A post-hoc test of Tukey was made whenever there was a significant effect of factor studied by ANOVA.

RESULTS AND DISCUSSION

Yield of extracts

The yield of extracts is presented in Fig 1. The highest yield is observed at methanol extract (13.1%) followed by aqueous (9%) and ethanol extracts (6.5%). The ANOVA showed a highly significant difference between the three extracts studied (ANOVA: $F = 49.562$; $ddl = 2$; $P \leq 0.001$).

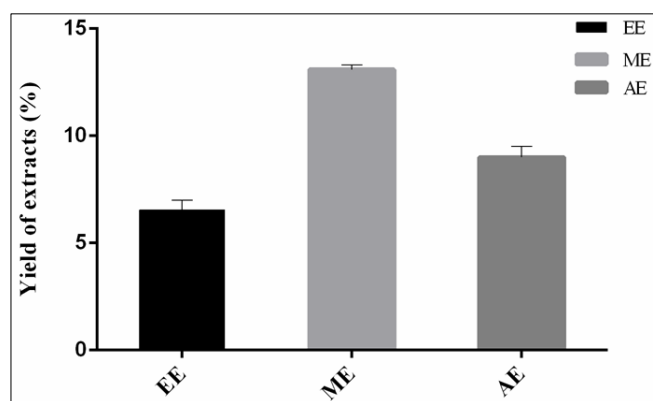


Figure 1: Yield of the different extracts studied (EE, ME and AE). Vertical bars correspond to SE for n = 3

This results are in disagreement with Djemai [20], which revealed that the yield of the aqueous extract of *Z. lotus* fruit is higher (40.4%) than methanol extract (6.3%). This could be explained by the variation of chemical constituents of the seeds and fruits. Bourgou *et al.* [21] researched on the effect of the solvent and the extraction method on the phenolic content and the antioxidant potential of *Euphorbia helioscopia*. They showed that the solvent has a significant influence on the yield. It can be noted that temperature could play a positive effect by improving the extraction of the compounds for aqueous extract, this justifies the choice of the Soxhlet apparatus [22]. Pélagie *et al.* [23] conducted a comparative study of the phenolic contents and the anti-radical activity of extracts (aqueous and ethanol) of *Garcinia kola* (Guttiferae) and *Cucumeropsis edulis* (Cucurbitaceae) seeds. According to their results, the aqueous extract of *Cucumeropsis edulis* has a higher yield (14%) compared to the ethanol extract (10%). However, for *Garcinia kola* seeds, the yield was higher in the ethanol extract (30%) than in the aqueous extract (8.8%).

Determination of secondary metabolites in the extracts

Phenols content

The phenolic content of methanol, ethanol and aqueous extracts is showed in fig 2. Results showed that the ethanol extract had a high total phenols content (50.67 ± 1.44 mg/g). On the other hand, the aqueous extract has the lowest concentration (23.54 ± 0.44 mg/g). The ANOVA showed a highly significant differences between the three extracts (ANOVA $F = 134.498$; $ddl = 2$; $P \leq 0.001$).

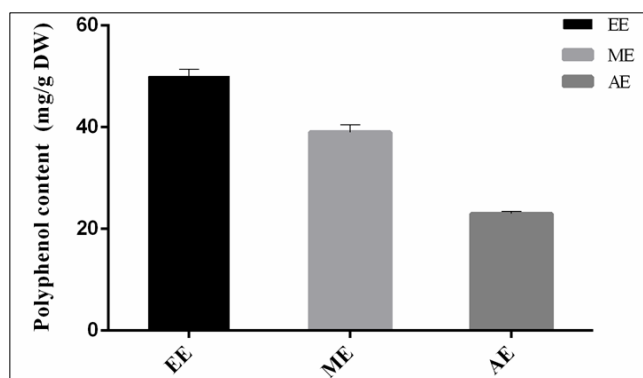


Figure 2: Polyphenol content of different extracts types of the *Z. lotus* seeds. Vertical bars correspond to SE for n = 3

This Results disagrees with Khouchlaa *et al.* [24], who show that the highest phenols content of *Z. Lotus* fruits was recorded in aqueous extract. In addition, a study carried out on the aqueous extracts of *Ziziphus mauritiana* and *Eriobotrya japonica* fruits, revealed that the phenols content of these extracts was largely low compared to our aqueous extract [25]. Several factors may influence the phenolic contents, such as extrinsic factors (geographical and climatic factors), genetic factors, and the degree of maturation and the duration of storage [26, 27].

