Journal of Coastal Life Medicine

journal homepage: www.jclmm.com

Original article doi: 10.12980/jclm.3.2015j5-96

Antihyperglycemic effect of *Juniperus phoenicea* L. on alloxan-induced diabetic rats and diterpenoids isolated from the fruits

Salma Ahmed El-Sawi[1], Hemaia Mohamed Motawae[1], Abdel-Rahman Omar El-Shabrawy[2], Mohamed Aboul-Fotouh Sleem[2], Amani Ameen Sleem[3], Maii Abdel Naby Ismail Maamoun[1*]

[1]*Pharmacognosy Department, National Research Centre, Cairo, Egypt*

[2]*Pharmacognosy Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt*

[3]*Pharmacology Department, National Research Centre, Cairo, Egypt*

ARTICLE INFO

Article history:
Received 26 Jun 2015
Accepted 28 Jul 2015
Available online 3 Nov 2015

Keywords:
Juniperus phoenicea
Antihyperglycemic
Abietane
Labdane
Diterpenoid

ABSTRACT

Objective: To explore the traditional use of *Juniperus phoenicea* L. (*J. phoenicea*) growing in Egypt as antidiabetic herb.

Methods: The antihyperglycemic activities of the crude 80% ethanol and successive extracts of leaves and fruits of the plant were investigated in alloxan-induced diabetic rats after collecting blood samples through retro-orbital puncture technique. As a consequence of the biological results, phytochemical investigation of the chloroform fraction of fruits was carried out by column chromatography and thin layer chromatography.

Results: Results revealed the reduction in blood glucose levels in rats, which were significantly different from control at 4 and 8 weeks ($P < 0.01$). The highest antihyperglycemic activity was exhibited by the crude extracts of fruits and leaves of which the potency was 83.6% and 81.9%, respectively, after 8 weeks, comparing to metformin drug (100% potency). Chloroform fractions of leaves and fruits were the most potent fractions (potencies were 70.3%, 71.4%, respectively, along with ethyl acetate fraction of fruits (71.4%). Phytochemical investigating of the chloroform fraction of fruits resulted in the isolation and identification of 5 abietane diterpenoids. Ferruginol, 7-dehydroabietanone, sugiol, 6-α-hydroxy-7-oxoferruginol, totarolone and a labdane diterpenoid, varodiol were isolated for the first time from the fruits of *J. phoenicea* growing in Egypt. The identification of these compounds was based on spectroscopic analysis: 1hydrogen-nuclear magnetic resonance and electron impact mass spectrometry, comparing the results with the literature.

Conclusions: It has become clear that leaves and fruits of the Egyptian *J. phoenicea* provide effective antihyperglycemic action in diabetic rats as was reported in folk medicine. The high contents of terpenoids in the non-polar fractions may attribute to the antidiabetic effect of the plant.

1. Introduction

People have used plants for medicine for thousands of years. Among developed countries, people are seeking alternative medicines to avoid the side effects and expenses of synthetic medications and have turned back to herbs to treat a wide array of ailments. The genus *Juniperus* is the main member of family Cupressaceae. There are between 50 and 67 species of juniper, widely distributed throughout the Northern Hemisphere, from the Arctic, south to tropical Africa in the Old World, and to the mountains of Central America. Junipers have needle-like leaves, their fruits called berries. The berries of junipers, especially *Juniperus communis*, are used as a spice, particularly in European cuisine[1]. Native Americans have used juniper berries as a female contraceptive[2]. Also, some indigenous peoples of America have traditionally used juniper to treat diabetes[3].

In Turkey, berries of *Juniperus oxycedrus* L. subsp. *oxycedrus* have been ingested or decoctions of berries have been taken as tea to lower blood glucose levels[4].

The berries of *Juniperus phoenicea* L. (*J. phoenicea*) and *Juniperus oxycedrus* have been found in ancient Egyptian tombs at multiple sites[5].

J. phoenicea, the Phoenician juniper or Arâr[6], is the juniper growing in Egypt in Sinai near the Red Sea (Yelleg, Halal and Maghara mountains) in Rocky Ridges, Mediterranean region, extending to Central Arabia[7].

Phytochemical screening of leaves and fruits of the plant revealed

*Corresponding author: Maii Abdel Naby Ismail Maamoun, Pharmacognosy Department, National Research Centre, El-Buhouth St., Dokki 12311, Cairo, Egypt.
Tel: (+202) 33371362, 33371211
Fax: (+202) 33370931
E-mail: maimaamoun0nrc@gmail.com

that they are rich in essential oil, carbohydrates, and/or glycosides, sterols and/or triterpenes, and flavonoids[8], and they showed significant diuretic action as its use in folk medicine[9].

