SENRA Academic Publishers, Burnaby, British Columbia
Vol. 2, No. 1, pp. 115-122, 2008
ISSN: 1715-9997

Canadian Journal of pure&applied sciences

LABDANE, PIMARANE AND ABIETANE DITERPENES FROM THE FRUITS OF *JUNIPERUS PHOENICEA* L. GROWN IN EGYPT AND THEIR ACTIVITIES AGAINST HUMAN LIVER CARCINOMA

*Salma A El Sawi and Hemaia M Motawe
Department of Pharmacognosy, National Research Centre, Dokki, Cairo, Egypt

ABSTRACT

Globally 70 *Juniperus spp*. grow and only one species *J. phoenicea* is found in Egypt, which spreads in Sinai. Continuing our studies on searching for extracts and compounds with cytotoxic activities, with the aim of finding new natural compounds with anticancer activities, we present herein the study of the pet. ether extract of fruits of *J. phoenicea* grown in Egypt. Seven diterpenes were isolated and identified from the fruit petroleum ether extract together with β-sitosterol. These terpenes were belonging to pimarane, labdane and abietane groups. The isolated diterpenes were identified as; sandaracopimaric acid (**I**); pimaric acid (**II**), 3α-acetoxy-labda-8(17),13(16)-14- triene-19-oic acid (juniperexcelsic acid) (**III**), 3α-hydroxy-labda-8(17), 13(16)-14-triene-19-oic acid (isolated for the first time) (**IV**), 4-epi-abietic acid (**V**), 4-epi-abietol (**VI**) and 3β, 12-dihydroxyabieta-8,11,13-triene-1-one (**VII**). Cytotoxic activity of fruit successive extracts was tested against Erlich Ascitis carcinoma. It was found that the cytotoxic activity was confined to pet. ether extract, which inhibited 80% of the viable cells at concentration =100 μg/ml. Fruit pet. ether extract was also highly active against 3 human tumor cell lines; liver carcinoma cell line (0.4 μg/ml), lung carcinoma cell line, (IC50= 0.54 μg/ml) and breast carcinoma cell line (4.72 μg/ml). The isolated compounds were tested against human liver carcinoma cell line. Four of the isolated compounds have higher activity than cisplatin (IC50= 9.82μg/ml). The activity of these compounds was as follows: compound **I**; IC50= 7.39μg/ml, compound **III**; IC50= 7.49 μg/ml. compound **V**; IC50= 5.72 μg/ml and compound **VI**; IC50= 5.52 μg/ml.

Keywords: *Juniperus phoenicea*, berries, diterpenes, labdane, pimarane, abietane, cytotoxic, Cupressaceae.

INTRODUCTION

Juniperus phoenicea (Phoenicean Juniper or Arâr) is a juniper found throughout the Mediterranean region, from Morocco and Portugal east to Turkey and Egypt, and also on Madeira and the Canary Islands, and on the mountains of western Saudi Arabia near the Red Sea. It mostly grows at low altitudes close to the coast, but reaches 2,400 m altitude in the south of its range in the Atlas Mountains (Adams, 2004). Although 70 *Juniperus* spp. (Family Cupressaceae) grow throughout the world, only *J. phoenicea* is found in Egypt in Sinai (Boulus, 1999). Several chemical compounds have been isolated from leaves and berries of *J. phoenicea* grown in different countries as volatile oils (Cosentino *et al*., 2003; Angioni *et al*., 2003; El-Sawi *et al*., 2007), flavonoids (AboulEla *et al*., 2005a), fatty acids, sterols and hydrocarbons (AboulEla *et al*., 2005b), lignans (Cairnes *et al*., 1980), furanon glycosides (Comte *et al*., 1996), isovalerate derivatives of p-methoxycinnamyl alcohol and linalool (Barrero *et al*., 2004), diterpenes (Barrero *et al*., 2004; Tabacik and Laporthe, 1971; Tabacik and Poisson, 1971) and phenylpropanoids (Comte *et al*., 1997). The mixture of leaves and cones of *J. phoenicea* is used as an oral hypoglycemic, whereas the leaves are used against bronchopulmonary diseases and as diuretic (Bellakhder, 1997). The Essential oils of leaves and cones have been proven to possess antimicrobial activity (El-Sawi *et al*., 2007).

