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Study of the Enzymatic and Anti-Inflammatory Activities of Phenolic Extracts of *Atriplex halimus* L. and *Haloxylon scoparium* Pomel

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ABSTRACT

Several of experimental studies and molecular investigations highlight the power of herbs to evidence-based medicine. In Algeria, a number of medicinal plants have been studied for the treatment of diabetes such as Haloxylon scoparium pomel and Atriplex halimus L., growing in arid and subarid areas, known for its hypoglycemic activity and widely used by the local population as remedy to treat diabetes mellitus. Reported to be used for anti-inflammatory. The objective of this review is assessing these plants extracts effect in vitro on the activity of α-amylase to give value to this important traditional medicinal plant as antidiabetic, and evaluate the anti-inflammatory potential.

the inhibitory activity of the dichloromethane fraction A. halimus L. *of α-amylase has a remarkable inhibition percentage, equal to 61.43% with an IC50 value equal to 0.5 mg/ml followed by the butanol fraction of rods haloxylon scoparium pomel 56.21% and the IC50 equal to 0.73 mg/ml. In fact, the dichoromethane extracts of the stems and flowers of the species Haloxylon scoparium pomel exhibit very high NO inhibition percentages at a concentration equal to 160 µg/ml The dichoromethane extract of the species Atriplex halimus* L. *demonstrates the With 97.6% inhibition of NO production at 160 µg/ml.*

Keywords: Medicinal plants, Bioactive compounds, Polyphenols, α-amylase, *In vitro*

INTRODUCTION

Besides the traditional treatment of several diseases, including diabetes mellitus, cardiovascular diseases and other pathologies is a custom known in Algeria, which has a rich heritage of medicinal and food agricultural resources. What makes the Algerian flora a very interesting track for the development of therapeutic activity of these plants? In this context, the objective of our study is focused on *Haloxylon scoparium Pomel* and *Atriplex haimus* The Algerian endemic plant widely used in traditional medicine. The design of this study is summarized in two parts, a study that looks at the effect tested *in vitro* extracts prepared on the activity of α-amylase and evaluation of anti-inflammatoty potential.

Plant. *H. scoparium Pomel* [=*Hammada scoparia (Pomel)* Iljin., *Arthrophytum scoparium (Pomel)* Iljin., *Salsola articulata Cav., Haloxylon articulatum* (Cav.)] [1-3], belongs to the family *Chenopodiaceae*, has 120 genera and more than 1300 species. They are distributed worldwide especially in desert and semi-desert areas in soils Containing much salt. The plants are herbs, shrubs, and subshrubs rarely small trees. The genus *Haloxylon* Bunge (Incl. Hammada) comprised of about 25 species. It is distributed from Western Mediterranean region to Arabia, Iran, Mangolia, Burma and Southwest of China [4]. Two species of the genus were recorded in the literature to-have folkloric uses. *H. salicornicum* is reported to be used for diabetes [5], as antiseptic and anti-inflammatory [6]. In Oman the stems of this species are used as a mordant for dyeing wool in traditional weaving. In addition, *Haloxylon scoparium={Haloxylon articulatum}* is used to treat eye disorders [7]. Infusion and powder infusion of aerial portion

of *H. scoparium* are used for Their antidiabetic effects [8,9]. The crude extracts from some *Haloxylon* species biologically were evaluated. The ethanol extract of *H. salicornicum* was found to-have antidiabetic [5] and anticoagulation activity in experimental animals [10].

A. halimus L. (*Chenopodiaceae*) is a perennial shrub growing on arid and saline soils being a typical mediterranean halophytic plant [11]. As a response to abiotic stress, several halophytic species Produce high levels of phenolic compounds [12]. *A. halimus* L. Was Previously Studied for it antioxidant [13], antiacetylcholinesterase [14] and hypoglycaemic [15] activities, furthermore previous investigations on the chemical species from the genus *Atriplex* Revealed the presence of saponins, alkaloids, betains, proteins, amino acids, mineral salts [16] and phytoecdysteroids [17].