The aim of the current investigation is to evaluate the efficiency of *J. phoenicea* extracts for the antihyperglycemic activity in alloxan-induced diabetic rats in order to explore its use in folk medicine as antidiabetic herb. Also, work continues to isolate and characterize the active compound that is responsible for the activity, providing the rationale behind its use as a useful drug for diabetes.

2. Materials and methods

2.1. Plant materials

Fresh aerial parts of *J. phoenicea* (family: Cupressaceae) were collected from North Sinai, Maghara Mountain, Egypt and were kindly identified by Prof. Dr. Nabeel El-Hadidi, Botany Department, Faculty of Science, Cairo University. The plant was dried, ground and a voucher specimen (No. 21882) was kept at Pharmacognosy Department, National Research Centre, Cairo, Egypt.

2.2. Preparation of extracts

2.2.1. Crude extracts

About 500 g of air-dried powdered leaves and fruits of *J. phoenicea* were exhaustively extracted by reflux with 80% ethanol. The combined extract was evaporated under reduced pressure using rotatory evaporator (Heidolph, Germany).

2.2.2. Successive extracts

About 500 g of air-dried powdered leaves and fruits of *J. phoenicea* were exhaustively and successively extracted in Soxhlet apparatus using petroleum ether, chloroform, ethyl acetate and methanol. These extracts were evaporated to dryness under vacuum at 40 ˚C.

2.3. Chemicals and drugs

Alloxan (Sigma Co.) was used for the induction of diabetes in rats. Metformin (Cidophage®, Chemical Industries Development Co., Giza, Egypt) was used as standard anti-diabetic drug.

2.4. Experimental animals and diet

Adult albino rats, of Sprague-Dawley strain weighing 130–150 g, were obtained from the animal house colony of National Research Centre, Dokki, Egypt. They were kept under the same hygienic conditions and well-balanced diet and water. Diet was consisted of vitamin mixture (1.0%), mineral mixture (4.0%), corn oil (10.0%), sucrose (20.0%), cellulose (0.2%), casein 95% (10.5%) and starch (54.3%). Samples were administered orally by gastric tube. The experiments were done after taking the permission from the Research Ethics Committee, National Research Centre.

2.5. Acute toxicity studies

In a previous study by the authors, the acute toxicity test was carried out in albino mice (25–30 g) and the LD_{50} of the crude 70% ethanol and successive extracts of leaves and fruits of *J. phoenicea* were determined[8]. All the extracts were safe showing LD_{50} more than 5 g/kg body weight. So the effective dose was fixed at 100 mg/kg body weight for evaluation of antihyperglycemic activity.

2.6. Determination of acute antihyperglycemic activity

Rats were injected intraperitoneal with alloxan (150 mg/kg body weight) to induce diabetes mellitus[10]. Hyperglycemia was assessed after 72 h. Animals were divided into seven groups. First group was diabetic rats that served as positive control. Groups from second to sixth were diabetic rats that received 100 mg/kg body weight of crude 70% ethanol, petroleum ether, chloroform, ethyl acetate and methanol extracts of leaves, respectively. The seventh group was diabetic rats that received 150 mg/kg body weight of metformin as reference antidiabetic drug. At the end of each study period, blood samples were collected from the retro-orbital venous plexus through the eye canthus of anesthetized rats after an overnight fast. Serum was isolated by centrifugation and the blood glucose level was measured[11]. The experiment was repeated for fruit extracts.

2.7. Methods for diterpenoids isolation

Half kilogram of the air-dried powdered fruits of *J. phoenicea* was exhaustively extracted with chloroform several times. The combined extract was evaporated to dryness under vacuum at 40 ˚C. The chloroform extract (10 g) was fractionated by column chromatography using neutral alumina (aluminium oxide neutral, Sisco Research Laboratories Pvt. Ltd.) and stepwise gradient elution by ether/chloroform/methanol in successive proportions were done. Three main fractions were taken into consideration. Fraction 1 yielded from 75% ether\$CHCl_3$ and upon further fractionation by preparative thin-layer chromatography (TLC) (silica gel 60 F254 for TLC, Fluka Chemie AG, Switzerland) using chloroform-acetone 9:1 yielded compounds (T1-T3); Fraction 2 yielded from 100% ether and upon further fractionation by preparative TLC/benzene-ethyl acetate 3:1 yielded compound (T4) and Fraction 3 yielded from 75% $CHCl_3$/MeOH and upon further fractionation by preparative TLC/chloroform-acetone 9:1 yielded compounds (T5, T6). Plates were visualized before and after spraying with 10% H_2SO_4 and heating. The isolated compounds were subjected to spectroscopic analysis techniques: electron impact mass spectrometry (EI-MS) (Finnigan model 3200 at 70 eV) and 1hydrogen-nuclear magnetic resonance (1H-NMR) (Bruker DRX 500 spectrometer, Biospin, Rheinstetten, Germany).

