Maghrebian Journal of Pure and Applied Science
ISSN : 2458-715X
http://revues.imist.ma/?journal=mjpas&page=index

Phytochemical and antimicrobial screening of fruits and leaves of *Zizyphus lotus* L. collected in North West of Algeria

N. Chelli Chentouf[(1)*], a. Tir Touil Meddah[(1)], a. Belgharbi[(1)], H. Benfreha Temmouri[(1)], B. Meddah [(1)].

(1) Laboratory of Bioconversion, Microbiological Engineering and Sanitary Safety (LBMESS).University Mustapha Stambouli of Mascara. Algeria. BP 305, Route Mamounia, 29000.Mascara.Algeria

Corresponding author: N. Chelli Chentouf, Email: chelinad@yahoo.fr; Phone: +213799164345;

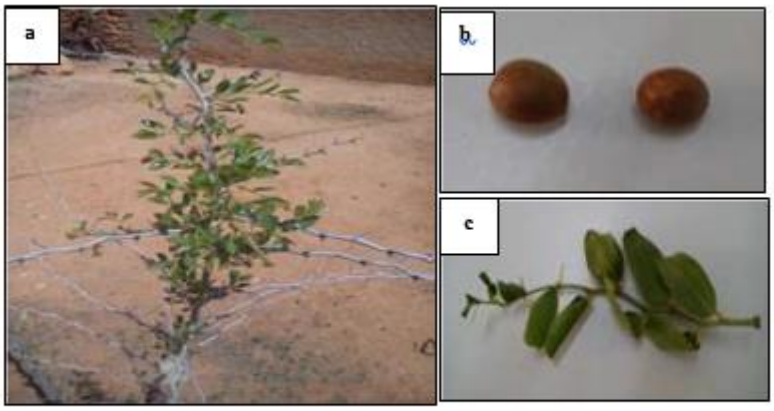
Received 14 March 2018, Revised 15 April 2018, Accepted 27 May 2018

Abstract. The present study was conducted on medicinal plant, called *Zizyphus lotus* L. (Rhamnaceae) which is known as Sedra in Mascara city (North West of Algeria). This plant is very well-known in the traditional medicine to cure gastro-intestinal tract, liver and other different respiratory infections. It is communally used for its anti-inflammatory, analgesic, anti-ulcer and antidiabetic properties. In this context, this study was aimed at investigating the *in vitro* antimicrobial activity of ethereal extract fruits and leaves of *Zizyphus lotus*, collected in the locality of Bouhanifia (Mascara city). Phytochemical screening of ethereal extract revealed the presence of free quinons, entraquinons, flavonoids and tannins, alkaloids. Fruits and leaves polyphenolic amount was 563.17 and 542.93µgEGA/mgMS respectively, and 47.62 and 45.19 µgEGA/mg MS for flavonoids and 213.74 µgEQ/gdm and 170.66 µgEQ/dm. Evaluation of the antimicrobial activity of the ethereal extract showed significant activity on the different tested bacteria.

Keywords: *Zizyphus lotus*, etheric extract, polyphenols, tanins, antimicrobial activity

1. Introduction

Because of public health problems, like the toxicity of some chemical drugs and the emergence of microorganisms resistant to many antibiotics much studies was conducted towards the development of new therapeutic agents to fight against the phenomena of the antibiorésistance [1]. For this, the investigation of the plants represented a priceless potential for the recherch of new active molecules with antimicrobial power. These last years, we witnessed to renewed interest of the consumers for natural products. Therefore, the industrialists develop them more and more, proceeded implementing extracts and active vegetable ingredients [2]. The medicinal and aromatic plants sources of these substances were largely widespread in the world [3] and more especially in Algeria with a rich and diversified floristic cortege. Among the medicinal herbs that constitute this one, our study was focused on an endemic plant *Zizyphus lotus* L., commonly called Sedra belonging to the Rhamnaceae. It is very largely used in traditional pharmacopeia used as antidiabetic, sedative, bronchitis, and antidiarrheal by local population [4]. Several scientific reviews for health benefit and biological potential of bioactive compounds from this shrub have been reported including antidiabetic, anti-inflammatory, antifungal, antibacterial, anti-ulcerogenic, antipyretic, antiviral and immunomodulatory effects [4- 6]. Thus, we carried out a phytochemical study and evaluated the antimicrobial activity of the etheric extract from fruits and leaves of *Zizyphus lotus L.* (Sedra) collected in Bouhanifia (wilaya of Mascara, North west of Algeria).



