BIOCHEMICAL CHARACTERIZATION AND NUTRITIONAL PROPERTIES OF *Zizyphus lotus* L. FRUITS IN AURES REGION, NORTHEASTERN OF ALGERIA

Mohamed **Abdeddaim**, Ourida **Lombarkia**, Ali **Bacha**, Djamel **Fahloul**, Djoumana **Abdeddaim**, Radhia **Farhat**, Mouna **Saadoudi**, Yassine **Noui** and Adel **Lekbir**

Food Science Laboratory (LSA), Department of Food Technology, Veterinary and Agriculture Institute
University Hadj Lakhdar Batna, Algeria
Email: dzhamoudi@gmail.com

Abstract
The small fruits of Zizyphus lotus L., found in Aures region, northeastern of Algeria have been studied. The study was realized on the pulp (P) and almond (A) separately. Levels of some nutrients in these parts were determined using standard analytical methods. Crude protein, crude fat, crude fiber, ash, carbohydrate, pectin, moisture contents and calorific values were in the (P) 3.80%, 1.32%, 8.41%, 3.28%, 65.90%, 3.78%, 12.27% and 16.341 Kj/g. In the (A) they were 24.22%, 31.73%, 16.57%, 3.12%, 11.10%, 2.35%, 9.57% and 17.271Kj/g respectively. The (P) has a hard consistency, with a sweet taste and very specific flavor. The TLC showed the presence of glucose, fructose and sucrose like sugars. The profile of amino acids revealed the presence of cystein, glycin, arginin, serin, leucin, histidin and alanin. Moreover, the almond is richer in fats. The fatty acids analysis by GC-MS revealed the presence of 15 fatty acids; the unsaturated ones were at 71% with 49% of oleic acid of the total mixture, followed by 22% of linoleic acid. The elemental analysis of the two parts of the fruit in mg/100g dry matter (DM) indicated that the (P) contained appreciable levels of zinc (0.44), potassium (134.99), sodium (11.45), phosphorus (10.62), manganese (2.17), magnesium (397.91) and iron (1.33). The (A) appears to be richer in these elements, mainly in phosphorus (24.00) and magnesium (1349.06). These results revealed that this fruit presents a high nutritional value. Therefore, it should be included in diets to satisfy needs of the body.

Keywords: *Zizyphus lotus* L., Jujube, Small fruit, Nutritional properties, Arid zone.

Submitted: 19.01.2014 Reviewed: 05.03.2014 Accepted: 10.04.2014

1. INTRODUCTION

In the arid zone, the fruits of *Zizyphus lotus* L are consumed by the autochthon population and it constitutes a considerable food resource for humans and animals in the food shortage periods. The *Zizyphus lotus* L. is an (*Rhamnaceae)* which grows in arid and semiarid zone in the north of Sahara, its endemic specie of the Aures region, northeastern of Algeria. The plant called Sedra, yields small fruits, the jujubes, they are named locally N'Beg. Several parts of the plants of *Ziziyphus* species are widely used in traditional medicine for the treatment of several diseases like gastrointestinal disturbance, liver troubles, urinary infections, skin infections, insomnia, diarrhea, and diabetes (Renault et al., 1997; Glombitza et al., 1994; Le Croueour et al., 2002). A cyclopeptidic alkaloids and saponins are responsible for an antifungal and antibacterial activities of the roots extract of *Z lotus* L (Ghedira et al., 1995; Renault et al., 1997). These roots are used for treatment of lung diseases, rheumatisms, arthritis, febrile state and for healing in a folk medicine. Leaves have a hypoglycemic effect and an antiseptic activity (Epfraim et al., 1998; Abdel-Zaher et al., 2005). The flowers infusion is used as febrifuge and disinfectant for eyes (Sudhersan and Hussain, 2003). Several effects of the fruits of zizyphus have been reported like an anti-age and anti-tumoral effects (Perdue and Hartwell, 1976; Oh et al., 2004), the anti-diarrheic and antiulcerogenic (Adzu et al., 2002; Borgi et al., 2007), the antibacterial effects (Ali et al., 2001; Nazif, 2002, Lahlou et al., 2002). In the Aures region this fruit is commonly used in food like a crudity, especially by children. The aim of this study was to investigate the nutritional quality of these fruits.