Total flavonoids content

The total flavonoids content for each extract is showed in Fig 3. The results revealed that the ethanol extract had a high content of flavonoids (27.68 ± 0.04 mg EQ/g), followed by the methanol extract (21.25 ± 0.20 mg EQ /g) and the aqueous extract with a low concentration (3.85 ± 0.35 mg EQ/g). The ANOVA showed a highly significant difference for all studied extracts (ANOVA: $F = 8,240$; $ddl = 2$; $P \leq 0.001$).

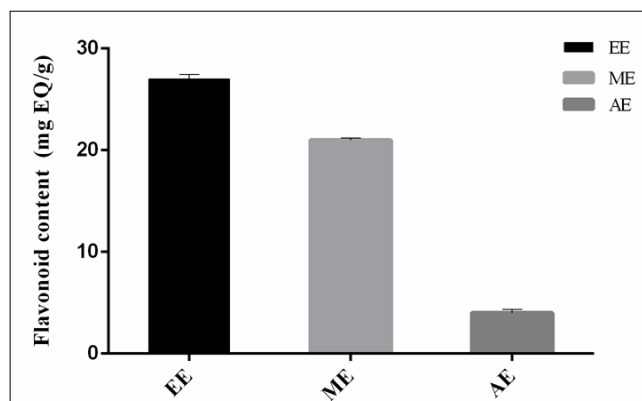


Figure 3: Flavonoides content of Different *Z. lotus* seed extracts types. Vertical bars correspond to SE for n = 3

Khouchlaa *et al.* [24] found that the n-hexane extract of the *Ziziphus lotus* L. fruit is rich in flavonoids (33.44 mg EQ / mg dry extract) compared to the methanol extract (26.79 mg EQ/mg dry extract).

Content of condensed tannins

The analysis of the results showed a high condensed tannin content of the methanol extract (9.12 ± 1.07 mg/g) compared to the other two extracts studied (Fig 4). The ANOVA showed a highly significant differences between the condensed tannin contents of the three extracts (ANOVA: $F = 17.61$; $ddl = 2$; $P \leq 0.001$).

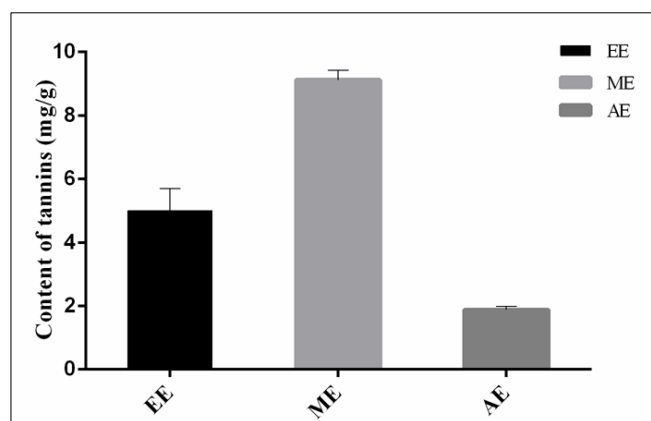


Figure 4: Content of condensed tannins of different types of *Z. lotus* seed extract. Vertical bars correspond to SE for n = 3

Some studies on the phytochemical and biological activity of *Ziziphus*

lotus L. fruit showed that the aqueous extract is richer in tannins ($6.77 \pm 1.95 \mu\text{g ECT/mg}$ of extract) followed by the methanol extract ($4.57 \pm 0.94 \mu\text{g ECT/mg}$ of extract) [20].