a: *Zizyphus lotus* L. **b: fruits , c: Leaves**

Figure 1: Specimen of *Zizyphus lotus L.*collected from Bouhanifia locality (wilaya of Mascara*)*

2. Materials and methods

2.1. Plant material

The plant material used was fruits and leaves of "*Ziziphus lotus (L) Desf.*" These parts were collected in June 2016 in area "Bouhanifia", located 20 km west of Mascara city. The identification of this plant was confirmed by a botanist in the department of Biology of Mascara University. A referenced specimen (RH00001) was introduced in the WAMAP-base of the Laboratory of Bioconversion, Microbiological Engineering and Sanitary Safety (LBMESS) of our university. After separation of the cores, the pulp was ground by using an electric grinder,

2.2 Preparation of etheric extract

An etheric maceration was carried out on 50g of powder (leaves or fruits) of *Zizyphus lotus L.* with 400 mL of petroleum ether, then the mixture was placed under agitation during 24h. The extracts were filtered using Whatman paper and concentrated under Vacuum with Rotary Evaporator to 40 °C. Then sterilized using a 0.22 µm filter and conserved at+4°C until use [7].

2.3 Phytochemical screening

2.3.1 Qualitative screening

Z. lotus leaves and fruits etheric extracts were subjected to qualitative phytochemical analysis for the presence of various classes of active chemical constituents such as tannins, saponins, glycosides, flavonoids, alkaloids, terpenes, steroids, etc. using standard procedures [8].

2.3.2 Quantitative screening

2.3.2.1 Determination of total phenolics

The total phenolic compounds were quantified by using reagent Folin-Ciocalteu colorimetric method [9] .The total phenolics amount was deduced from the established calibration curve based on Gallic acid (0-200 g/ml) and were expressed in micrograms of Gallic acid equivalent per mg of dry matter (GAE µg /g dw).

2.3.2.2 Determination of total flavonoids

The flavonoids in the extracts from different parts of *Zizyphus lotus* L. were mesured and estimated according to $AlCl_3$ method [10]. The concentrations of flavonoids were deduced from the established calibration curve based on quercitrin (0-100µg/ml) and were expressed as micrograms of catechin equivalent per gram of dry matter (µg QE/g dw).

2.3.2.3 Determination of tannins

The dosage of condensed tannins in the extract of *Zizyphus lotus* was determined according to the method of Heimler et al. [11]. The concentrations of tannins were deduced from the established calibration curve based on catechin (0-300µg/ml) and were expressed in micrograms of catechin equivalent per milligram of dry matter (CE µg/g).

3. Evaluation of the antibacterial activity

3.1 Tested Bacteria

The team of Microbial Ecology and Health of our laboratory provided the bacteria species. They were isolated from oral diseases in young students (aged from 17 to 30 years).

Gram positive: *S. aureus, Lactobacillus sp., Staphylococcus sp, Streptococcus sp.*

Gram negative: *E. coli, Serratia liquenfaciens, Enterobacter agglomerans*

3.2 Qualitative antibacterial susceptibility

The antimicrobial activity of the etheric extract was evaluated by qualitative screening of the susceptibility spectrum of tested strains to the etheric extract solubilized in sterile distilled water according to the diffusion method. 100µL of the bacterial inoculum (2.10^8 CFU/mL or 0,5 McFarlend) [12] was spread on the Muller Hinton Agar. Wells were then bored into the agar using a sterile 6 mm diameter cork borer and filled with the solution of the sterile etheric extract of *Z .lotus* L. (50- 25- 12,5 -6,25 and 3,14mg/ml). Plates were left at room temperature for 2 h for equal-diffusion of the extract into the media and afterwards Petri dishes were incubated at 37°C for 24 h [13]. Inhibition zones were then measured and recorded as the mean diameter (mm) of complete growth inhibition. 20 mg of gentamycin (20µg/mL) was used as a positive control. The evaluation of the activity was assessed on diameters of inhibition zone (Ø) and according to the following scale according to Rsaissi (2013) [7]