Adams *et al*. (2006) reported for the geographic variation in *Juniperus phoenicea* from the Canary Islands, Morocco and Spain. Also, Meloni *et al*. (2006) reported for the genetic variation in five Mediterranean populations of *Juniperus phoenicea* as revealed by Inter-Simple Sequence Repeat (ISSR) Markers.

Continuing our studies on searching for extracts and compounds with cytotoxic activities, with the aim of finding new natural compounds with anticancer activities, we present herein the chemical and biological studies of the pet. ether extract of fruits of *J. phoenicea*. This is the first report on fruits of *J. phoenicea* L grown in Egypt.

MATERIALS AND METHODS

Plant materials

J. phoenicae berries were collected from Sinai, Egypt from fully grown plants when berries were at the ripening stage. They were identified in Herbarium Department Faculty of Sciences, Cairo University. Voucher specimens

*Corresponding author email: salmaelsawi@yahoo.com

have been deposited in the Department of Pharmacognosy, National Research Centre, Cairo, Egypt.

Extraction and isolation

Crushed plant material was extracted with petroleum ether in a soxhlet apparatus, the extract was evaporated in vacuum. The residue was fractionated on neutral alumina column (5.5X100 cm) eluting with pet. ether, followed by a gradient of chloroform up to 100% and then a gradient of methanol up to 25%. After TLC application, similar frs. were combined and further separated and purified on prep. TLC plates using pet. ether: ethyl acetate (8:2) as solvent system.

Measurement of cytotoxicity against Erlich ascites carcinoma

Extracts were screened *in vitro* using a single tumor (Erlich acites carcinoma cells). Two mgs of each extract were dissolved in dimethylsulfoxide (DMSO) to give concentration of 100 µg/0.1 ml. the tumor was maintained in the laboratory by weekly intrapretonial transplantation in female albino mice. A set of sterile test tubes were used for each test solution where 2.5X106 tumor cells/ml were suspended in phosphate buffer. 0.1 ml of different dilutions of each test solution were added separately to the suspension and kept at 37° C for two hours. Trypan blue dye exclusion test (El-Hossary *et al*., 2000) was carried out to calculate the percentage of nonviable cells which stained blue. Concentrations causing less than 30% nonviable cells in the suspension are considered not active

Measurement of potential cytotoxicity by SRB assay

Potential cytotoxicity of petroleum ether extract and the isolated compounds was tested using the method of Skehan and Storeng (1990). Cells were plated in 96-multiwell plate (10^4 cell/well) for 24 h before treatment with the tested samples to allow attachment of cell to the wall of the plate. Different concentrations of the samples (0, 1, 2.5, 5 and 10 µg/ml) were added to the cell monolayer. Triplicate wells were prepared for each individual dose.

Monolayer cells were incubated with the samples for 48 hours at 37˚ C and in atmosphere of 5% CO_2. After 48 hours, cells were fixed, washed and stained with sulforhodamine B stain. Excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer.

Color intensity was measured in an ELISA reader. The relation between surviving fraction and sample concentration is plotted to get the survival curve of the tumor cell line after the specified compound.

Apparatus

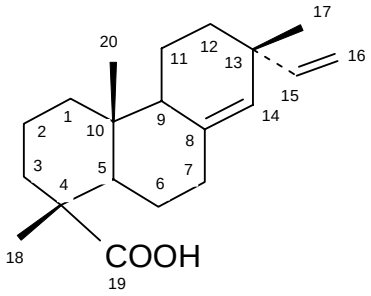
- Mass spectrometer finnigan Mat SS Q 7000, digital DEC EL, 70 eV.
- ^{1}HNMR spectrometer Jeol EX-300 NMR spectrometer.
- ^{13}CNMR spectrometer Jeol EX-300 75 MHz.
- ultra violet absorption spectrometer (Shimatzo).
- IR Bruker Victor-22.