MARERIALS AND METHODS

Extraction of phenolic compounds

The plant material dried in air was extracted using methanol-water solvent system (8/2). The crude extract was collected and concentrated in a rotary evaporator at 40ºC and partitioned successively with petroleum ether, dichloromethane, ethyl acetate and butanol, so as to fractionate the compounds in the raw materials according to their polarity**.**

Enzyme activity

The analysis of the alpha-amylase inhibitors is performed by quantification of the released reducing sugar in the medium (maltose). This method is based on the reducing power of free aldehyde groups and ketone sugars. Alkali and heat, oxidation of these functions simultaneously causes the reduction of 3,5-dinitrosalicylic acid yellow-orange in 3-amino-5 nitrosalicylic acid orange red color that absorbs at 540 nm. The intensity of the color depends on the amount of reducing sugars present in the reaction medium. The inhibitory activity of the enzyme is expressed by the decrease in units of maltose liberated in the reaction medium. The method followed is that of, it is based on the 3,5-dinitrosalicylic (NDS) which stops the enzyme reaction (pH change) and forms a complex with maltose giving a red color, whose absorbance is measured at 530 nm. From an aqueous stock solution of maltose mass concentration 0.03 mol/l of the test solutions are so prepared, in a tube is introduced 2 ml of the maltose solution then 1 ml DNS reagent was added (1 g DNS, 200 mg of crystalline phenol, double sodium tartarate 20 g and 50 mg sodium sulfite dissolved in the sodium hydroxide 1%) which is heated to 100°C for 5 min, after cooling the mixture was made up to 10 ml with distilled water. Reading the absorbance of each solution is performed using a spectrophotometer at a 530 nm wavelength longueure against a white (same solution without the maltose solution).

Alpha amylase activity

The reaction mixture contained 100 mu.l of phenolic extracts, 50 mu.l of alpha-amylase are pre-incubated for 30 min, 1 ml of starch solution 1% (w/v) is added and then incubated at 37°C for 10 min, thereafter the medium is treated in the same way that the maltose calibration curve and reading is carried out at 530 nm. A blank was placed without phenolic extract and again without alpha amylase**.**

Anti-inflammatory activity

This test is based on the measurement of inhibition of Nitric Oxide (NO) produced by mouse macrophages (RAW 264.7). This test is carried out according to the protocol described by Green [18] with minor modifications.

Protocol

Samples at different concentrations and mouse macrophages are initially incubated in 24-well microplates. LPS (interferon gamma) is then added to the plates to stimulate NO production and thus trigger an inflammatory reaction in macrophages. The supernatants recovered after incubation is contacted with the Griess reagent which becomes red in the presence of NO [18]. The absorbance of the contents of each well is measured using a plate reader (Varioskan). The cell mat remaining at the bottom of the wells is used to perform a metabolic activity test with resazurin. A plate reader (Fluoroskan) makes it possible to read fluorescence generated. The results of the anti-inflammatory test are systematically compared with L-NAME (methyl ester of N (G)-nitro-L-arginine), an inhibitor of the enzymatic protein "Nitric Oxide Synthase inducible" (iNOS).

The anti-inflammatory test is carried out on a 24-well plate. The cells used are RAW 264.7, which are mouse macrophages from ATCC. The cells are seeded in 400 µL of DMEM+10% FBS at a concentration of 500,000 cells per ml. The cells adhere for 24 h. Thereafter, the water and maple syrup extracts are added at a non-toxic concentration determined by the WS-1 cells of the anticancer test. Four 1: 2 dilutions are made and 400 µL of diluted extract is added to the cells and in a plate without cells already containing 400 µL of medium which will serve as blank during the analysis of the results. Two 250 µM and 1 mM L-Name controls are also added on the cells and in the control plate in 400 µL medium. Subsequently 200 µL of LPS (lipopolysaccharide) or interferon gamma is added as an inflammatory stimulus. The plates are subsequently incubated 24 hrs at 37°C 5% CO_2. Subsequently, the cell-containing plate supernatant is transferred to a new 24-well plate so that a resazurin test is performed on the cells to ensure that the extracts have no toxicity. Then, 100 µL of supernatant of the plates with and without cells is transferred into a 96-well plate to which 100 µL of Greiss reagent will be added. The plates are then incubated for 30 min at room temperature in the dark and finally read at the spectrophotometer for plate (Varioskan, Thermo) at 550 nm.