- No activity (-) Ø=0
- 10 to12 mm : significant activity
- 12 to 15mm : moderately significant activity
- 15 to 20mm : very significant activity
- ≥ 20 mm : highly significant activity
-

3.3 Quantitative antibacterial activity

The quantitative assay of the antibacterial activity was performed by broth microdilution method in 96–well microplates in order to establish the minimal inhibitory concentration (MIC). The MIC of extracts was determined in 96 well microtiter plate using the microdilution broth method [14]. The standard drugs were dissolved in sterile distilled water. The dilutions of the extract were prepared in the test medium at the required concentration (binary dilutions). Tested bacteria was calibrated at (2.10^8 CFU/mL or 0,5 McFarlend) [12]The microtiter plates were incubated at 37°C for 24 H where the kinetics growth of bacteria was followed to various time intervals (every 2H until 18H) comparatively to control wells (nutrient broth +inoculum). Growth was estimated as LogCFU (colonies forming unit). Gentamycin (20µg/mL) was used as a positive control.

Minimum inhibitory concentration (MIC) represented the concentration that completely inhibits the growth of microorganisms, while *Minimum Bactericidal concentration (MBC)* was the lowest concentration of the bactericidal extract, which lyses bacteria (less than 0, 01% of survivors). MBC/MIC was calculated, to determine if a substance was bactericidal (MBC/MIC <4) or bacteriostatic (MBC/MIC ≥ 4) [15].

4. Statistical analysis

All experiments were made in duplicate. All data are presented as means ±SD. For *in vitro* antibacterial activity, we consider Log CFU≤ 1 as significant [16].

5. Results and discussion

5.1. Yield of extract

The etheric extract of the leaves was dark green. However, that of the fruits was dark brown. The yields of extraction were 2.73% and 7, 93% respectively. Our results were in accordance with study of Ghalem (2014) [17] where the etheric extract of fruits of *Zizyphus lotus* represented the highest value (51% compared to the total weight). According to works of Elhanafi (2012) [5],

among the four extracts of *Zizyphus lotus*, aqueous extract (AQ) represented the highest yield (50.5%), whereas the etheric (0.36%) has the lowest one. This variation can be due to the use of various solvents with different polarity; also, the exhaustion of solvent with reduced pressure makes it possible to obtain the maximum of the compounds and to prevent their denaturation or probable modification due to the high temperatures used in other methods of extraction [2].

5.2 Phytochemical screening

5.2.1 Qualitative screening

Photochemical analyses of etheric extracts of jujube fruits, and leaves showed the presence of quinons, combined anthraquinons, condensed tanins and flavonoids (Table 1).

Table 1. phytochemical screening of of *Zizyphus lotus* L. etheric extract

Chemical groups	Fruits	Leaves
Free Quinons	+	+
combined anthraquinons	-	+
Saponosids	++	++
Tanins	+++	+++
Flavonoïds	++	++
Alcaloïds	+	+

+: coloring little abundant, + +: medium coloring; + + abundant coloring; + + + very abundant coloring.