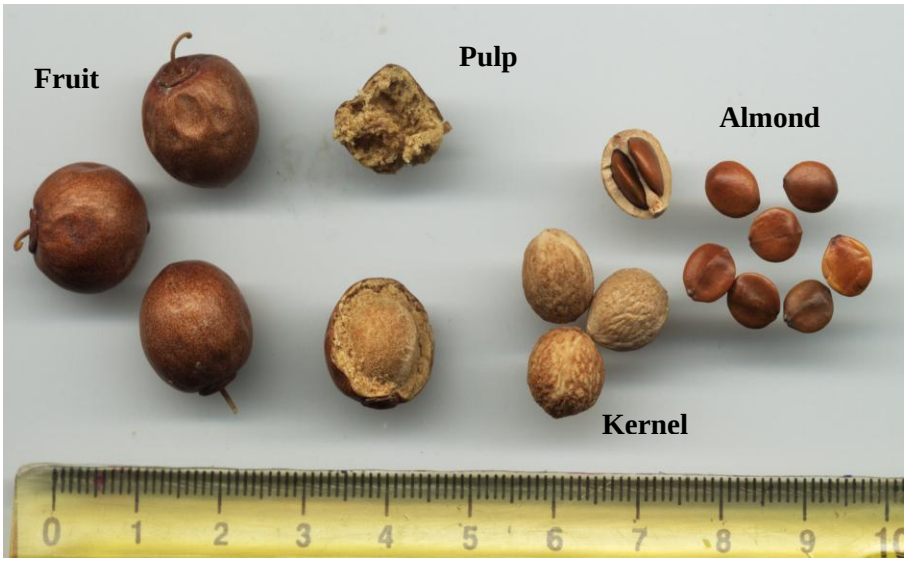

Figure 1. Fruit compartments of *Z.lotus* L

2. MATERIALS AND METHODS

2.1.Plant material

The fruits of *Z. lotus* L were collected from El Mader region (Aures) during September 2009, where this plant grows spontaneously. It was kindly identified in the Agriculture Institute of Batna. Edible parts, seeds and almonds were manually isolated (Fig.1).

2.2. Physical characterization

Fifty fruits were aleatory chosen, the weight of each fruit and its parts have been realized, pulp, kernel and almond were weighed with a precision scale Sartorius 1702. The measures were determined with a digital caliper. The dry mater was obtained by dehydration in oven Memmert SLE 400 at 103±2 °C (Audigie et al., 1984). The total energy has been realized with an adiabatic calorimeter IkaWerk 5003 on the dried powder. The refractive index of oil has been determined with an Abee refractometer Schmidt-Haensch**.**

2.3. Chemical characterization

A visible spectrophotometer, Shimadzu UV 120-01 was used to analyze the sample for soluble sugars, and phosphorus. The extraction apparatus Fibertec System Tecator 1010 has been used to evaluate total fibers. A flame spectrophotometer Jenwey PEP7 permited the quantification of natrium and kalium.

The atomic absorption spectrometer Pye Unicam sp 2900 with air acetylene Flame, was used for the determination of the following minerals; iron, magnesium, manganese and zinc. The automatic extractor, Soxtec System HT 1043 permited the extraction of fats from the sample. A gas chromatograph GC-MS Hewlett Packard HP 6890, coupled with a mass spectrometer detector Hewlett Packard MSD 5973 with electronic impact was used for the separation and identification of fatty acid methyl esters.

2.3.1. Protein content

Total proteins were determined according to the standard method of Kjeldahl (AFNOR-DGCCRF, 1995). The protein content is obtained by the multiplication of total nitrogen by 6.25 (Deymie et al.,1981).

2.3.2. Amino-acids identification

The identification of amino acids was realized by thin layer chromatography (TLC) on silica gel 0.25mm (Merck, Darmstadt, Germany). The used mobile phase was butanol : acetic acid: water (4 /1 /4). The sample was the hydrolysat of protein in HCl 6N according to the method reported by Audigie et al. (1984). The revelation is realized by pulverization of plate with 3% of Ninhydrine in acetone. It