3. Results

3.1. Antihyperglycemic activity

Results listed in Tables 1 and 2 revealed that there was significant antihyperglycemic activities exhibited by all extracts of *J. phoenicea* leaves and fruits as indicated by the significant reduction in blood glucose levels in alloxan-induced diabetic rats. Effects of extracts were significantly different from control group at 4 and 8 weeks. Fruit crude extract was more potent (83.6%) than leaf crude extract (81.9%) after 8 weeks compared to metformin drug (100.0% potency). Pertaining to the successive extracts of leaves, it was indicated that chloroform extract was the most potent one where its relative potency was 70.3% followed by petroleum ether extract (65.0%), then ethanol extract (54.6%), while ethyl acetate extract showed the lowest activity (38.0%), after 8 weeks compared to metformin drug. Similar experiment was done for fruit successive extracts and it has shown that chloroform and ethyl acetate extracts were the most potent ones where their relative potencies were 71.4% followed by ethanol extract (57.9%), while petroleum ether extract

Table 1
Relative potencies of leaves extracts *J. phoenicea* on blood glucose level of diabetic rats in comparison to metformin.

Group	Value	Time			RP (%)
		Zero	4 Weeks	8 Weeks	
Group 1	M ± SE	246.8 ± 9.2	257.9 ± 10.3	262.4 ± 10.8	-
Group 2	M ± SE	257.6 ± 8.2	126.9 ± 4.5*	112.4 ± 3.9*	81.9
	% Change[a]	-	50.7	56.4	
Group 3	M ± SE	249.9 ± 11.3	153.4 ± 6.2*	138.2 ± 5.1*	65.0
	% Change[a]	-	38.6	44.7	
Group 4	M ± SE	255.1 ± 8.9	169.4 ± 7.3*	131.5 ± 6.1*	70.3
	% Change[a]	-	33.6	48.45	
Group 5	M ± SE	261.0 ± 9.4	134.5 ± 5.1*	114.3 ± 4.1*	38.0
	% Change[a]	-	48.5	26.2	
Group 6	M ± SE	238.0 ± 11.4	171.2 ± 8.3*	148.9 ± 6.5*	54.6
	% Change[a]	-	28.3	37.6	
Group 7	M ± SE	265.5 ± 11.2	101.4 ± 4.6*	82.7 ± 3.8*	100.0
	% Change[a]	-	61.8	68.9	

[a]: % Of change calculated as regard the control group; RP: Relative potency; Potencies are calculated relative to metformin after 8 weeks. *: Significantly different from zero time at $P < 0.01$.

Table 2
Relative potencies of fruit extracts *J. phoenicea* on blood glucose level of diabetic rats in comparison to metformin.

Group	Value	Time			RP (%)
		Zero	4 Weeks	8 Weeks	
Group1	M ± SE	246.8 ± 9.2	257.9 ± 10.3	262.4 ± 10.8	-
Group 2	M ± SE	251.5 ± 9.6	131.2 ± 5.1*	106.4 ± 4.7*	83.6
	% Change[a]	-	47.8	57.6	
Group 3	M ± SE	236.9 ± 11.2	188.2 ± 9.3*	151.7 ± 8.4*	52.2
	% Change[a]	-	20.6	36.0	
Group 4	M ± SE	241.7 ± 8.6	176.1 ± 7.2*	143.5 ± 6.1*	71.4
	% Change[a]	-	27.1	49.2	
Group 5	M ± SE	243.8 ± 9.2	161.3 ± 5.7*	123.8 ± 4.6*	71.4
	% Change[a]	-	33.8	49.2	
Group 6	M ± SE	252.6 ± 10.3	182.2 ± 8.4*	151.7 ± 6.3*	57.9
	% Change[a]	-	27.9	39.9	
Group 7	M ± SE	265.5 ± 11.2	101.4 ± 4.6*	82.7 ± 3.8*	100.0
	% Change[a]	-	61.8	68.9	

[a]: % Of change calculated as regard the control group; RP: Relative potency; Potencies are calculated relative to metformin after 8 weeks. *: Significantly different from zero time at $P < 0.01$.

showed the lowest activity (52.2%) after 8 weeks compared to metformin drug.