RESULTS AND DISCUSSION

Enzyme activity

From the literature review on the physiological function of α-amylase and the role of its inhibitors in the treatment of several diseases. We evaluated the inhibitory potency of our extracts on the activity of this enzyme in order to find natural inhibitors. The tests are performed with the same concentration of extract because of comparison. The percentages of inhibition obtained are recorded in the table below.

The results obtained in Table 1 shows that the inhibitory capacity of the dichloromethane fraction of *A. halimus* L. of the α-amylase has a powerful inhibition percentage, equal to 61.43% with an IC50 value equal to 0.5 mg/ml followed by the butanol fraction of rods haloxylon scoparium 56.21% and the IC50 equal to 0.73 mg/ml, other extracts inhibit the α-amylase at least 50% is 35.63 and 33.00% respectively. The butanol fraction of the extract *A. halimus* L. has a low percentage of inhibition of the order of 9.83%.

Table 1: The percentage of inhibition of α-amylase by the extracts of the Halimus atriplex L. and Haloxylon scoparium pomel

	Extraits des plants	PI (%)	IC50 (mg/ml)
Atriplex halimus L. (Top)	AHAE	33,00	-
	AHDCM	61,43	0.5
	AHB	9,83	-
Haloxylon scoparium pomel (stems, leaves)	HTFAE	35,89	-
	HTDCM	21,51	-
	HTB	56,21	0.73
Haloxylon scoparium pomel (flowers)	HFAE	31,02	-
	HFDCM	35,56	-
	HFB	18,27	-

AHAE: *Atriplex halimus* L., ethyl acetate fraction, AHDCM: *Atriplex halimus* L. dichloromethane fraction, AHB: *Atriplex halimus* L, butanolic fraction, HTFAE: *Haloxylon scoparium pomel* (stems, leaves), ethyl acetate fraction, HTDCM:*Haloxylon scoparium pomel* (stems, leaves), dichloromethane fraction, HTB: *Haloxylon scoparium pomel* (stems, leaves), butanolic fraction, HFAE: *Haloxylon scoparium pomel* (flowers), ethyl acetate fraction, HFDCM: *Haloxylon scoparium pomel* (flowers), dichloromethane fraction.

By analyzing these results, it was found that all of the phenolic extracts acetate fractions have a powerful inhibition of the α-amylase with more or less significant percentages of inhibition. In addition, we noticed that the AHB and HFB fractions have very low percentages of inhibition although they are rich in phenolic compounds according to the quantification test using the Folin but poor in flavonoids [19]. This can be explained by the fact that the extracts are rich in gearboxes molecules like amino acids, sugars or other compounds that are not known by their inhibitory power for the α-amylase. However, acetate fractions have a satisfactory inhibitory capacity which can be attributed to flavonoid compounds, proven by several studies for their inhibitory power of various enzymes [20]. The synthesis of various results for the activity of our extracts clearly shows that the inhibition of power of the α-amylase is a specific character for each extract which can varies according to its composition and rich in molecules responsible the activity. In order to know the IC50 of our extracts on enzyme activity of α-amylase, we performed inhibition assays to calculate the inhibition constants of our extracts and to determine their type of inhibition. To do this we have chosen only two excerpts that gave percentages above 50% The results obtained in the table shows that the inhibitory activity of the dichloromethane fraction *A. halimus* L. vis-à-vis the α-amylase has a remarkable inhibition percentage, equal to 61.43% with an IC50 value equal to 0.5 mg/ml followed by the butanol fraction of rods *haloxylon scoparium pomel* 56.21% and the IC50 equal to 0.73 mg/ml, other extracts inhibit the α-amylase at least 50% is 35.63 and 33.00% respectively. The butanol fraction of the extract *A. halimus* L. has a low percentage of inhibition of the order of 9.83%. The values found show that the inhibition rate is independent of the concentration of phenols, one can conclude that enzyme inhibition depends on the type of phenolic compounds (structure - activity). It is found that the least extracts phenolics and flavonoids are the most active extracts.