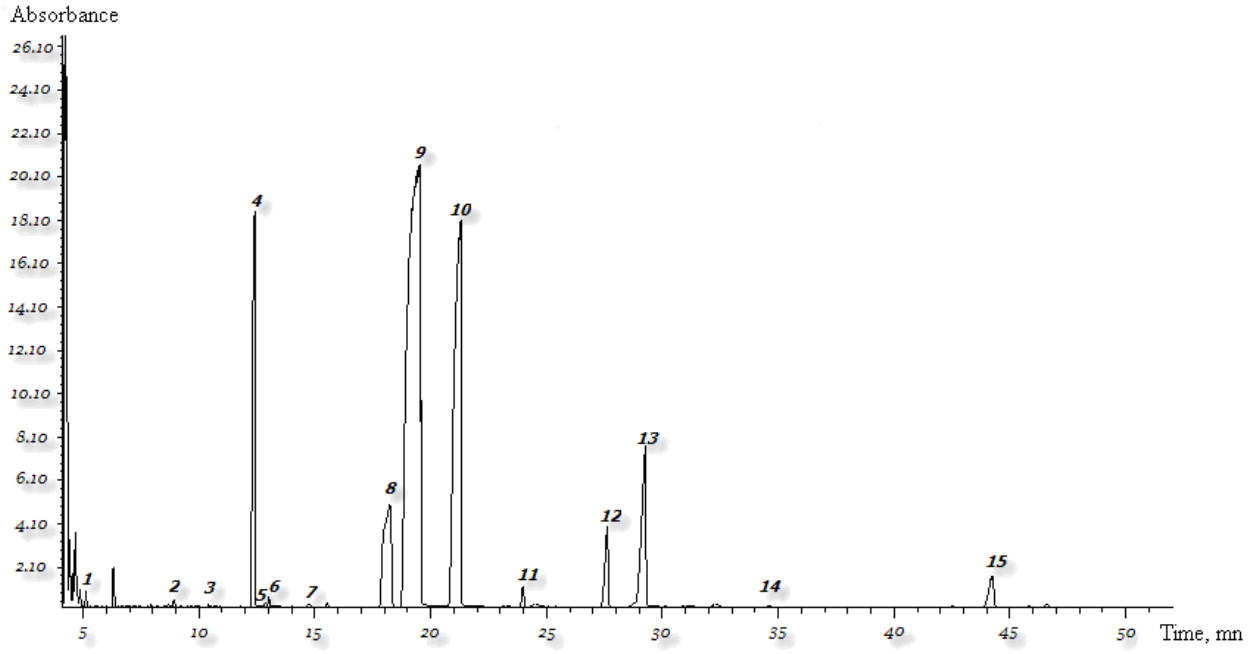

Figure 2. GC-MS Chromatogram of fatty acid methyl esters of almond oil of *Z.lotus* L fruit.

(1) C_{10}: 0 – **(2)** C_{14}: 0 – **(3)** C_{15}: 0 – **(4)** C_{16}: 0 – **(5)** C_{16}: 1^{7} – **(6)** C_{16}: 1^{9} – **(7)** C_{17}: 0 – **(8)** C_{18}: 0 – **(9)** C_{18}: 1^{9} – **(10)** C_{18}: $2^{9,12}$
(11) C_{18}:$3^{9,12,15}$ – **(12)** C_{20}: 0 – **(13)** C_{20}: 1^{11} – **(15)** C_{22}: 0

shows the amino acids in blue purple spots on the plate. The identification of amino acids is based on the comparison of front reference R_f of the amino-acids references (Merck, Darmstadt, Germany) with those of samples.

2.3.3. Extraction of lipids

About 50g of dried and powdered pulp or almonds were extracted with 150ml of hexane (Biochem Chemopharma, Georgia, USA) using an automatic extractor (Soxtec System HT 1043) during 90 mn. The extract was collected and the solvent evaporated in the water bath at 75 °C, the evaporation residue was dried in oven up to constant weight. The saponification index is determined in the usual way with ethanolic KOH. An aliquot of 0.4g of oil is dissolved in 20 ml of ethanolic KOH and boiled under reflux during 45 mn. The excess of potash was titrated with HCL at 0.2 N. The refractive index of oil was obtained using the Abee refratometer.

