

ANALYTICAL AND PHYTOCHEMICAL STUDIES ON ZIZYPHUS LOTUS

Zaid Najah¹ and Khaled Elsherif^{2*}

¹Chemistry Department, Faculty of Science, Al-Mergheb University, Libya.

²Chemistry Department, Faculty of Science, Benghazi University, Libya.

* Corresponding Author: Dr. Khaled Elsherif

Chemistry Department, Faculty of Science, Benghazi University, Libya.

Article Received on 20/05/2016

Article Revised on 10/06/2016

Article Accepted on 30/06/2016

ABSTRACT

Phytochemicals and heavy metals analysis of *Zizyphus lotus* were carried out with a view to assess their therapeutic values. The results showed that the bioactive compounds like alkaloids, flavonoids, phenolics, glycosides, carbohydrates, phytosterols, proteins and saponins were present. The tests were performed on different extracts with different polarity; the ethyl acetate extract was the richest extract. Heavy elements concentrations in *Zizyphus lotus* were also investigated. The zinc concentration was found to be high; 69.18 ppm. The micronutrients levels in *Zizyphus lotus* was in the order: Zn > Cu > Fe > Mn. The toxic metals levels were in following order: Pb > Cd.

KEYWORDS: Phytochemical Screening, *Zizyphus Lotus*, Heavy Metals.

INTRODUCTION

For a long time, plants are being used for the development of new drugs and many medicinal plants are used in modern medicine where they occupy a very significance place as raw material for important drugs; about 61 % of the total 877 drugs are from natural sources.^[1-3] A large number of the plants are claimed to possess the antibiotic properties in the traditional system and are also used extensively by the tribal people worldwide. Even the World Health Organization (WHO) supports the use of medicinal plants, provided it is proven to be efficacious, safe, less toxic, available and reliable natural resource.^[4] More than 80 % of population in the developing countries makes use of medicinal plant extracts for a good health.^[5] The basic components of herbal medication are diverse bioactive compounds like alkaloids, flavonoids, terpenoids, phenolic compounds, glycosides etc.^[6]

Extraction of the bioactive plant constituents has always been a challenging task for the researchers and there are many research articles that focused on the medical importance of plants and also to identify the basic components of these plants.^[7-11]

Zizyphus lotus (Figure 1) is abundantly present in the Mediterranean region, throughout Libya to Morocco, and southern European countries like Spain, Sicily, Greece and Cyprus.^[12] In Libya, it is known as 'Sedr'. The fruit is the edible part of the plant by local population. Several parts of *Zizyphus* have been used by traditional and ancestral medicine, both in North Africa and Middle

East, for the treatment of several pathologies including digestive disorders, weakness, liver complaints, obesity, urinary troubles, diabetes, skin infections, fever, diarrhea and insomnia.^[13,14] The medicinal properties of this plant depend on the part of the plant concerned (root, leaf stalk and pulp or fruit) and the extract used (ethanolic, butanolic etc.). Fruit has been used for its emollient properties, and a mixture of dried leaves and fruits is applied topically in the treatment of boils. Interestingly, the root bark is known for its antidiabetic activity.^[15]

Preliminary phytochemical screening of the plants is an important aspect in finding the chemical constituents of the plant materials. Therefore, this study will focus on chemical qualitative analysis of secondary metabolite in addition to quantitative evaluation of heavy metals in *Zizyphus lotus*.



Figure 1. *Zizyphus Lotus*

EXPERIMENTAL**Herbal Material Collection And Preparation Of Extracts**

Herbal material was collected from Gaser Khair city during Summer 2015. The plant was identified by Biology department, Science Faculty, Almergheb University. The collected leaves were dried in shade for one month dried then cut to small fragments before was grinded into fine powder using electrical blender (Hommer, 350 Watt).