RESULTS AND DISCUSSION

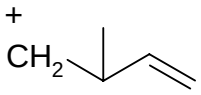
Seven diterpens were isolated from the petroleum ether extract together with one sterol (β-sitosterol). This is the first report of isolation of compound IV in nature. Compound II was isolated for the first time from the genus *Juniperus* and compounds III and VI and VII were isolated for the first time from the species *phoenicea*. The last three compounds (I, V and VI) were isolated from *J. phoenicea* grown in France and Morocco (Barrero *et al*., 2004; Tabacik and Laporthe, 1971; Tabacik and Poisson, 1971). Data of ^{1}H-NMR and ^{13}C-NMR are listed in Tables 1and 2. The isolated compounds are:

Compound 1 (sandaracopimaric acid):

IR(cm^{-1}): 3080, 1690 (C=O),1630, 913 and 994 for vinyl group (R-CH=CH_2), 825 for (C=C). UV (methanol) λ_{max} nm: 229. EI-Ms m/z (rel. int) 302 [M^+] ($C_{20}H_{30}O_2$) (12), 257 [M-COOH]$^+$, 276 [M-2 CH]$^+$ (10), 233 [276-C_3H_7]$^+$ (15), 81 (90), 69 (100).



Sandaracopimaric acid



C_5H_9

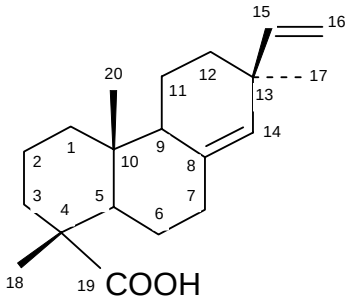
m/z 69

Base peak of sandaracopimaric acid

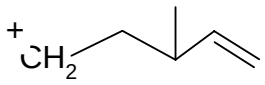
Compound II (pimaric acid):

IR(cm^{-1}): as in compund I, UV (methanol) λ_{max} (log ε) nm: 232.

EI-Ms m/z (rel. int) 302 [M$^+$] ($C_{20}H_{30}O_2$) (2), 287 [M-CH_3]$^+$(15), 257[M- COOH]$^+$ (6), 242 [M-COOH-Me]$^+$ (12), 236 (15), 207 (14), 175 (16), 112 (22), 83 (100), 77 (16), 69 (26).



Pimaric acid (II)



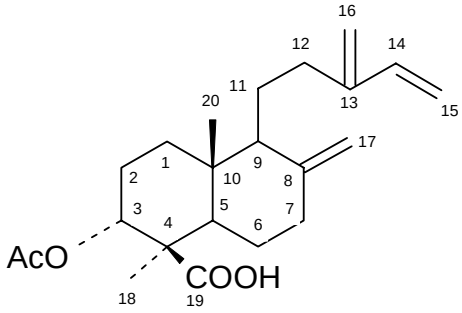
C_6H_{11}

m/z 83

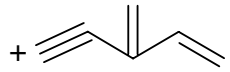
Base peak of pimaric acid

Compound III (juniperexcelsic acid):

IR (cm^{-1}): 3420 (OH), 2920 (CH), 2500-2600 (sh) (acid), 1735 (C=O), 1705 (acid), 1640 (C=C), 1595, 1465 (methyl gp.), 1450, 1380, 1250 (acetyl), 1035, 900, 760. UV (methanol) λ_{max} nm: 243. EI-Ms m/z (rel. int) 360 [M$^+$] ($C_{22}H_{32}O_4$)(2), 300 [M-AcOH]$^+$(2), 285 [M-AcOH-Me]$^+$ (4), 255 [300-COOH]$^+$ (4), 121 (20), 105 (24), 91 (32), 87 (26), 85 (28), 83 (22), 77 (100), 69 (36).



Juniperexcelsic acid



C_6H_5

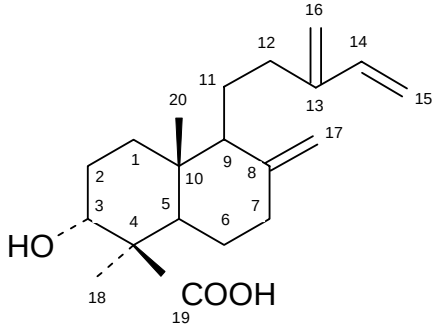
m/z 77

Base peak of juniperexcelsic acid

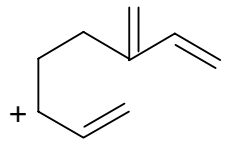
Compound IV (3α-hydroxy-labda-8(17), 13(16)-14-triene-19-oic acid):