5.2 Quantitative screening

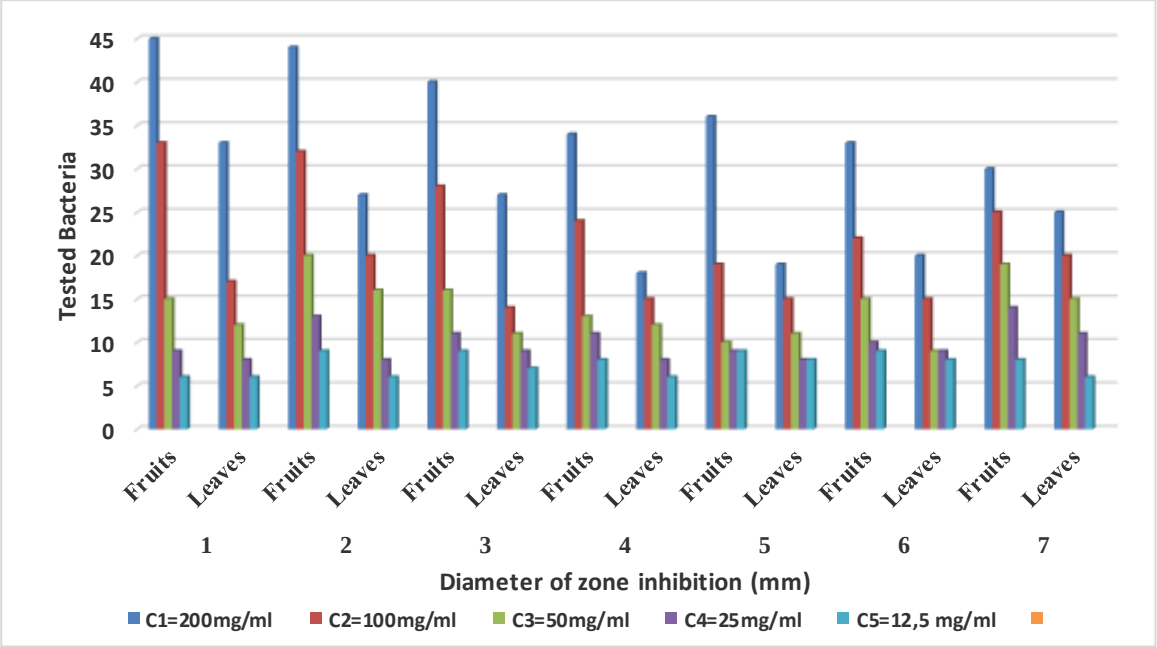
The amount of phenolic compounds showed that the etheric extract was very reached in polyphenols with 563.17 and 542.93µg EAG/g dm for the fruits and the leaves respectively. Our result was similar with those of Ghalem (2014) [17], which found that the etheric extract of the fruits of *Zizyphus lotus* contains 500 µg EAG/g dm .For flavonoids, the amount was 47.62 and 45.19 µg EC/g dm for the fruits and the leaves respectively. Also, a high amount of tannins was recorded for the fruits and the leaves 213.74µg EQ/g dm and 170.66 µg EQ/g dm respectively. This results may be related not only to the variety, but also to influences of the extraction methods and conditions, the stage of maturity and fruits harvest, storage conditions after harvest , the environmental factors, the dosage of reagents and type of spectrophotometer used [18- 20].

6. Evaluation of antibacterial activities

6.1 Qualitative antibacterial susceptibility

Results of the antimicrobial activity of the etheric extract of fruits and leaves of *Zizyphus lotus* L. were represented in figure 2. A remarkable activity was enregistred against tested bacteria responsible of buccal pathologies but with different degrees. We showed that the diameters of growth inhibition zones varied depending on the type and quantity of extracts and species tested bacteria [7]. The etheric extract has a good activity against the Gram-positive bacteria. The concentrations of 200 and 100 mg/mL presented the highest diameter of inhibition zone (40,75-29,25 mm) and (26,25-16,50mm) for the fruits and the leaves respectively (figure 2).Moreover, the Gram negative bacteria seem also very sensitive to the etheric extract of *Z. lotus* L. with diameters inhibition of (33 - 22mm) and (21,33-16,67) for the two parts of *Z. lotus* already cited. This sensitivity can be due to the morphology, the physiology or the type of studied strains.

The variation of the inhibition power between the leaves and the fruits was probably due to the difference in the chemical composition between them.



1: Staphyloccus aureus 2: Staphylococcus sp. 3: Streptococcus sp 4: Lactobacillus sp. 5: Serratia liquenfaciens 6: Enterobacter agglomerans 7: Escherichia coli

Figure 2: Evaluation of the antibacterial activity (diameter of the inhibition) of etheric extract of fruit and leaves of *Zizyphus lotus L.*

Our results had coincided perfectly with the results of Elhanafi (2012) [5], who judged that among the 04 extracts, which he tested, the etheric extract seems to have the most powerful antimicrobial effect.