The extraction started by soaking 5.0 g of powder in 1.0 L of 80% aqueous ethanol for 7 days at room temperature, the extract was reduced on hot plate, partitioned with gradual polarity increasing solvents using hexane, chloroform and ethyl acetate. Extracts were evaporated to dryness on hotplate then cooled to room temperature before conserved in fridge for subsequent phytochemical analysis. Aqueous extract was

prepared by heating 1.5 g of the powder in 150 ml of distilled water at 50°C for 15 minutes, the mixture cooled to room temperature, filtered and stored in fridge for next step.

Wet Digestion Procedure For Heavy Metals, Na, K Analysis^[16]

To 0.50 g of sample powder in 250 ml conical flask was added 3 ml of conc. HNO₃. The mixture was heated on hot plate at 100°C for 5 minutes till near dryness then left to cool down, another 3 ml of conc. HNO₃ was added and the process was repeated till evolving brown fumes stopped and clear light brown solution was obtained. The mixture was left to cool to room temperature, filtered, diluted to 250 ml with distilled water then introduced to atomic absorption machine Shimadzu AA7000 for heavy metals analysis. Sodium and Potassium analysis was performed on spectrophotometer 6300.

RESULTS AND DISCUSSION**Table 1 Phytochemical screening of Z. Lotus**

<i>Extract</i>	<i>Water</i>	<i>EtOH</i>	<i>EtOAc</i>	<i>CHCl₃</i>	<i>Hexane</i>
<i>Test</i>					
Alkaloids	-	-	+	+	+
Carbohydrates	-	-	+	+	+
Saponins	+	+	+	+	-
Phytosterols	+	+	+	+	-
Tannins	-	-	-	-	-
Phlobatinnins	-	-	-	-	-
Flavonoids	+	+	+	+	+
Phenolics	+	+	-	-	-
Protiens	-	+	+	+	+
Diterpenes	+	+	+	-	+

Phytochemical evaluation revealed the presence of some important secondary metabolites in the studied plant as shown in Table 1. As seen in Table 1, alkaloids and carbohydrates were found in EtOAc, CHCl₃ and hexane extracts while were absent in the heights polarity water and ethanol extracts. Most of Alkaloids have pharmacological importance in preventing and treating many diseases.^[17-21]

Saponins and Phytosterols were observed in all extracts except hexane extract, Phytosterols (plant sterols) are natural constituents of cell membrane of plants, their structural similarity with cholesterol accounts for their lipid-lowering effect humans.^[22]

All tests for tannins and phlobatannins were negative indicating the absence of these classes in this plant. The investigation revealed the presence of flavonoids in all extracts while the similar class, phenolics were noticed only in the most polar extracts, aqueous and ethanolic extracts. Anti-oxidant activity of wide array of herbal extracts is attributed to presence of flavonoids.^[23] The important nutrient, proteins and their basic components amino acids were observed in all extracts except aqueous extract. Other interesting secondary metabolites found in

Z. lotus is diterpenes, tests indicated the presence of diterpenes in all extracts except chloroform extract. Diterpenes are well known for their antibacterial, antifungal, antimycobacterial, cytotoxic, antitumor, cardiovascular, antifeedant, insecticidal and antileishmanial activities.^[24]

Metal Content Analysis**Table 2 The content of heavy metals in Zizyphus Lotus plant**

<i>Metal</i>	<i>Metal Content, ppm</i>
Cd	0.2633 ± 0.1113
Cu	35.31 ± 1.51
Pb	1.78 ± 0.32
Zn	69.18 ± 7.14
Fe	8.73 ± 0.29
Mn	1.07 ± 0.07

The growth of medicinal plants not only need nutrients for normal plant growth, but also can selectively uptake and accumulate some trace elements which are good and may also be toxic for human health if there not within the limits. The heavy metals analysis showed that Zizyphus Lotus plant contains variable levels of these elements

such as Cadmium (Cd), Copper (Cu), Lead (Pb), Zinc (Zn), Iron (Fe) and Manganese (Mn), results were summarized in Table 2 and shown in Figure 2.