Antimicrobial activity

Minimal inhibitory concentration

The MIC values of extracts against target strains are given in Table 2. Ethanol extract showed a low MIC about 50 mg/ml against *E. coli*, *P. aeruginosa* and *E. faecalis*. For methanolic extract, the concentration corresponding to 50 mg/ml inhibited growth of *P. aeruginosa* and *E. faecalis*. Similarly, aqueous extract had an antimicrobial activity against the different strains studied and the low MIC obtained was 100 mg/ml detected against *P. aeruginosa* and *E. faecalis*. It can be concluded that the ethanol, methanol and aqueous extracts had an antibacterial effect against the four bacterial strains tested. *E. coli* showed high sensitivity to ethanolic extract, whereas *Candida tropicalis* was more resistant.

Table 2: MIC of *Z. lotus* seed extracts on the tested microbial strains.

| | | Ethanol Extract (EE) | | | | | | | | | | | |
|----------------------|--|-------------------------|-----|----|----|------|------|------|------|------|------|------|---|
| [C] (mg/ml) | | 200 | 100 | 50 | 25 | 12,5 | 6,25 | 3,12 | 1,56 | 7,07 | 0,39 | 0,19 | T |
| Strain | | | | | | | | | | | | | |
| <i>E. coli</i> | | - | - | - | + | + | + | + | + | + | + | + | + |
| <i>P. aeruginosa</i> | | - | - | - | + | + | + | + | + | + | + | + | + |
| <i>S. aureus</i> | | - | - | + | + | + | + | + | + | + | + | + | + |
| <i>E. faecalis</i> | | - | - | - | + | + | + | + | + | + | + | + | + |
| <i>C. tropicalis</i> | | + | + | + | + | + | + | + | + | + | + | + | + |
| | | Methanolic Extract (ME) | | | | | | | | | | | |
| [C] (mg/ml) | | 200 | 100 | 50 | 25 | 12,5 | 6,25 | 3,12 | 1,56 | 7,07 | 0,39 | 0,19 | T |
| Strain | | | | | | | | | | | | | |
| <i>E. coli</i> | | - | - | + | + | + | + | + | + | + | + | + | + |
| <i>P. aeruginosa</i> | | - | - | - | + | + | + | + | + | + | + | + | + |
| <i>S. aureus</i> | | - | - | + | + | + | + | + | + | + | + | + | + |
| <i>E. faecalis</i> | | - | - | - | + | + | + | + | + | + | + | + | + |
| <i>C. tropicalis</i> | | + | + | + | + | + | + | + | + | + | + | + | + |
| | | Aqueous Extract (AE) | | | | | | | | | | | |
| [C] (mg/ml) | | 200 | 100 | 50 | 25 | 12,5 | 6,25 | 3,12 | 1,56 | 7,07 | 0,39 | 0,19 | T |
| Strain | | | | | | | | | | | | | |
| <i>E. coli</i> | | - | + | + | + | + | + | + | + | + | + | + | + |
| <i>P. aeruginosa</i> | | - | - | + | + | + | + | + | + | + | + | + | + |
| <i>S. aureus</i> | | - | + | + | + | + | + | + | + | + | + | + | + |
| <i>E. faecalis</i> | | - | - | + | + | + | + | + | + | + | + | + | + |
| <i>C. tropicalis</i> | | + | + | + | + | + | + | + | + | + | + | + | + |

The bioactive molecules of Ethanol Extract of *Ziziphus lotus* appear to have the strongest inhibitory effect against most studied strains. Our results are consistent with Ertürk [28] who found that the ethanol extract of cummin is more active against *Escherichia coli* and *Pseudomonas aeruginosa*, compared with methanol extract. Similarly, the results of Mohsen and Ammar [29] proved that ethanol was the best solvent for the extraction of phenolic compounds, followed by methanol and finally water. This could explain the richness of ethanolic extract in phenols

contents, and its effectiveness against most studied strains compared to other extracts.