3.2. Identification of the isolated compounds

The isolated compounds were identified based on the spectral analysis of data. EI-MS fragmentation was listed below and ^{1}H-NMR was listed in Table 3 as well as comparing the data with those available in the literature[12,13]. Chemical structure of the isolated diterpenoidal compounds are shown in Figure 1.

EI-MS of compound T1: *m/z* 286 (M^+, 4% calculated for the formula $C_{20}H_{30}O$), *m/z* 55, 100%, C_4H_7), *m/z* 271 [M^+-CH_3] and *m/z* 215 [M^+-CH_3-C_4H_7].

EI-MS of compound T2: *m/z* 284 (M^+, 5% calculated for the formula $C_{20}H_{28}O$), *m/z* 55, 100%, C_4H_7), *m/z* 269 [M^+-CH_3] and *m/z* 213 [M^+-CH_3-C_4H_7].

EI-MS of compound T3: *m/z* 300 (M^+, 4% calculated for the formula $C_{20}H_{28}O_2$), *m/z* 55, 100%, C_4H_7), *m/z* 286 [M^+-CH_3], *m/z* 230 [M^+-CH_3-C_4H_7] and *m/z* 202 [M^+-CH_3-C_4H_7-C = O].

EI-MS of compound T4: *m/z* 316 (M^+, 4% calculated for the formula $C_{20}H_{28}O_3$), *m/z* 55, 100%, C_4H_7), *m/z* 288 [M^+-CO], *m/z* 233 [M^+-CO, -C_4H_7] and 199 *m/z* [M^+-CO, -C_4H_7-2OH].

EI-MS of compound T5: *m/z* 300 (M^+, 7% calculated for the formula $C_{20}H_{28}O_2$), *m/z* 55, 100%, C_3H_3O), *m/z* 245 [M^+-C_3H_3O], *m/z* 203 [M^+-C_3H_3O-$CH_3C{\cdot}CH_3$] and *m/z* 175 [M^+-C_3H_3O-$CH_3C{\cdot}CH_3$-$2CH_2$].

EI-MS of compound T6: *m/z* 322 (M^+, 5% calculated for the formula $C_{20}H_{28}O_2$), *m/z* 45, 100%, C_2H_5O), *m/z* 277 [M^+-C_2H_5O], *m/z* 262 [M^+-C_2H_5O-CH_3] and *m/z* 230 [M^+-C_2H_5O-$2CH_3$, -OH].