2.3.4. Fatty acids identification

The fatty acid methyl esters were obtained by the dissolution of 0.5g of fatty extract in 0.2 ml of methanolic KOH 2N. The obtained methylic esters were analysed by GC-MS, chromatograph under the following conditions: Polar capillary column Stabilwax (60 m, 0.25 mm, 0.25 µm). The column oven temperature was programmed from 200 to 220 °C at 2 °C/minute and maintained for 30 mn. The quantity of injected sample was 1µl. Helium was the carrier gas with 0.5 ml/mn as flow. The injection was realized in split less mode; the injector temperature 250 °C, the transfer line and the bloc were respectively at 280 °C and 230 °C. The mass spectrum was obtained in ionization mode with electronic impact at 70 eV and scanning field from 50 to 600 uma at 2.83 scan/s. The recorded chromatogram is represented in figure 2 and the results which resume the fatty acids composition of the sample were presented in table 3.

2.3.5. Free sugars analysis

The soluble sugars were obtained by maceration of 2g of powder of edible part or almonds in 50 ml of distilled water during one hour with stirring. A 0.5g of $CaCO_3$ were added, the mixture was heated up to boiling. After cooling, 0.5g of lead acetate was added to the filtrate. After filtration, the extract was

used for the identification of soluble sugars by TLC on silica gel 0.25mm using Methylethylcetone / acetic Acid / Methanol (3/1/1) as mobile phase. The revelation was realized by spraying the plate with a mixture of α-naphtol (Merck, Darmstadt, Germany) 0.25 g, absolute ethanol 50ml and 50ml sulfuric acid 20% and heating for 5 min. The comparison of front reference of samples and the references (Fluka Chemie GmbH, Germany) permits the identification of soluble sugars (Multon, 1991). The total fibers were determined with an automatic apparatus by exhaustion using in first a solution of sulphuric acid (Cheminova International SA, Spain) 0.26 N during 30mn, after cleaning with water a second exhaustion with NaOH solution (Cheminova International SA, Spain) 0.23N was realized. The difference between the dry weight of the extract and the weight after incineration gave the weight of total fibers in the sample. The pectin was determined by extraction on 10g of powdered sample by 500ml of chlorhydric acid 0.1 N and heating at 90 °C for one hour with a continuous stirring. Then, the solution was left to settle for 12 hours and 5g of aluminium sulfate were added to precipitate the pectin. The pH was adjusted to 4 with ammonium hydroxide 1N. The precipitate was filtered, dried and weighted, results were summarized in table 2.

2.3.6. Mineral analysis

The ashes were obtained by incineration of 1g of samples in muffle furnace Heraeus (MR.170) at 550 °C (Pinta et al., 1980). The obtained ash was slowly moistened with 3 ml of bidistilled water and 3 ml of concentrated fluorhydric acid (Biochem Chemopharma Georgia, USA). The filtrate was adjusted to 100ml with bi-distilled water. The analysis of minerals was realized by a visible spectrophotometer (Shimadzu UV 120-01) for the phosphorus, a flame photometer for natrium and kalium and with atomic absorption spectrometer Pye Unicam sp 2900 for magnesium, manganese, iron and zinc using an air acetylene flame atomization and a single element lamps. For each element, a standard curve is realized in the same analysis conditions. The results were summarized in table 4.

2.3.7. Statistical analysis

Results regarding physical characteristics, proximate analysis and elemental composition were represented as means ± standard deviation.

3. RESULTS AND DISCUSSIONS

The physical characteristics (figure 1) show that the pulp constitutes 50% of fruit weight and the almond 14% of seed weight (Table1).

Table 1: Physical characteristics of *Z.lotus* L fruits.

Parameters	Values*
Fruit length (cm)	1.19±0.04
Fruit section (cm)	1.26±0.18
Pulp thickness(cm)	0.22±0.01
Kernel length(cm)	0.93±0.021
Kernel section(cm)	0.79±0.015
Pulp crud energy kJ/100g DM	1727.17±32
Almond crud energy kJ/100g DM	1625.73±28
Pulp proportion /Fruit (%)	50.36 ± 1.50
Almond proportion /Kernel (%)	14.58 ± 1.95

* Means± SD of five determinations.

The dry mater in level of 87% shows that the edible part has a hard consistency and can be stored for a long time. Measurements showed that the jujube of *Z.lotus* L is a small fruit with spherical form and brown color at maturity. The pulp can yield 17.271kJ/g, a very interesting quantity in regard to conventional fruits. The refraction index showed that the oil of *Z. lotus* L. is rich in oleic acid which was confirmed by the GC MS analysis. The total protein content is essentially present in seeds with 14.22 % and shows a small quantity in the pulp with 1.18% (Table 2). The amino acids identified with TLC were serine, glycine, alanin, leucin, histidin, arginin and cystein. The lipids in the *Z. lotus* L fruit are localized in seeds with a content of 29.73% of dry weight, when a small amount is detected in the pulp with 0.79%. The estimated unsaponifiable fraction is 3.02% of dry weight.