6.2 Quantitative antibacterial activity

The etheric extract of fruits and leaves of *Z. lotus* exerted inhibiting growth of the totalities of tested germs (figure 3, 4). Generally, the etheric extract of fruits of *Z. lotus* was active against the total oral bacteria. The first concentration of this extract (200 mg/ ml) was more active than the others. For Staphylococci, a significant reduction in growth was assessed after 2 h of incubation of *E.coli*. Our findings are in accordance with other studies. According to Guiraud (1998) [21], the activity of the antimicrobial agent depends on the physiological state of bacteria, bacteria in the exponential phase of growth being more sensitive. Our extracts are revealed rich in polyphenols, one can say whereas the antimicrobial activity of is due, to the presence of polyphenols, primarily tannins and the flavonoïds.

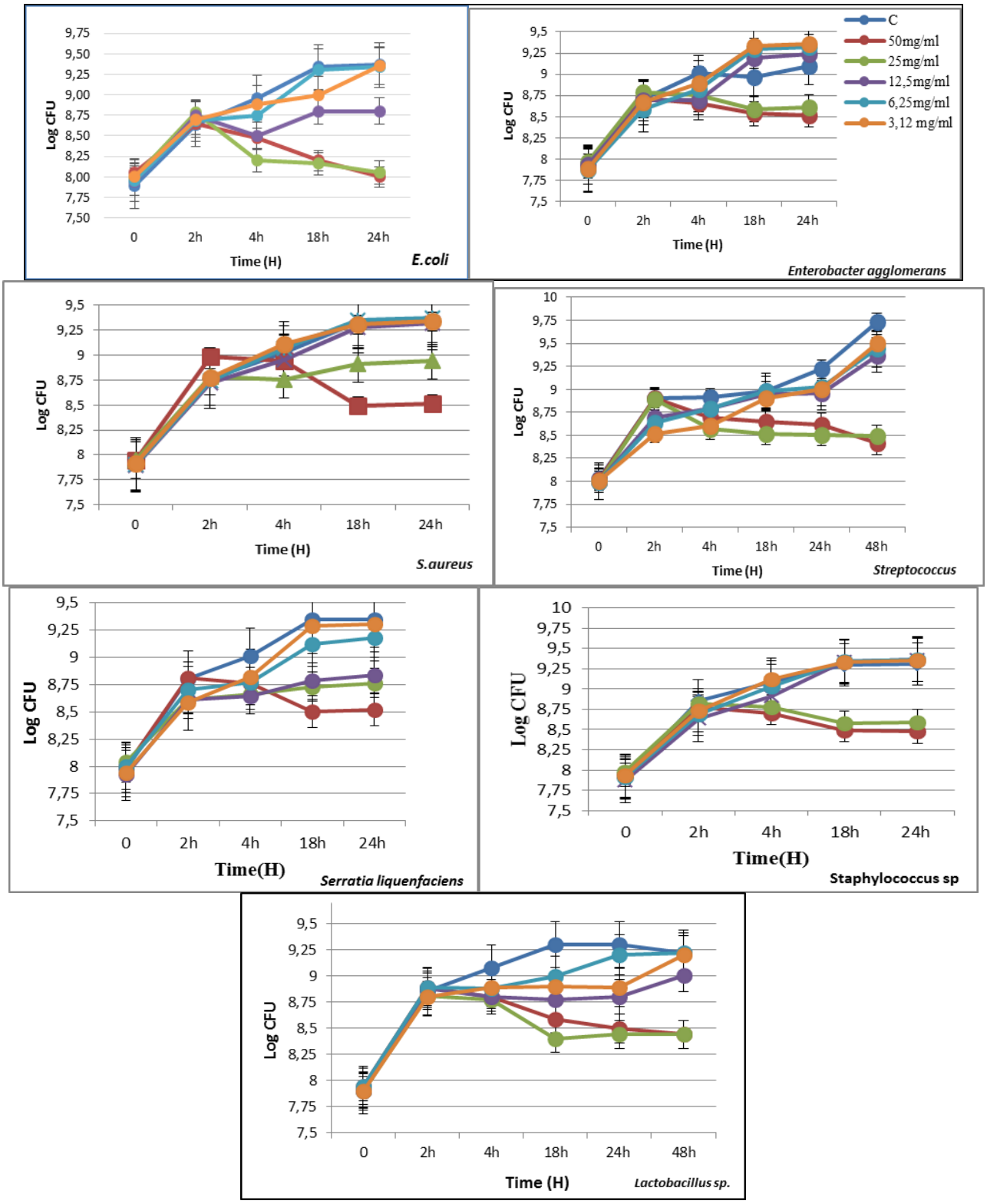


Figure 3: Antimicrobial effect of etheric extract of leaves of *Zizyphus lotus* L. on oral species bacteria