From the results it was concluded that compounds from T1 to T5

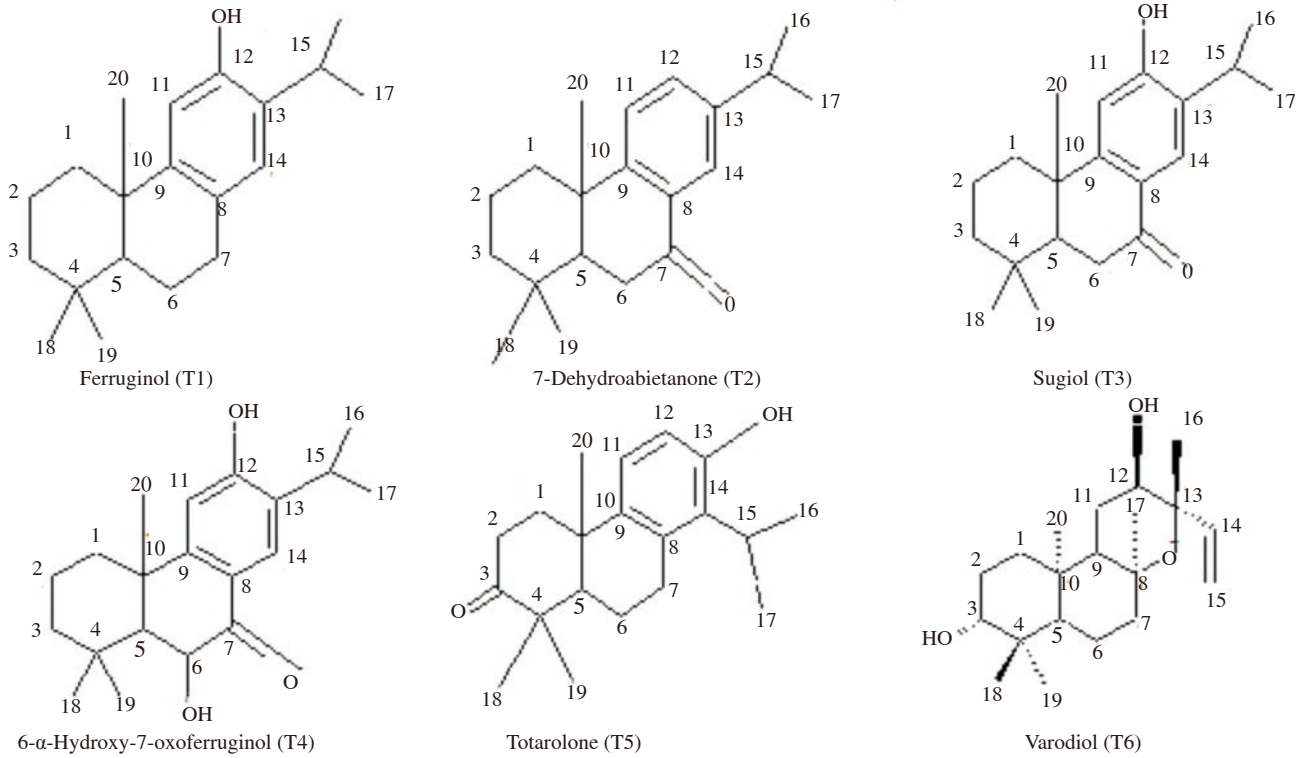

Figure 1. Chemical structures of the isolated diterpenoids.

Table 3
^{1}H-NMR data of diterpenoids isolated from chloroform extract of *J. phoenicea* fruit.

Proton	δ ^{1}H (ppm) (*J* in Hz)					
	T1	T 2	T3	T4	T5	T6
1	4H, m, 1.80	4H, m, 1.80	4H, m, 1.80	2H, t, 1.70	2H, d, 2.10	2H, d, 1.4, 1.41
2	4H, m, 1.80	4H, m, 1.80	4H, m, 1.80	2H, m, 1.30	2H, d, 2.40	2H, d, 1.73, 1.75
3	2H, t, 1.60	2H, t, 1.36	2H, t, 1.36	2H, t, 1.60	-	1H, dd, 3.65
5	1H, t, 1.45	1H, t, 2.30	1H, t, 2.30	1H, t, 2.60	1H, t, 1.69	1H, s, 1.45
6	2H, d, 2.02	2H, d, 2.83	2H, d, 2.90	1H, d, 5.20	2H, m, 1.50	2H, d, 1.93, 1.94
7	2H, t, 2.30	-	-	-	2H, t, 3.60	2H, d, 1.46, 1.47
9	-	-	-	-	-	1H, s, 1.90
11	1H, s, 6.60	1H, d, 6.50	1H, s, 6.45	1H, s, 6.80	1H, d, 6.30	2H, d, 2.1, 2.2
12	-	1H, dd, 6.80	-	-	1H, d, 6.20	1H, dd, 3.76
14	1H, s, 6.80	1H, d, 7.03	1H, s, 7.00	1H, s, 7.30	-	1H, dd, 4.90
15	1H, m, 3.50	1H, m, 3.00	1H, m, 3.00	1H, m, 3.40	1H, m, 3.50	1H, t, 4.44 (axial) & 1H, d, 4.23 (equatorial)
16, 17	6H, s, 1.22	6H, s, 1.39	6H, s, 1.39	6H, s, 1.22	6H, s, 1.25	6H, s, 0.90
18	6H, s, 1.12	3H, s, 1.41	3H, s, 1.41	6H, s, 1.10	6H, s, 1.13	3H, q, 1.25
19	6H, s, 1.12	3H, s, 0.94	3H, s, 0.94	6H, s, 1.10	6H, s, 1.13	3H, q, 1.10
20	3H, s, 1.30	3H, s, 0.92	3H, s, 0.92	3H, s, 1.30	3H, s, 1.50	3H, s, 0.66

were tricyclic diterpenoid with abietane skeleton and identified as ferruginol (T1), 7-dehydroabietanone (T2), sugiol (T3), 6-α-hydroxy-7-oxoferruginol (T4), totarolone (T5), in addition to a bicyclic diterpenoid with labdane skeleton; varodiol (T6) (ent-13-epi-manoyl oxides).