Table 2. Proximate analysis of *Z.lotus* L fruits.

Parameters	*Pulp	*Almond
Dry mater (%)	87.73 ± 0.20	92.43 ± 0.30
Ash%	3.20±0.15	92.43 ± 0.30
Crud Fats (%)	0.79 ± 0.02	29.73 ± 0.24
Soluble sugars (%)	10.55 ± 0.26	4.10 ± 0.23
Total fibres (%)	4.84 ± 0.55	16.57 ± 0.19
Pectins (%)	2.07 ± 0.33	1.35 ± 0.26
Crud protein (%)	1.18 ± 0.36	14.22 ± 0.89

* Means± SD of three determinations.

The fatty acids methyl esters analysis, by GC-MS revealed the presence of 15 fatty acids. Unsaturated fatty acids represent the major components with 79.78 %. These unsaturated fatty acids give the oil of *Z. lotus* L seeds a high nutritive value, while the saturated ones represent 20.22 % (Table 3). The major fatty acids in this oil are in decreasing value, oleic acid with 49.88 %, linoleic acid 22.97 %, palmitic acid 9.05 %, stearic acid 7.10, gadoleic acid 6.32 and arachidic acid 2.36 %. Other fatty acids characterize this oil, like the behenic acid. The soluble sugars represent 10.15 % in the edible part and 4.10% in the almond. Their identification by TLC shows that they are a mixture of glucose, fructose and saccharose. The total amount was 16.57 % in the edible part, probably is responsible for its hardness. In the almond, fibers reached 16.57 % and are the important constituents of the cuticles which cover the almond. The pectin is present with 2.07 % in the edible part and 1.35% in the almond; it's a poor fruit in pectic compounds. The mineral matter present in the edible part is 3.20 %, characterized by its richness in phosphorus, natrium, kalium and magnesium (Table 4).

Table 4. Elemental analysis of *Z.lotus* L fruit.

Minerals mg/100g DM	*Pulp	*Almond
Phosphorus	10.62±1.70	24.00±0.63
Natrium	11.45±1.20	17.41±1.63
Kalium	134.99±6.70	97.92±7.86
Manganese	2.17±0.09	7.84±0.93
Magnesium	397.91±18.81	1349.06±66.94
Iron	1.33±0.04	1.21±0.07
Zinc	0.44±0.02	1.38±0.05

* Means± SD of three determinations.

Table 3. Fatty acids composition of almond oil of *Z.lotus* L fruit.

Retention Time (mn)	Chemical denomination	Usual nouns		Proportion %	Mass
5.581	Decanoic	Caprylic	C_{10}	0.014	186
8.919	Tetradecanoic	Myristic	C_{14}	0.084	242
10.401	Pentadecanoic	Pentadecylic	C_{15}	0.024	256
12.411	Hexadecanoic	Palmitic	C_{16}	9.025	270
12.874	7-Hexadecenoic	Hypogenic	$C_{16}:1^{7}$	0.058	268
13.010	9-Hexadecenoic	Palmitoleic	$C_{16}:1^{9}$	0.134	268
14.753	Heptadecanoic	Margaric	C_{17}	0.077	284
18.150	Octadecanoic	Stearic	C_{18}	7.106	298
19.306	9-Octadecenoic	Oleic	$C_{18}:1^{9}$	49.882	296
21.233	9-12-Octadecadienoic	Linoleic	$C_{18}:2^{9,12}$	22.973	294
23.984	9-12-15-Octadecatrienoic	Linolenic	$C_{18}:3^{9,12,15}$	0.409	292
27.624	Eicosanoic	Arachidonic	C_{20}	2.367	326
29.261	cis11-Eicosenoic	Gadoleic	$C_{20}:1^{11}$	6.328	324
34.615	Heneicosanoic	/	C_{21}	0.047	340
44.225	Docosanoic	Behenic	C_{22}	1.409	354