It appears that *Enterococcus faecalis* (Gram+) was the most susceptible bacteria in comparison with the other strains (Gram-), this can be attributed to the difference in the structure of the cell wall between Gram+ and Gram- bacteria. The cell walls of Gram+ bacteria consist of a single layer while the Gram- cell wall has a multilayer structure,

bound by an outer cell membrane [30]. This makes Gram- bacteria more resistant. generally, the results of phenols contents, flavonoids and condensed tannins revealed that EE was rich in these metabolites compared to methanolic extract and aqueous extract. This could explain its antimicrobial activity.

Minimal bactericidal concentration

The MBC values of the three studied extracts are showed in Table 3. In fact, the most interesting MBC was 100 mg/ml, showed against *P. aeruginosa*, *E. faecalis* and *E. coli* by Ethanolic Extract. For the methanolic extract, the MBC of *S. aureus* and *P. aeruginosa* were 200 mg/ml, whereas the MBC of *E. Coli* and *E. faecalis* were greater than 200 mg/ml. AE had a MBC of 100 mg/ml against *E. faecalis* and 200 mg/ml against *P. aeruginosa*, *E. coli* and *S. aureus*. According to MBC/MIC ratio, it is appeared that the extracts of the *Ziziphus lotus* seeds had a bactericidal activity on the studied strains.

Table 3: Antibacterial parameters of extracts and their interpretation

| | Ethanolic Extract (EE) | | | |
|----------------|------------------------|-----|---------|----------------|
| | MIC | MBC | MBC/MIC | Interpretation |
| <i>E. coli</i> | 50 | 100 | 2 | Bactericidal |
| <i>S.A</i> | 100 | 200 | 2 | Bactericidal |
| <i>P.A</i> | 50 | 100 | 2 | Bactericidal |
| <i>E.F</i> | 50 | 100 | 2 | Bactericidal |

| | Méthanolic Extract (ME) | | | |
|----------------|-------------------------|------|---------|----------------|
| | MIC | MBC | MBC/MIC | Interpretation |
| <i>E. coli</i> | 100 | >200 | -- | -- |
| <i>S.A</i> | 100 | 200 | 2 | Bactericidal |
| <i>P.A</i> | 50 | 200 | 4 | Bactericidal |
| <i>E.F</i> | 50 | >200 | -- | -- |

| | Aqueous Extract (AE) | | | |
|----------------|----------------------|-----|---------|----------------|
| | MIC | MBC | MBC/MIC | Interpretation |
| <i>E. coli</i> | 200 | 200 | 1 | Bactericidal |
| <i>S.A</i> | 200 | 200 | 1 | Bactericidal |
| <i>P.A</i> | 100 | 200 | 2 | Bactericidal |
| <i>E.F</i> | 100 | 100 | 1 | Bactericidal |

Antioxidant activity

DPPH radical scavenging assay

The antioxidant activity of the methanol, ethanol and aqueous extracts of *Ziziphus lotus* seeds is showed in Fig 5. It can be noted that the percentage of inhibition for the extracts was lower than the standard. At a concentration of 4 mg/ml, the highest percentage of DPPH inhibition was detected in the methanol extract (90.12% ± 1.73), while the aqueous extract has the lowest concentration. (54.29% ± 1.13). The IC₅₀ detected for BHT (0.24 ± 0.001 mg/ml) was superior in comparison with the different extracts studied. The ethanol and methanol extracts respectively had an IC₅₀ in the order of 1.33 ± 0.008 and 1.32 ± 0.09 mg/ml. On the other hand, the aqueous extract showed a higher concentration (1.19 ± 0.01 mg/ml). This allowed us to emphasize that the ethanol extract represents the most active extract.