Anti-inflammatory activity

The evaluation of the anti-inflammatory potential is carried out by measuring the inhibition of the production of nitric oxide (NO), a pro-inflammatory agent produced by mouse macrophages (RAW 264.7). The results of the test are compared with those of L-name (1-ng-Nitroarginine Methyl Ester), an inhibitor of the enzyme responsible for the production of this mediator of the inflammatory process: "Nitric oxide Synthase inducible" or INOS.

If we observe the anti-inflammatory potential of the extracts of the two plants, it is noted that the percentage of inhibition of nitric oxide is higher in the case of the dichloromethane extracts followed by the ethyl acetate extracts (II.30).

The butanol extracts resulting from the extraction of the two plants have a low anti-inflammatory activity (3% inhibition of NO at 160 µg/ml).

Case of dichloromethane extracts

Overall, the dichloromethane extracts show a very interesting anti-inflammatory activity. Concerning the species Atriplex halimus the extract expresses a very significant potential with inhibition of NO at 97.6% followed by the extract of the stems of Haloxylon scoparium pomel which inhibits the production of NO at 85.6 % And finally the extract of the flowers of the same species with 76.5% inhibition of NO at a concentration of 160 µg/ml (Figure 1).

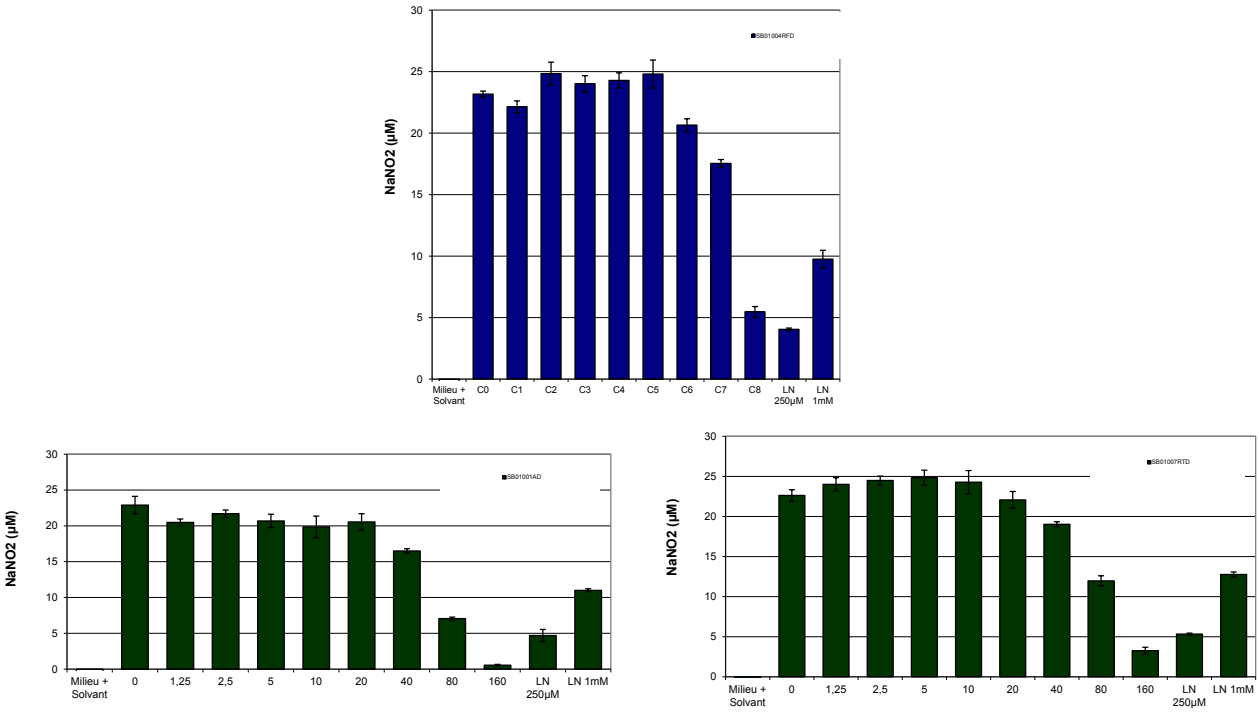

Figure 1: Anti-inflammatory activity of dichloromethane extracts

Case of ethyl acetate extracts

An interesting anti-inflammatory activity was observed for the extracts of the flowers of the species Haloxylon scoparium pomel with an inhibition of production of NO equal to 64.6% at 160 µg/ml, for the stems of the same species the inhibition Of NO production is 54.3% at 160 µg/ml. This activity is less significant in the *Atriplex halimus* L. species which demonstrates 40.2% at 160 µg/ml (Figure 2).