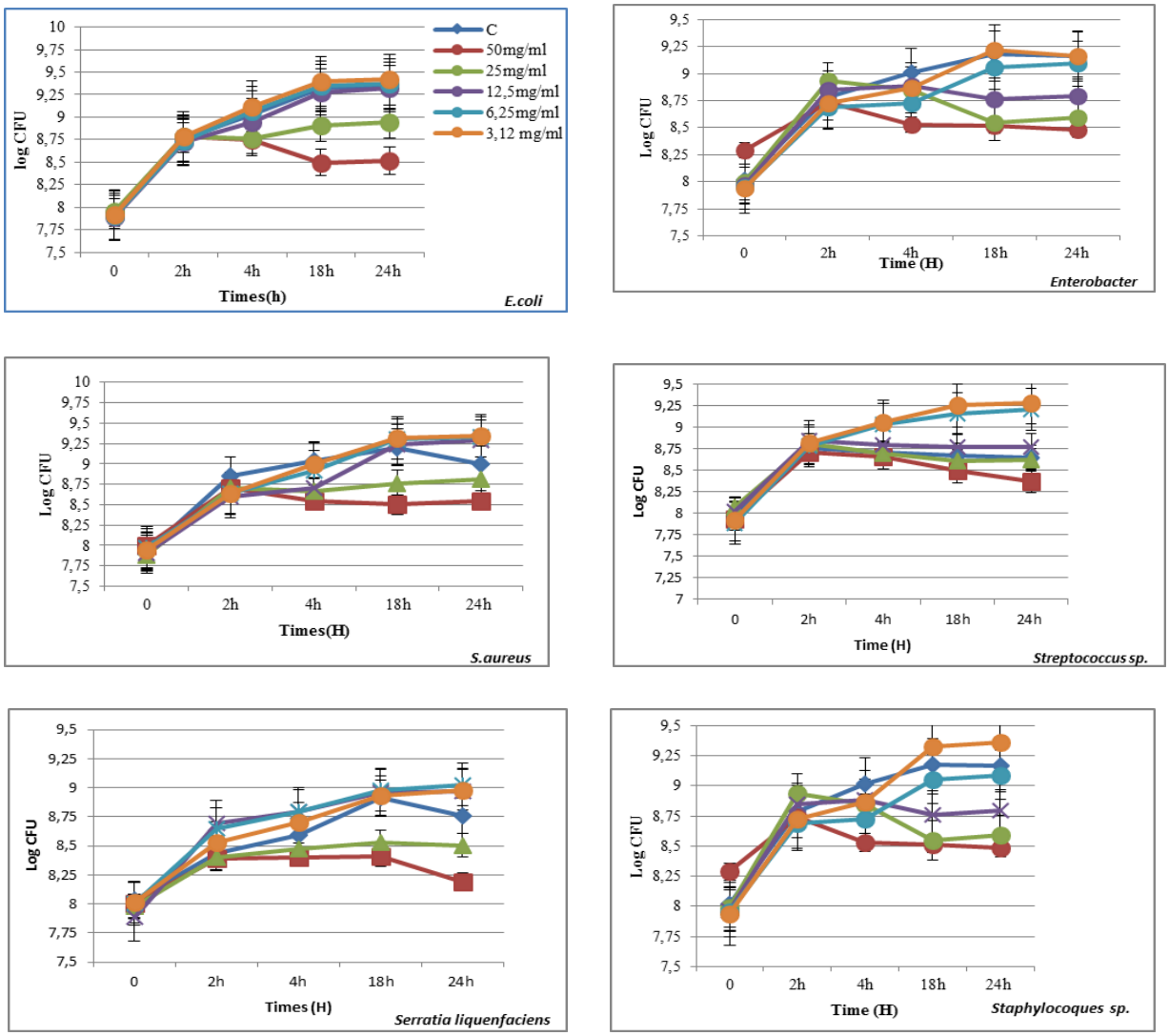


Figure 4: Antimicrobial effect of etheric extract of fruits of *Zizyphus lotus* L. on oral species bacteria