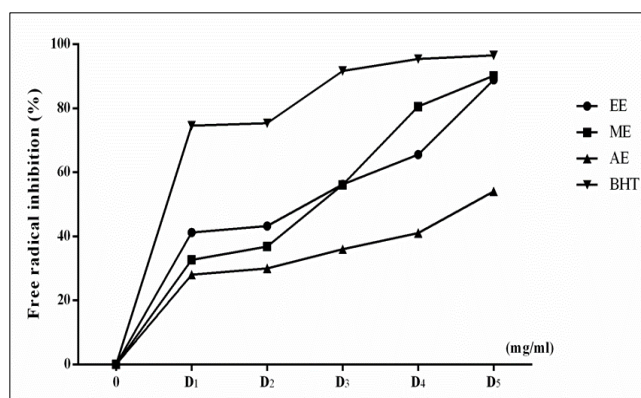


Figure 5: inhibition percentage of the DPPH radical of the reference antioxidant and the three extracts tested. MS: Stock solution: 4mg / ml; D1: 2mg / ml; D2: 1mg / ml; D3: 0.5 mg / ml; D4: 0.25mg / ml. Vertical bars correspond to SE for n = 3.

The ANOVA showed a highly significant difference between the ethanol, methanol and aqueous extracts (ANOVA: F = 1907,163; dd1 = 2; P<0.001).

Rajinder *et al.* [25] tested the antiradical activity of the aqueous extract of the *Ziziphus mauritiana* fruit and *Erio botryajaponica* with a concentration of 200 mg/ml. The inhibition percentage for *Z. mauritiana* was 74.89% and 51.07% for *Eriobotryaja ponica*. This activity appears weak compared to our aqueous extract, which showed a strong inhibition (54.29%) at 4 mg/ml. The strong activity of our methanol extract could be due to the presence of phenolic compounds in high concentration, especially the condensed tannins (9.12 mg/g). Similarly, Bougandoura and Bendimerad [31] showed that antioxidant molecules such as ascorbic acid, tocopherol, flavonoids and tannins, reduce and discolor the DPPH due to their ability to liberate hydrogen. On the other hand, it is well established that the antioxidant activity is positively correlated with the structure of the phenols. Generally, phenols contents with a high number of hydroxyl groups have the highest antioxidant activity [32], due to their ability to give more atoms to stabilize the free radicals [33]. Thus, the antioxidant activity is not only dose-dependent but also structure-dependent [34].

Table 4: Antioxidant potency (expressed as IC₅₀ in mg/ml) of the reference antioxidant

| | IC ₅₀ ±Ecart type (mg /ml) |
|--------------------|--|
| BHT | 0,24±0,001 |
| Ethanolic Extract | 1,33±0,008 |
| Methanolic Extract | 1,32±0,09 |
| Aqueous Extract | 3,11±0,05 |

Total antioxidant capacity

The phosphomolybdate test of the various extracts was presented in Fig 6. The estimation of the total antioxidant capacity showed a variability as a function of the nature of solvent. (ethanol, methanol and aqueous). It can be noted that for the concentration 4 mg/ml, the methanol extract has a strong total antioxidant capacity, followed by the ethanol and aqueous extracts. This result confirms the DPPH test. The ANOVA showed a highly significant differences between ethanol, methanol and aqueous extracts (ANOVA: F = 1907.163; dd1 = 2; P<0.001).

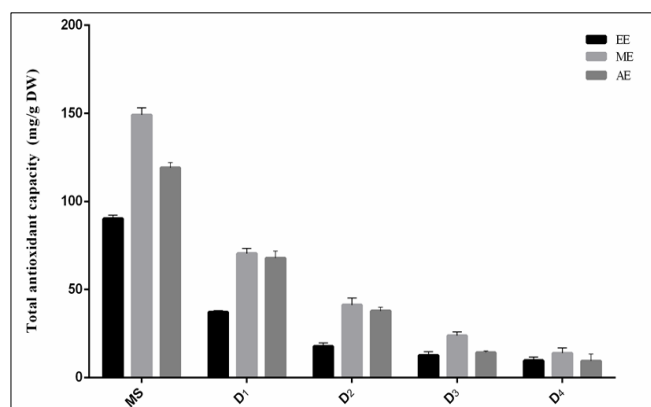


Figure 6: Total antioxidant capacity of the three tested extracts of *Z. lotus*. MS: Stock solution: 4mg / ml; D1: 2mg / ml; D2: 1mg / ml; D3: 0.5 mg / ml; D4: 0.25mg / ml. Vertical bars correspond to SE for n = 4

CONCLUSION

The results show that the ethanol extract is the richest in phenolic contents compared to the other studied extracts. The evaluation of the antimicrobial activity showed a bactericidal activity of the ethanol, methanol and aqueous extracts of the *Ziziphus lotus* seeds against the microbial strains tested, with resistance of *Candida tropicalis*. MIC and MBC vary according to the extraction solvent. In addition, the methanol extract showed a very important antioxidant activity. It can be indicated that the seeds of *Z. lotus* appear an antioxidant reservoir that can be used in the fight against free radicals.