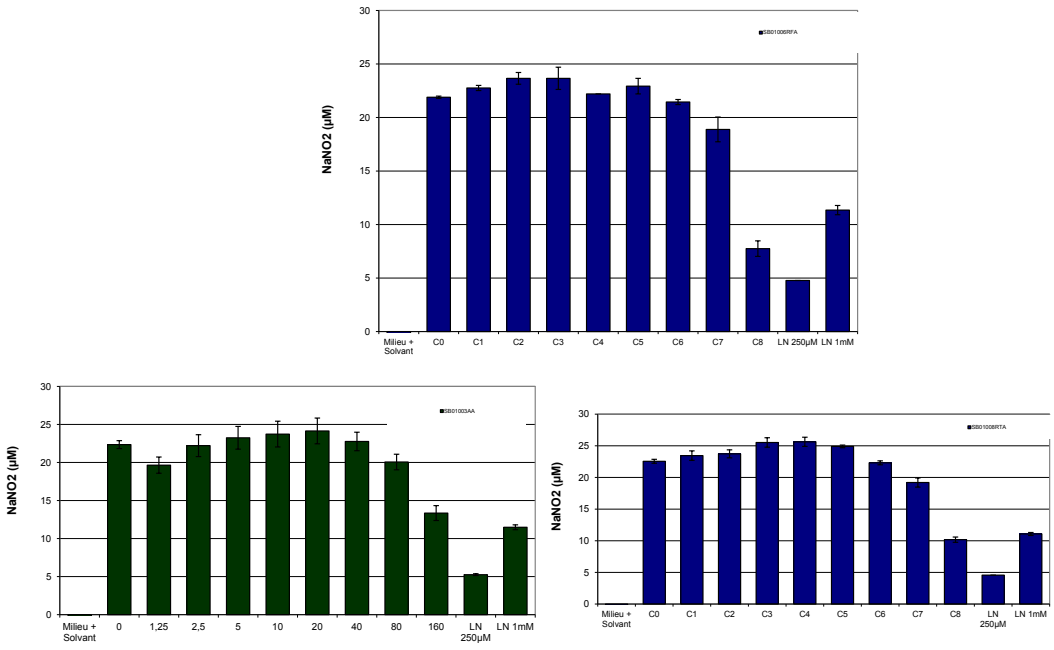

Figure 2: Anti-inflammatory activity of ethyl acetate extracts

Case of butanol extracts

An anti-inflammatory activity of the two plants studied (Figure production of NO is 36.4 even more significant in the case of the inhibition species at 40 µg/ml compared to 67 µg/ml.

The flower extract of the species of Haloxylon scoparium pomel has a low anti-inflammatory potential which is 15.8%, then the species *Atriplex halimus* L. appear to be inactive against the production of NO with an equal inhibition percentage to 3% for 160 µg/ml (Figure 3).