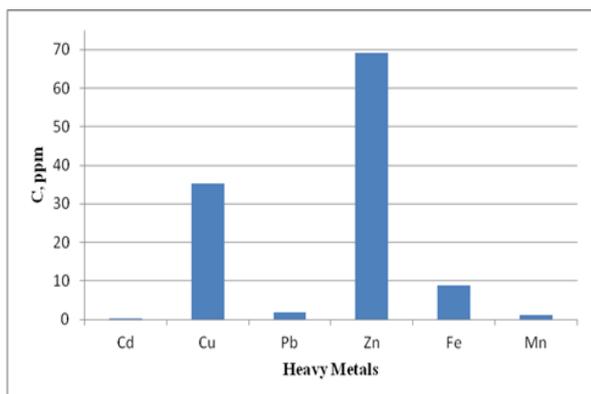


Figure 2 Concentrations of heavy metals in Zizyphus Lotus plant

It can be noted from Table 2 that Zn is the major constituent of Zizyphus Lotus and formed the bulk of total minerals which is higher than permissible limit set by WHO for edible plants (27.60 ppm). Zinc is trace essential element and plays an important role in basic bio-processes for the plant and its deficiency could lead to serious growth problems.^[8] The next major constituents are Cu and Fe. Cadmium is the least found in Zizyphus Lotus. No limits values were set by WHO for copper in medicinal plants, although China and Singapore have permissible limits 20 ppm and 150 ppm respectively.^[25] Toxicity with copper could cause hair and skin discoloration, dermatitis, irritation of the upper respiratory tract, metallic taste in the mouth and nausea. Copper deficiency is rare but it causes anemia, malnourished infants can have copper deficiency.^[26] Cadmium concentration was 0.26 ppm; the least between all measured metals (less than 0.30 ppm); the WHO limit for medicinal herbs. The increase of cadmium level in human body could cause serious problems in kidney and liver, vascular and immune system.^[27]

Lead is non-essential trace and highly poisonous element. Pb induces various toxic effects in humans at low doses and mainly targeting nervous system in both adults and children.^[28] The permissible limit set by FAO / WHO in edible plants was 0.43 ppm while in WHO herbal medicine is 10 ppm.^[28] Lead concentration was found 1.780 ppm which is lower than WHO limit.

Sodium And Potassium Contents

The sodium and potassium contents in Zizyphus Lotus were 89 and 64 ppm, respectively. From a nutritional point of view, Zizyphus lotus seed may be considered a good source of minerals, especially magnesium, potassium and calcium.^[29] Macromineral elements, such as potassium, calcium and sodium and trace minerals, such as iron, copper, zinc, and manganese, play a critical role in biological systems. These elements maintain normal physiological reactions, induce general

metabolism, germination, circulatory systems and influence reproduction as catalysts of various biochemical reactions.^[30]

CONCLUSIONS

The results revealed the presence of medicinally important constituents in the plant studied. Many evidences gathered in earlier studies which confirmed the identified phytochemicals to be bioactive. Therefore, extracts from these plants could be seen as a good source for useful drugs. Further work should be carried out to isolate, purify, and characterize the active constituents responsible for the activity of these plants.