Moreover, it seems of great importance to extend the range of antimicrobial tests, as well as the isolation and characterization of the active compounds in the various extracts, in order to identify the elements responsible for the biological activities of this plant.

REFERENCES

- Abalaka E, Daniyan SY, Mann A. Evaluation of the antimicrobial activities of two *Ziziphus* species (*Ziziphus mauritiana* L. and *Ziziphus spinachristi* L.) on some microbial pathogens. *Afr J Pharmaco*. 2010; 4(4):135-139.
- Le-Floc'h E. Contribution à une étude ethnobotanique de la flore tunisienne, Publications Scientifiques Tunisiennes, Tunisia. 1983; 390-402.
- Borgi W, Ghedira K, Chouchane N. Antiinflammatory and analgesic activities of *Ziziphus lotus*. *Fitotherapia*. 2007; 78(1):16-19.
- Benammar C, Hichami A, Yessoufou A. *Ziziphus lotus* L. (Desf.) modulates antioxidant activity and human T-cell proliferation. *BMC Compl Alter Med*. 2010; 10(54):1-9.
- Seyfried HE. Oxidative stress and antioxidants: a link to disease and prevention. *J Nutr Bio chem*. 2007; 18(3):168-171.
- Goodman M, Bostick RM, Kucuk O, Jones DP. Clinical trials of antioxidants as cancer prevention agents: Past, present, and future. *Free Radical Bio Med*. 2011; 51(5):1068-1084.
- Mukhopadhyay P, Horváth B, Zsengellér Z, et al. Mitochondrial-targeted antioxidants represent a promising approach for prevention of cisplatin-induced nephropathy. *Free Radical Bio Med*. 2012; 52(2):497-506.
- Surh YJ, Chun KS, Cha HH, et al. Molecular mechanisms underlying chemopreventive activities of antiinflammatory phytochemicals: down-regulation of COX-2 and iNOS through suppression of NF-κB activation. *Mutat Res*. 2001; 480-481:243-268.
- Aruoma OI. Methodological considerations for characterizing potential antioxidant actions of bioactive components in plant foods. *Mutat Res*. 2003; 523-524:9-20.
- Eloff JN. Which extractant should be used for the screening and isolation of antimicrobial compounds from plants. *J Ethnopharmacol*. 1998; 60(1):1-8.
- Mitscher L, Segaran P, Gentry EJ, Shankel DM. Multiple Drug Resistance. *Medic Res Rev*. 1999; 19(6):477-496.
- Rollinger JM, Haupt S, Stuppner H, Langer TJ. Combining ethnopharmacology and virtual screening for lead structure discovery: COX-inhibitors as application example. *J Chem Inf Comput Sci*. 2004; 44(2):480-488.