The amount of phosphorus is more important in the almond than in the edible part with 24.00 mg/100g DM, the same conclusion was found by Largois (1994). This value is higher than that found by Murdock (2002) with 65 mg/kg of DM. Other varieties like *Z.mauritiana* and *Z. jujuba* show a respective content of phosphorus as 89.3 and 105mg/100g of DM (Li et al., 2007). The magnesium content of *Z.lotus* L as 397.91mg/100g of DM is more important than that found by Murdock (2002). The jujubes of *Z.lotus* L content of natrium is 11.45 mg/100g of DW. The presence of this important quantity of natrium can be explained by the presence of this plant in the dry and salty soils in arid and semiarid zones. Kalium in edible part of *Z.lotus* L fruit is in amount of 134.99mg/100g DW. This value is small with regard to those found by Murdock (2002) for the fresh jujube, of *Z.mauritiana* 250 mg/100g.The fruit of *Z.lotus* L can yield an appreciable amount of oligoelements, iron, manganese and zinc. The iron concentration in the edible part of *Z.jujuba (*Zhao et al., 2006; Zhao et al., 2007) is important with 3mg/100g DW.The manganese content is higher than that found by Murdock (2002) as 1mg/100g of DW but it is in concordance with values reported by Pinta et al.(1980). The zinc content of *Z.lotus* L fruit is lower than that found by Murdock (2002) in the fresh jujube. The proximate biochemical composition in the Chinese jujube was reported by Wei et al. (2007).

4. CONCLUSIONS

The study shows that the *Z.lotus* L. fruits are dry product and the edible part is interesting representing 50 % of fruit weight. The proportion of almond in kernel is also interesting, it could be valorized as a food or like oleaginous. Because of its high energy potential, this fruit can be considered as a high energetic fruit. The moisture content is a good parameter for its storage for a long time. The study of pulp shows that the major compounds of the biomass are sugars and they are also important in the almond. The fiber content permits to classify this fruit as a functional food. The essential oil constitutes a very characteristic flavor of this fruit where it can be used in food or in cosmetics. The study of fatty fraction indicates that the oil in the fruit is located in almond and has standard characteristics of vegetable oils. The biochemical characterization shows that this oil is distinct by its richness in essential fatty acids, thus it can be used as a food, in pharmacology or in cosmetics. The edible part cannot be considered as a source of protein because its low content in this fraction but the almond can be considered as a proteinous seed. The studied proteins can compensate in part the foodstuffs deficit shown in arid regions. The studied fruit represents an appreciable source of minerals, very appreciated and consumed by children of local populations as crudeness in the shortage period. This unconventional and underutilized fruit has an interesting biochemical composition which can be valorized. This work can help to preserve the specie by its integration as an agro-resource in the sustainable development in arid regions of the Sahara.

5. ACKNOWLEDGMENTS

The authors are grateful to Professor B. Oudjehih, from Agriculture Institute of Batna for the identification of specie, and to Drs Y. Badja and R. Tir, from Haouari Boumedienne University for the GC-MS analysis.

6. REFERENCES

[1] Abdel-Zaher, A.O., S.Y. Salim, M.H. Assaf and R. Abdel-Hady, 2005. Antidiabetic activity and toxicity of *Zizyphus spina-christi* leaves. *J. Ethnopharmacol.*, 101: 129-138.

[2] Adzu, B., S. Amos, S. Dzarma, C. Wambebe and K. Gamaniel, 2002. Effect of *Zizyphus spina-christi* Wild aqueous extract on the central nervous system in mice. J. Ethnopharmacol., 79 : 13 -16.

[3] AFNOR-DGCCRF, 1995. Contrôle de qualité des produits alimentaires, méthodes d'analyses officielles. Tome 1. Paris.

[4]Ali, N.A., W.D. Julich, C. Kusnick and U. Lindequist, 2001. Screening of Yemeni medicinal plants for antibacterial and cytotoxic activities. J. Ethnopharmacol., 74 : 173 -179.

[5] Audigie, C.l., J. Figarella and F. Zonszain, 1984. Manipulation D'analyse Biochimique. Doin, Paris,

ISBN-13: 9782704004287, pp: 25-37.

[6] Borgi, W., Bouraoui. A and Chouchane. N, 2007. Antiulcerogenic activity of *Zizyphus lotus* L. extracts. *J. Ethnopharmacol.*, 112: 228-231.