Moreover, the results got for the MIC (Table 2) are for the majority in agreement with the diameters of the zones of inhibition observed in the test of diffusion agar disc. In addition, the etheric extracts of the leaves and the fruits tested have shown a bactericidal activity with promising results of BMC and a report MBC/MIC lower than 4 for all tested bacteria [22]. Lastly, the mode and the mechanism of action of the extract and its compounds have not been completely understood or not yet found until now, and research continued. For this, chemical studies were necessary for identification of active molecules doted of an antibacterial activities, for using them in the future as therapeutically agents [23].

Table 2: Antibacterial profile of selected oral pathogenic bacteria treated with the etheric extract of *Zizyphus lotus* L.

Tested bacteria		**MIC (mg/ml)**	**MBC (mg/ml)**	**MBC/MIC**	**Antibacterial effect**
E.coli	**Leaves**	25	50	2	Bactericidal
	Fruits	25	50	2	
Streptococcus sp.	**Leaves**	25	50	2	Bactericidal
	Fruits	25	50	2	

Staphylococcus sp.	**Leaves**	25	50	2	Bactericidal
	Fruits	25	50	////	
S.aureus	**Leaves**	12,5	25	2	Bactericidal
	Fruits	25	50	2	
Lactobacillus sp.	**Leaves**	12,5	25	2	Bactericidal
	Fruits	25	50	2	
Serratia liquenfaciens	**Leaves**	25	50	2	Bactericidal
	Fruits	25	50	2	
Enterobacter agglomerans	**Leaves**	25	50	2	Bactericidal
	Fruits	25	50	2	

6. Conclusion

The experimental results reported in this paper revealed that fruits and leaves of *Zizyphus lotus* L. was considered as potential sources of nutrient. The etheric extract was reached in tannins, polyphenols and flavonoids, it showed better antibacterial activities. Further detailed investigations on the isolated compounds are needed to identify the active one responsible for this antibacterial power.

References

1. Benabdallah F.Z., Kouamé R.O. , El Bentchikou M. , Zellagui A. and Gherraf N. Études ethnobotanique, phytochimique et valorisation de l'activité antimicrobienne des feuilles et de l'oléorésine du pistachier de l'atlas (*Pistacia atlantica* Desf.). **Lavoisier SAS. Phytothérapie 2 (2015).**
2. Djahra A. B., Etude phytochimique et activité antimicrobienne, antioxydante, antihépatotoxique du Marrube blanc ou *Marrubium vulgare* L. Thesis of Doctorat. University Badji Mokhtar – Annaba. (2014).
3. Lamamra M. Contribution à l'étude de la composition chimique et de l'activité antimicrobienne des huiles essentielles de *Tinguarrasicula*(L**.) Parl. et de *Filipendula hexapetala* Gibb.(2007)**

4. Souleymane Abdoul-Azize. Potential Benefits of Jujube (*Zizyphus Lotus* L.). **Bioactive Compounds for Nutrition and Health .13 (2016)**
5. Elhanafi L. Activités antimicrobienne et antioxydante d'extrait aqueux du fruit de *Zizyphus lotus* et de l'écorce du fruit de *Punica granatum*. **Ecole supérieure de technologie Fes. (2012).**
6. El Hachimi F. ; El Antari A. ; Boujnah M. ; A. Bendrisse et Alfaiz C. Comparaison des huiles des graines et de la teneur en acides gras de différentes populations marocaines de jujubier, de grenadier et de figuier de barbarie, **Tétouan, Maroc. *Sci.* 6 (5) 1488-1502. (2015). ISSN : 2028-2508**
7. Rsaissi.N , EL Kamili, B. Bencharki , L. Hillali M., Bouhache. Antimicrobial activity of fruits extracts of the wild jujube "*Ziziphus Lotus* (L.) Desf. ***Inter.J. of Scient.& Engineer. Res*. 4(9), 152 (2013).**
8. Essam Nabih Ads, Saravanan Rajendrasozhan, Syed Imran Hassan, Sherif Mohamed Sayed Sharawy, Jamal Ragheb Humaidi. Phytochemical, antimicrobial and cytotoxic evaluation of *Ziziphus spinachristi* (L.) stem bark***. Biomed. Res. 28 (15) 6646-6653 (2017).***
9. Singleton V.L., Rossi J.A. 'Colorimetry of total phenols with phospho molybdic phosphotungstic acid reagents'. ***American J. of Enol. and Viticulture,* 16 144-158 (1965).**
10. Bahorun, T., Grinier, B., Trotin, F., Brunet, G., Pin, T., Luncky, M., Vasseur, J., Cazin, M ., Cazin, C. and Pinkas, M. 'Oxygen species scavenging activity of phenolic extracts from hawthorn fresh plant organs and pharmaceutical preparations'. ***Arzneimittel-Forsching*, 46(11) 1086-1089 (1996).**
11. Heimler, D., Vignolini, P., Dini, M.G., Vincieri, F.F., Romani, A. 'Antiradical activity and polyphenol composition of local Brassicaceae edible varieties'. ***Food Chemistry*, 99 (3) 464–469 (2006).**