- Singleton VL, Rossi JA. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *J Enol Vitic*. 1985; 16(3):144-158.
- Barros L, Carvalho AM, Ferreira CFRI. Exotic fruits as a source of important phytochemicals: Improving the traditional use of *Rosa canina* fruits in Portugal. *Food Res Int*. 2011; 44(7):2233-2236.
- Ribéreau-Gayon P, Stonestreet E. Le dosage des tanins dans le vin rouge et détermination de leur structure. *Chim Anal*. 1966; 48(4):188-192.
- Bouhdid S, Abrin J, Zhiri A, et al. Investigation of fractional and morphological changes in *Pseudomonas aeruginosa* and *Staphylococcus aureus* cells induced by *Origanum compactum* essential oil. *J appl microbial*. 2009; 106(5):1558-1568.
- Levison ME. Pharmacodynamics of antimicrobial drugs. *Infectious Disease Clinics of North America*. 2004; 18(3):451-465.
- Hebi M, Eddouks M. Evaluation de l'activité antioxydante de *Stevia rebaudiana*. *Phytothérapie*. 2016; 14(1):17-22.
- Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal Biochem*. 1999; 269(2):337-341.
- Djemai ZS. Etude de l'activité biologique des extraits du fruit de *Ziziphus lotus* L. 2009.
- Bourgou S, Serairibeji R, Medini F, Ksouri R. Effet du solvant et de la méthode d'extraction sur la teneur en composés phénoliques et les potentialités antioxydantes d'Euphorbiahelioscopia, *Journal of new sciences*. *Agri & Biotechnol*. 2016; 28(12):1649-1655.
- Majhenic L, Knez Z, Kerget MS. Antioxidant and antimicrobial activity of guarana seed extracts. *Food Chem*. 2007; 104(3):1258-1268.
- Pélagie Y, Alexis T, Koudoro Y, et al. Etude comparative des Composés phénoliques et activité antiradicalaire des extraits des Graines de *Garcinia kola* (Guttiferae) et de *Cucumeropsis edulis* (cucurbitacées) du Bénin. *Int J Innov Sci Res*. 2015; 15(1):217-227.
- Khouchlaa A, Talbaoui A, El Yahyaoui El Idrissi A, et al. Détermination des composés phénoliques et évaluation de l'activité litholytique *in vitro* sur la lithiase urinaire d'extrait de *Ziziphus lotus* L. d'origine marocaine. *Phytothérapie*. 2017; 1-6.
- Rajinder K, Upralrit K, Harpreet W. Evaluation of Free Radical Scavenging Activities of Aqueous Extracts of Fruits of *Ziziphus mauritiana* and *Eriobotrya japonica* through *in vitro* Antioxidant Assays, *Glob J Res Rev*. 2015; 2393-8854.
- Aganga AA, Mosase KW. Tannins content, nutritive value and dry matter digestibility of *Lonchocarpus capassa*, *Ziziphus mucronata*, *Sclerocarya birrea*, *Kirkia acuminata* and *Rhus lancea* seeds. *Anim Feed Sci Technol*. 2001; 91:107-13.
- Bouzi W, Yahia M, Abdeddaim M. Evaluation de l'activité antioxydante et antimicrobienne des extraits de l'aubépine monogyne. *Leban Sci J*. 2011; 12:1.
- Ertürk Ö. Antibacterial and antifungal activity of ethanolic extracts from eleven spice plants. *Biologia Bratislava*. 2006; 61(3):275-278.
- Mohsen SM, Ammar ASM. Total phenolic contents and antioxidant activity of corn tassel extracts. *Food Chem*. 2009; 112(3):595-598.
- Ali Shtayeh MS, Yaghmour RMR, Faidi YR, et al. Antimicrobial activity of 20 plants used in folkloric medicine in the Palestinian area. *Journal of Ethnopharmacol*. 1998; 60(3):265-271.
- Bougandoura N, Bendimerad N. Evaluation de l'activité antioxydante des extraits aqueux et méthanolique de *Satureja calamintha ssp Nepeta* (L.) Briq. *Rev Nat Technol*. 2012; 5(2):14-19.
- Heim EK, Tagliaferro AR, Bobilya DJ. Flavonoids antioxidants: chemistry; metabolism and structure-activity relationships *J Nutr Biochem*. 2002; 13(10):572-584.
- Torres De Pinedo A, Pen Alver P, Morales JC. Synthesis and evaluation of new phenolic-based antioxidants: structure-activity relationship *Food Chem*. 2007; 103(1):55-61.
- Rodriguez-Bernaldo AQFS, Frecha PA, Vidal Perez AM, Lopez HJ. Antioxidant compounds in edible brown seaweeds. *Europ Food Res Technol*. 2010; 231(3):495-498.

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