IR (cm^{-1}): 3420 (OH), 2920, 2500-2600 (sh), 1705, 1640, 1595, 1465, 1450, 1380, 1035, 900, 760. UV (methanol) λ_{max} nm: 243, 227 (shoulder). EI-Ms m/z (rel. int) 318 [M$^+$] ($C_{20}H_{30}O_3$)(2), 303 [M-CH_3]$^+$(70), 287 [M- OH-CH_2]$^+$ (33), 232 [287-COOH]$^+$ (20), 121 (100), 105 (80), 105 (95), 91 (90), 83 (46), 77 (48), 67 (63).

^{1}H NMR: Table 1, ^{13}C NMR: Table 3.



3α-hydroxy-labda-8(17), 13(16)-14- triene-19-oic acid



C_9H_{13}

m/z 121

Base peak of compound IV

The EIMS of compound IV gave a molecular ion peak at m/z) 318 corresponding to a molecular formula ($C_{20}H_{30}O_3$). The ^{13}C NMR spectrum displayed 20 signals indicated four methine, nine methylene, two methyl and five quaternary carbon atoms. The signals at δ 182.1 was

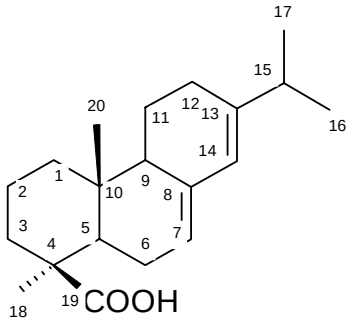
attributed to the presence of acid group which appeared in IR spectrum at 1705 and 2500-2600 cm^{-1}. The presence of one exocyclic and two terminal methylene groups followed from the ^{13}C NMR at δ 115.5, 113.19 and 106.8, as well as the three pairs of methylene signals in ^{1}H NMR spectrum at δ 4.48, 4.45 (1 H, br s , H-17, H-17'), δ 4.85, 4.83 (1 H, br s , H-16, H-16') and δ 5.1 (1 H, br d, *J*= 10.5 Hz, H-15), 4.88 (1H, br d, *J*= 17.5 Hz, H-15'). The methyl signals were observed at δ 0.6 and 1.2 as singlets. The presence of α hydroxyl at C-3 was indicated by the resonance of a methyl group at C-4 at δ 23.8, otherwise it would be at δ 28 with no substitution at C-3. The chemical shifts of the methylene carbons also indicated the location of the hydroxyl group which affects the signals at C-1 and C-2 (Topcu *et al*., 1999). All the ^{1}HNMR data suggested a labdane diterpene structure for IV. The UV spectrum gave a maximum at 243 nm indicating conjugation in the molecule.

Compound V (4-epi-abietic acid):

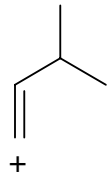
IR (cm^{-1}):1725 (C=O), 1230 (CO), 1150, 1190, instaurations at 3020, 1660, 1380 and 1390 (gem methyl gps), 990, 895 cm^{-1}

UV (methanol) λ_{max} nm. 241, 234.

EI-Ms m/z (rel. int) 302 [M^+] ($C_{20}H_{30}O_2$) (0.1), 287 (25), 269 (13), 255 (16), 201, (36), 187 (56), 97 (40, 83 (50), 69 (100).



4-epi-abietic acid



C_5H_9

m/z 69

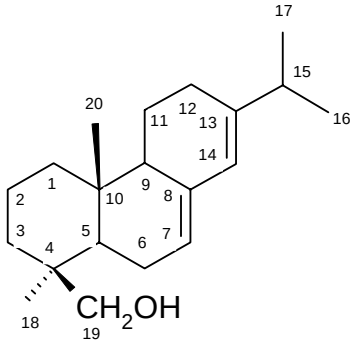
Base peak of 4-epi-abietic acid

Compound VI (4-epi-abietol):

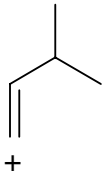
IR (cm^{-1}): 3630 (OH), 1040 (CO), 3020, 1380 and 1390 (gem methyl gps), 980, 890 (instaurations).

UV (methanol) λ_{max} (nm): 241, 234.