12. Al-Bayati, F.A., Sulaiman, K.D. In vitro antimicrobial activity of Salvadora persica extracts against some isolated oral pathogens in Iraq. ***Turkish J.of Bio.*** **32 57–62 (2008).**
13. Damintoti, K., Mamoudou, H.D., Simpore, J., Traore, A.S. Antioxidant and antibacterial activities of polyphenols from ethnomedicinal plants of Burkina Faso. ***African J.of Biotech.*****4 823–828 (2005).**
14. Howaida, A.F., Skaug, N., Francis George, W. In vitro antimicrobial effects of crude miswak extracts on oral pathogens. ***Saudi J. of Dentistry*** **14 26–32 (2002).**
15. N. Chelli-Chentouf, Aicha Tir Touil Meddah, Catherine Mullie´, Abdelkader Aoues,Boumediene Meddah. *In vitro* and *in vivo* antimicrobial activity of Algerian Hoggar Salvadora persicaL. extracts against microbial strains from children's oral cavity. ***Journal of Ethnopharmacol*****.144 57–66 (2012).**
16. Ouattara, A., Ouattara, K., Coulibaly, A., Adima, A..Phytochemical screening and evaluation of the antibacterial activity of bark extracts of *Pericopsis* (Afrormosia) *laxiflora* (Benth) of *Escherichia coli* and *Klebsiella pneumoniae* ESBL, ***J. Chem. Pharma. Res*****., 5(1) 86-90 (2013).**
17. Ghalem M. Effets antioxydants et anti-inflammatoires des extraits de *Zizyphus lotus* et *Anthyllis vulneraria*. **Thesis of Doctorat Physiology et Biochimestry of Nutrition (2014).**
18. Shahidi, F., & Naczk, M. 'Phenolics in food and nutraceuticals'. ***Boca Raton, FL: CRC Press*** **. 131–155 (2004).**
19. N'guessan A.H.O., Deliko C.E.D., Bekro J.A.M., Beko Y.Y. Teneurs en composés phénoliques de 10 plantes médicinales employées dans la tradithérapie de l'hypertension artérielle, une pathologie émergente en Côte d'Ivoire'. ***Revue de génie industriel*****, 655-61 (2011).**
20. Pawlowska A. M., Camangi F., Bader A., Braca A. Flavonoids of *Zizyphus jujuba* L. and *Zizyphus spina-christi* (L.) Willd (Rhamnaceae) fruits'**.** ***Food Chem.*** **(February), 112 (4) 858-862 (2009).**
21. Guiraud E. Microbiologie Alimentaire Technique et Ingénierie. **Série agroalimentaire258-261 (1998).**
22. Doughari J. H., Ndakidemi P. A., Human I. S. and Benade S. Antioxidant, antimicrobial and antiverotoxic potentials of .extracts of Curtisia dentata. ***J. Ethnopharmacol*****; 1411041-1050 (2012).**
23. Benzeggouta N. Etude de l'Activité Antibactérienne des Huiles Infusées de quatre plantes médicinales connues comme aliments **(2005).**