REFERENCES

- Iwu M, Hand book of African Medicinal plants, CRC press, Boca Raton, Fl., 1993.
- Cseke L. J., Kirakosyan A., Kaufman P. B., Warbe S. L., Duke J. A., Briemann H. L., Natural Products from Plants, Second Edition, CRC Press, New York, 2006.
- Audu S. A., , Mohammed I., Abdul Kaita H., Life Sci J, 2007; 4(4).
- World Health Organization (WHO): World Health Report. Bridging the gap. 1, WHO, Geneva, 1995; p118.
- World Health Organization (WHO): Traditional Medicine. Growing needs and Potential, WHO policy perspective on Medicines, Geneva, 2002; p1-6.
- Hasan S M R, Majumder J M, MM, Hossain A R, Mazumder M M, Alam M E H, Jahangir M A, Rana R, Rahman S., Am J of Pharmacol and Toxicol, 2009; 4(1): 1-7.
- Najah Z, Elsherif K M, Alshatewi M, and Attorshi H, J App Chem, 2015; 4(6): 1821-1827.
- Najah Z, Elsherif K M., Kawan E and Farah N, Ijppr. Human, 2015; 4(3): 82-91.
- Benkhayal A. A., Elsherif K, and El-turki K S, Der Chem Sin, 2013; 4(5): 1-6.
- Tiwari P, Kumar B, Kaur M, Kaur G, Kaur H, Int. Pharm. Sci., 2011; 1(1): 98-106.
- Eswari M L, Bharathi R V, Jayshree N, Int. J. Pharm. Sci. Drug Res., 2013; 5(1): 38-40.
- Pottier P, Alapetite G, Programme Flore et Végétation Tunisiennes. Flore de la Tunisie Publications Scientifiques Tunisiennes, Tunis, 1981.
- Adzu B, Amos S, Amizan M B, Gamaniel K, Acta Tropica, 2003; 7: 245-250.
- Lahlou M, El Mahi M, Hamamouchi J, Desf. Ann. Pharm. Fr., 2002; 60: 410-414.
- Glombitza K W, Mahran G H, Mirhom Y W, Michel K G, Motawi T K, Planta. Med., 1994; 60: 244- 247.
- Denton G.R.W. and Breck W G, Mar. Pollut. Bull., 1981; 12: 116-121.
- Kittakoop P, Mahidol C, Ruchirawat S, Curr. Top. Med. Chem., 2014; 14(2): 239–252.
- Russo P, Frustaci A, Del Bufalo A, Fini M, Cesario A, Curr. Med. Chem, 2013; 20 (13): 1686–1693.

19. Sinatra R S, Jahr J S, Watkins-Pitchford J M, The Essence of Analgesia and Analgesics, Cambridge, Cambridge University Press, 2010.
20. Cushnie T P, Cushnie B, Lamb A J, *Int. J. Antimicrob. Agents*, 2014; 44(5): 377–386.
21. Qiu S, Sun H, Zhang A H, Xu H Y, Yan G L, Han Y, Wang X J, *Chin. J. Nat. Med*, 2014; 12(6): 401–406.
22. Nijjar P S, Burke F M, Bloesch A, Rader D J, *J. Clin. Lipidol.*, 2010; 4: 248-58.
23. Sofowara A., *Medicinal plants and Traditional medicine in Africa*. Spectrum Books Ltd, Ibadan, Nigeria., 1993; p289.
24. Gulact T and Ahmet C. G, *Rec. Nat. Prod*, 2007; 1(1): 1-16.
25. WHO, *Quality Control Methods for Medicinal Plant Materials*, Geneva, 2005 Revised.
26. Ullah R., Khader J.A., Hussain I., AbdElsalam N.M., Talha M., and Khan N., *Afr. J. Pharm. Pharmacol.*, 2012; 6(25): 1829-1832.
27. Jabeen S., Shah M.T., Khan S., and Hayat M.Q., *Pak. J. Med. Plants Res.*, 2010; 4(7): 559-566.
28. Khan S.A., Khan L., Hussain I., Marwat K.B., and Ashtray N., *Pak. J. Weed Sci. Res.*, 2008; 14(1-2): 101-110.
29. Chouaibi M, Mahfoudhi N, Rezig L, Dons`ı F, Ferraria G, and Hamdi S, *J. Sci. Food Agric.*, 2012; 92: 1171–1177.
30. Alqarnia A S, Owayssa A A, Mahmoudb A A, Hannana M A, *J. Saudi Chem. Soc.*, 2014; 18(5): 618-625.