EI-Ms m/z (rel. int) 288 [M^+] ($C_{20}H_{32}O$)(1), 234 (12), 218 (11), 203 (12), 194 (28), 165 (28), 121 (24), 91 (40), 82 (56), 69 (100).



4-epi-abietol



C_5H_9

m/z 69

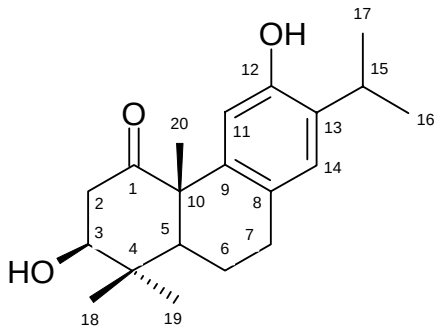
Base peak of 4-epi-abietol

Compound VII

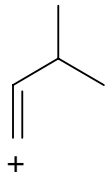
IR (cm^{-1}): 3500 and 3265 (OH), 1690 (CO), 1600, 1490, 1380 and 1390 (gem methyl gps) and 1270.

UV (methanol) λ_{max} (nm): 230 and 300.

MS m/z (rel. int): 316 [M^+] ($C_{20}H_{28}O_3$)(15), 301 [M-Me]$^+$ (1), 243 (2), 229 (2), (202 (10), 187 (10), 85 (36), 83 (30), 69 (100).



Compound VII



C_5H_9

m/z 69

Base peak of compound VII

Cytotoxic activities of *J. phoenicea* fruit extracts

Successive extracts of fruits of *J. phoenicea* were evaluated against Erlich ascites carcinoma cells. The only active extract was the pet. ether extract (Table 3). This extract was further tested against 4 human tumor cell lines and found to be particularly active against liver cell line (Hepg2) (IC50=0.40 µg/ml), as well as lung cell line (H460 (IC50=0.54 µg/ml) and (U251) (IC50= 4.72 µg/ml), while it was inactive against cervix cell line (Hela) (IC50>10 µg/ml). The cytotoxic activites of fruit pet. ether extract are shown in Table 4 and Fig. 1.

Since the activity of the pet ether extract was the highest against liver carcinoma, so the isolated compounds were tested against this cell line. Four of the isolated compounds have higher activity against liver carcinoma than cisplatin (IC50= 9.82µg/ml). The activity of these compounds was as follows: compound **I**; IC50= 7.39µg/ml, compound **III**; IC50= 7.49 µg/ml. compound **V**; IC50= 5.72 µg/ml and compound **VI**; IC50= 5.52 µg/ml Table 5 and Fig. 2.

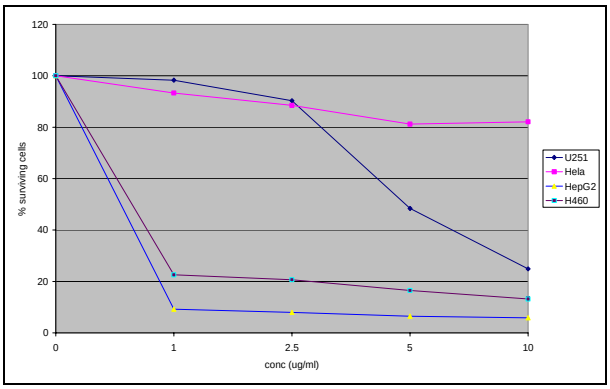


Fig. 1. Cytotoxic activities of pet. ether extract of fruits of *J. phoenicea* against human tumor cell lines.

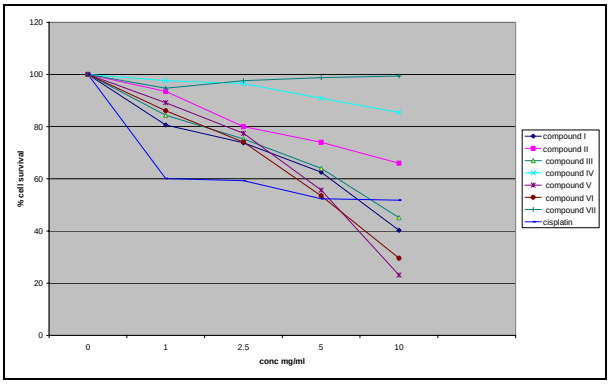


Fig. 2. Cytotoxic activities of isolated compounds from pet. ether extract of fruits of *Juniperus phoenicea* against human liver carcinoma.