[7] Deymie, B., J.L. Multon and D. Simon, 1981. Technique d'analyse et de contrôle dans les industries

agro-alimentaire . Lavoisier Tec et Doc. Vol 4. Paris, 409p.

[8] Epfraim, K.D., U.A. Osunkwo, P. Onyeyilli and A. Ngulde, 1998. Preliminary investigation of the possible antinociceptive activity of aquous leaf extract of *Zizyphus spina-christi* L. Desf. *Indian J. Pharmacol.*, 30: 271-272.

[9] Ghedira, K., R. Chemli, C. Caron, J.M. Nuzillard, M. Zeches and L. Le Lemen-Olivier, 1995. Four cyclopeptid alkaloids from *Zizyphus lotus* L. *Phytochemistry*, 38: 767-772.

[10] Glombitza, K.W., G.H. Mahran, Y.W. Mirhom, K.G. Michel and T.K. Motawi, 1994. Hypoglycemic and antihyperglycemic effect of *Zizyphus spina christi*. Planta Med., 60 : 244 -247.

[11] Lahlou, M., M. El Mahi and J. Hamamouchi, 2002. Evaluation of antifungal and molluscidal activities of Moroccan *Zizyphus lotus (L.)* Desf. *Ann. Pharm. Francaises*, 60(6) : 410-414.

[12] Largois, D., 1994. Alimentation Vivante. 2nd Edn., Doin, Paris, Pages: 59.

[13] Le creour, G., Thépenier P., Richard B., Petermann C., Ghédira K. and Zèches-Hanrol M., 2002. Lotusine G: A new cyclopeptide alkaloïd from *Zizyphus lotus*. *Fitoterapia*, 73 : 63-68.

[14] Li, J.W., L.P. Fan, S.D. Ding and X.L. Ding, 2007. Nutritional composition of five cultivars of Chinese jujube. *Food Chem.*. 103: 454-460.

[15] Multon, J.L., 1991. Technique d'analyse et de contrôle dans les industries agro-alimentaire. Lavoisier Tec and Doc (Ed). vol 2. Paris, 456p.

[16] Murdock, D.H., 2002. Encyclopedia of Food a Guide to Healthy Nutrition. APA Academic Press, San Diego, California, Pages: 501.

[17] Nazif, N.M., 2002. Phytoconstituents of *Zizyphus spina-christi* L. fruits and their antimicrobial activity. Food Chem., 76 : 77 -81.

[18] Oh, M.H., P.J. Houghton, K.W. Whang and J.H. Cho, 2004. Screening of Korean herbal medicines used to improve cognitive function for anti-cholinesterase activity. Phytomedicine, 11: 544 -548.

[19] Perdue, Jr, R.E. and J.L. Hartwell, 1976. Plants and cancer. Proc. 16th Annual Meeting Soc. Econ. Bot. *Cancer Treatment Rep.*, 60: 973-1215

[20] Pinta, M., B. Bourdon and F. Rousselet, 1980. Spectrophotometrie D'absorption Atomique. Masson, Paris, Pages: 478.

[21] Renault, J.H., K. Ghedira, P. Thepenier, C. Lavaud, M. Zeches-Hanrot and L. Le Men-Olivier,

1997. Dammarane saponins from *Zizyphus lotus*. *Phytochemistry*, 44(7): 1321-1327.

[22] Sudhersan, C. and J. Hussain, 2003. *In vitro* clonal propagation of a multipurpose tree, *Zizyphus spina Christi* (L.) Desf. Turk. J. Bot., 27 : 167 -171.

[23] Wei. L.I. J., Ping Fan. L., Dong Ding. S., Lin Ding. X., 2007. Nutritional composition of five cultivars of chinese jujube. Food Chemistry. elsevier. 103:454-460

[24] Zhao, J., S.P. Li, F.Q. Yang, P. Li and Y.T. Wang, 2006. Simultaneous determination of saponins and fatty acids in Zizyphus jujube (Suanzaoren) by high performance liquid chromatography-evaporative light scattering detection and pressurized liquid extraction. J. Chromatogr. A, 1108: 188–194.

[25] Zhao, Z., M. Liu and P. Tu, 2007. Characterization of water soluble polysaccharides from organs of Chinese Jujube *Zizyphus jujube* Mill. *Eur. Food Res.* Technol.,226: 985-989