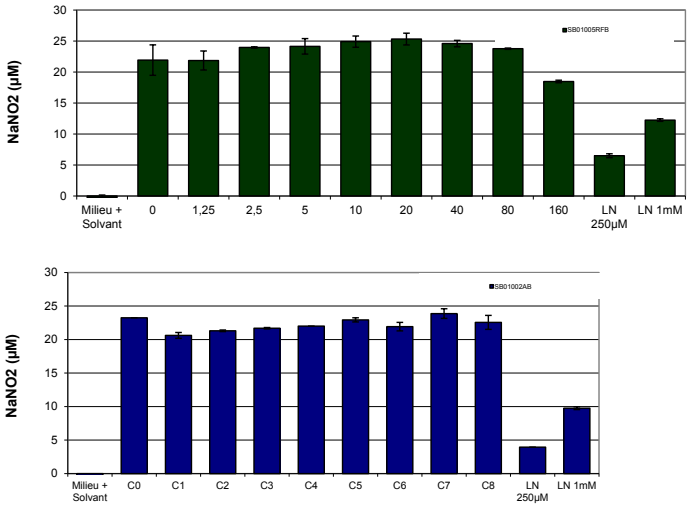

Figure 3: Anti-inflammatory activity of butanol extracts

CONCLUSION

The alpha amylase inhibitors are diverse and generally used as synthetic drug against diabetes, but still we look to nature to search for inhibitors of this enzyme, many medicinal plants are known in this field, our plants are Saharan plants that contain phenols known by their ability to inhibit enzymes and especially flavonoids in their chemical structures. Among all samples there is only an extract which has low enzyme inhibition percentages less than 20%, given that these plants have undergone the same procedure of extraction of phenolic compounds which explains that the inhibitor is capable of specific block the active site of the enzyme.

Some plants have an enzyme inhibitory activity include polyphenolic compounds and glyco-protein [21]. Many of these polyphenols have an action on α-amylase such as tannins, which are capable of binding to digestive enzymes and inhibit [22]. Depending on our results obtained on the inhibition of α-amylase, it is suggested that the extracts of the two plants may have a beneficial effect on post prandial hyperglycemic or diabetic hyperglycemic. The analysis of the alpha-amylase inhibitors was performed by the quantification of the released reducing sugar in the medium (maltose) [23-26].

The results obtained in this study shows that the inhibitory activity of the dichloromethane fraction of *A. halimus* L. has a remarkable inhibition percentage α-amylase, equal to 61.43% with an IC50 value equal to 2.5. The values found show that the inhibition rate is independent of the concentration of phenols.

Samples which demonstrate anti-dichloromethane and ethyl acetate activity. In fact, the dichoromethane extracts of the stems and flowers of the species Haloxylon scoparium pomel exhibit very high NO inhibition percentages at a concentration equal to 160 µg/ml The dichoromethane extract of the species *Atriplex halimus* L. demonstrates the With 97.6% inhibition of NO production at 160 µg/ml [27-30]. Desproprietes anti-inflammatory are also found for ethyl acetate extracts of flowers of the species haloxylon scoparium pomel. All the extracts studied apart from the butanol fractions shows a strong potential to inhibit the production of NO.

The presence of phenolic compounds of the flavonoid type could explain the anti-inflammatory activity noted for the dichloromethane and ethyl acetate extracts carried out on flavones, flavonols and isoflavones showed a negative regulation of the action of the iNOS of this family chemical property [31-39].

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REFERENCES

[1] M. Zohary, **1966**, 163.

[2] V. Täckholm, *Cairo Univ.,* **1974**, 127.

[3] L. Boulos, Flora of Egypt Vol. I. *Al Hadara Publishing*, **1999**, Cairo, Egypt, 123.

[4] D.J. Mabberley, *Cambridge University Press*, **1997**, UK, 326.

[5] M.A. Ajabnoor, M.A. Al-Yahya, M. Tariq, A.A. Ayyab, L.V. Fitoterapia, **1984**, 107

[6] M.A.A. Al-Shanawani, **1996**, 162.

[7] H.B. Salah, J. Raoudha, M. Marie-Thérèse, C.V. Nigel, J.G. Renée, S.J.S. Monique, *Chem. Pharm. Bull.*, **2002**, 50, 1268.

[8] M. Bnouham, H. Mekhfi, A. Legssyer, A. Ziyyat, *Int. J. Diabetes. Metabol*., **2002**, 10, 33.