4. Discussion

Diabetes mellitus, a metabolic disorder is characterized by increase in blood glucose level. The main objective of the drugs acting as antihyperglycaemic is to reduce the blood glucose level to normal so as to reduce the diabetes related complications. In the current study, diabetes was induced using alloxan. The results revealed that the crude and successive extracts of *J. phoenicea* have the potential to reduce the elevated blood glucose level in the tested rats at doses of 100 mg/kg body weight.

Following the bio-guided fractionation, the most promising fraction as antihyperglycaemic extract was the chloroform fraction. The phytochemical investigation of that fraction revealed the isolation and characterization of 6 terpenoidal compounds which may attribute to the herb antidiabetic effect.

In conclusion, it has become clear that leaves and fruits of the Egyptian *J. phoenicea* provide effective antihyperglycemic action in diabetic rats as was reported in folk medicine. Investigating the phytochemical contents of fruit chloroform extract revealed the isolation of 6 diterpenoidal compounds, five compounds of abietane skeleton identified as ferruginol, 7-dehydroabietanone, sugiol, 6-α-hydroxy-7-oxoferruginol, totarolone in addition to a bicyclic diterpenoid with labdane skeleton, varodiol. To our knowledge, this is the first report for the isolation of these diterpenoids from the fruits of *J. phoenicea* growing in Egypt. Such extracts and the isolated compounds should be more widely investigated for the antidiabetic effect in order to be considered as good sources for drug discovery from natural origin.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors express their sincere gratitude to the administration of the National Research Centre, Egypt, for funding and supporting this work.

References

[1] Food and Agriculture Organization of the United Nations. *Non-wood forest products from conifers*. Rome: Food and Agriculture Organization of the United Nations; 1998.
[2] Tilford GL. *Edible and medicinal plants of the West*. Missoula: Mountain Press Publishing Company; 1997.
[3] McCabe M, Gohdes D, Morgan F, Eakin J, Sanders M, Schmitt C. Herbal therapies and diabetes among Navajo Indians. *Diabetes Care* 2005; **28**: 1534-5.
[4] Orhan N, Aslan M, Orhan DD, Ergun F, Pektaş M, Bedir E. Antidiabetic effect of *Juniperus oxycedrus* subsp. *oxycedrus* berries by using bioactivity guided fractionation. *Planta Med* 2011; doi: 10.1055/s-0031-1282168.
[5] Manniche L. *Sacred luxuries: fragrance, aromatherapy, and cosmetics in ancient Egypt*. New York: Cornell University Press; 1999, p. 21.
[6] Farjon A. *Juniperus phoenicea*. IUCN Red List of Threatened Species; 2006. [Online] Available from: http://www.iucnredlist.org/details/42244/0 [Accessed on 29th October, 2015]
[7] Bolous L. *Flora of Egypt*. Cairo: Al Hadara Publishing; 1999.
[8] El-Sawi SA, Motawae HM, Sleem MA, El-Shabrawy AO, Sleem A, Ismail MA. Phytochemical screening, investigation of carbohydrate contents, and antiviral activity of *Juniperus phoenicea* L. growing in Egypt. *J Herbs Spices Med Plants* 2014; **20**: 83-91.
[9] Salma ESA, Hemaia MM, Mohamed SA, Abdel-Rahman ESE, Amani S. Investigation of lipoidal matter, antimicrobial and diuretic activities of leaves and fruits of *Juniperus phoenicea* L. growing in Egypt. *Aust J Med Herb* 2011; **23**(4): 174-9.
[10] Etuk EU. Animals models for studying diabetes mellitus. *Agric Biol J North Am* 2010; **1**: 130-4.
[11] Trinder P. Determination of glucose in blood using glucose oxidase with an alternative oxygen acceptor. *Ann Clin Biochem* 1969; **6**: 24-7.
[12] Fang JM, Sou YC, Chiu YH, Cheng YS. Diterpenes from the bark of *Juniperus chinesis*. *Phytochemistry* 1993; **34**(6): 1581-4.
[13] Garcia-Granados A, Martinez A, Molina A, Onorato ME, Rico M, de Buruaga AS, et al. Diterpenoids from *Sideritis varoi* subspecies *cuatrecasasii*: ^{13}C NMR of Ent-13-epi-manoyl oxides functionalized at C-12. *Phytochemistry* 1985; **24**(8): 1789-92.