[9] M. Eddouks, M. Maghrani, A. Lemhadri, M.L. Ouahidi, H. Jouad, *J. Etnopharmacol*., **2002**, 82, 97.
[10] A.S. Awaad, N.M. Sokkar, G.M. Soliman, *Cairo Univ*., **2001**, 39, 121.
[11] A.B. Hassine, M.E. Ghanem, S. Bouzid, S. Lutts, *J. Exp. Bot*., **2008**, 59(6), 1315-1326.
[12] R. Ksouri, W.M. Ksouri, I. Jallali, A. Debez, C. Magne, I. Iroko, C. Abdelly, *Crit. Rev. Biotechnol*., **2011**, 1-38.
[13] N. Benhammou, F.A. Bekkara, T.K. Panovska, **2009**, 12, 1259-1266.
[14]H. Benamar, W. Rached, A. Derdour, A. Marouf, *J. Biol. Sci.*, **2010**, 10(1), 1-9.
[15] H. Benamar, W. Rached, A. Derdour, A. Marouf, *J. Biol. Sci.*, **2010**, 10(1), 1-9.
[16] Z. Aharonson, J. Shani, F.G. Sulman, *Diabetologia*., **1969**, 5(6), 379-383.
[17] S. Emam, *J. Nat. Prod*., **2011**, 4, 25-41.
[18] L. Dinan, P. Whiting, A. Scott, *Biochem. Syst. Ecol*., **1998**, 26, 553-576.
[19] J. Green Shawn, S. Monte, B. John, *The J. Immunol*., **1990**, 144, 1, 278-283
[20] S. Belhadj Tahar, M. Hadj-Mahammed, M. Yousfi, *J. Chem. Pharm. Res*., **2015**, 7(11), 258-264.
[21] S. Akkarachiyasit, P. Charoenlertkul, S. Yibchok-anun, S. Adisakwattana, *Int. J. Mol. Sci*., **2010**, 11, 3387-3396.
[22] R. Tundis, M.R. Loizzo, F. Menichini, *Mini. Rev. Med. Chem*., **2010**, 10, 315-331.
[23] L. Kandra, G. Gyeman, A. Zajaez, G. Batta, *Biochem. Biophys. Res commun*., **2004**, 319(4), 1265-1271.
[24] Kim H. Pyo, K. Ho, Chang, H. Wook, J. Pharmacol. Sci., **2004**, 0411110005.
[25] S. Aboutabl El, A. Said, H. Abdallah, *Cairo Univ*., **1997**, 35, 221.
[26] A. Awaad, D. Maitland, R. Donia, S. Algasoumi, G. Soliman, *Pharma Biol*., **2007**, 50, 99-104.
[27] D. Barron, L. Vain, R.K. Ibrahim, J.B. Harborne, C.A. Williams, *Phytochem*., **1988**, 27, 2375-2395.
[28] R.J. Marles, N.R. Farnsworth, *Phytomed*., **1995**, 2, 137-189.
[29] A.Y. Oubré, T.J. Carlson, S.R. King, G.M. Reaven, *Diabetologia*., **1997**, 40, 614-617.
[30] R. Benkrief, M. Brum-Bousquet, F. Tillequin, M. Koch, *Annales Pharmaceutiques Francaises.,* **1990**, 48, 219
[31] W. Bylka, M. Stobiecki, R. Franski, *Acta. Phys. Plant*., **2001**, 23(3), 285-290.
[32] S.C. Sanderson, C. Ge-Ling, E.D. McArthur, H.C. Stutz, *Biochem. Syst. Ecol*., **1988**, 16, 143-149.
[33] W. Bylka, J.G. Diaz, J.G. Ruiz, B.R. Dias, J.A. Gavin Sazatornil, W. Herz, *Biochem. Syst. Ecol*., **2005**, 33(2), 201-205.
[34] P. Sathiyamoorthy, H. Lugasi-Evgi, P. Schlesinger, I. Kedar, J. Gopas, Y. Pollack, *Pharma. Biol*., **1999**, 37, 188.
[35] P. Sathiyamoorthy, H. Lugasi-Evgi, P. Van Damme, A. Abu-Rabia, J. Gopas, Y. Pollack, *Int. J. Pharmacog*., **1997**, 35, 265.
[36] P.A. De Smet, *N. Engl. J. Med*., **2002**, 347(25), 2046-2057.
[37] D.M. Eisenberg, R.C. Kessler, C. Foster, F.F. Norlack, D. Calkins, L. Delbanco, *N. Engl. J. Med*., **1993**, 328, 246-252.
[38] Y. Zhang, Y.M. Zhao, *Chin. J. Chin. Mater. Med*., **2005**, 30(9), 679-681.
[39] A. El-Shazly, M.Z. Wink, *Naturforsch*., **2003**, 58, 477.