Table 1. ^{1}H-NMR data of the isolated compounds in $CDCl_3$.

Position	I	II	III	IV	V	VI	VII
1	1.2 m	1.2 m	1.17 m	1.25 m	1.8 m	1.8 m	-
2	3.20dd (9.7,12.5) 2.91 dd (4.2.12.5)	3.20 dd (9.7,12.5) 2.91 dd (4.2, 12.5)	3.20 dd (9.7, 12.5) 2.90 dd ((4.2, 12.5)	3.20 dd (9.7, 12.5) 2.90 dd ((4.2, 12.5)	3.20dd (9.7,12.5) 2.91 dd (4.2.12.5)	3.20dd (9.7,12.5) 2.91 dd (4.2.12.5)	3.20dd (9.7,12.5) 2.91 dd (4.2.12.5)
3	1.59 m	1.59 m	5.29 t (2.5)	2.37 m	1.68 m	1.68 m	3.72 dd (4.2, 9.0)
4	-	-	-	-	-	-	-
5	2.5 d (10.8)	2.5 d (10.8)	2.8 d (10.8)	2.4 d (10.8)	1.70 d (10.8)	1.70 d (10.8)	1.71 d, br (10.8)
6	2.2 m	2.2 m	1.39 m	1.38 m	1.79 m	1.77 m	1.79 m
7	2.83 m	2.83 m	1.37 m	1.35 m	6.80 m	6.80 m	2.8 m
8	-	-	-	-	-	-	-
9	4.09 m	4.09 m	4.38 m	4.45 m	4.04 m	4.00 m	-
10	-	-	-	-	-	-	-
11	2.85 m	2.85 m	1.53 m	1.53 m	2.80 m	2.4 m	7.78 s
12	2.88 m	2.88 m	1.48 m	1.49 m	2.90 m	2.9 m	
13	-	-	-	-	-	-	
14	6.85 s	6.85 s	6.36 dd (17.5, 10.5)	6.21 dd (17.5, 10.5)	6.45 s	6.45 s	7.03 s
15	3.59 m	3.85 m	5.21 br d (17.5)	5.10 br d (17.5)	3.63 m	3.60 m	3.61 q (6.9)
15'	-	-	5.06 br d (10.5)	4.88 br d (10.5)			
16	4.20 b s	4.50 b s	5.00 br d	4.85 br d	1.30 d (7.0)	1.30 d (7.0)	1.40 d (7.0)
16'	-	-	4.99 br d	4.83 br d			
17	1.04 d (7.0)	1.04 d (7.0)	4.89 br s	4.48 br s	1.20 d (7.0)	1.20 d (7.0)	1.39 d (7.0)
17'	-	-	4.59 br s	4.45 br s			
18	1.12 s	1.17 s	1.21 s	1.21 s	1.10 s	1.10 s	1.19 s
19	-	-	-	-	-	2.10 s	1.25 s
20	1.59 s	1.59 s	0.60 s	0.60 s	1.6 s	1.50 s	1.61s
CH_3 of acetyl gp			2.09 s				

Coupling constants (*J* in Hz) in parentheses

Table 2. ^{13}C-NMR data of the isolated compounds in $CDCl_3$.

Position	I	II	III	IV	V	VI	VII
1	38.4	38.6	32.7	32.6	39.4	39.2	211.7
2	18.3	18.5	24.3	24.0	19.8	18.4	46.4
3	37.1	37.5	73.2	77.0	38.5	35.4	77.1
4	47.2	47.6	47.1	47.1	44.2	26.9	39.2
5	48.7	49.1	50.0	50.1	50.2	51	49.6
6	24.9	25.5	25.4	25.2	24.9	23.4	19.0
7	35.5	35.8	38.5	38.1	121.4	121.0	31.0
8	136.2	138.5	147.6	147.5	134.6	135.4	126.4
9	50.7	51.9	55.6	55.3	51.7	51.2	140.1
10	37.8	38.1	40.1	40.0	35.5	37.9	52.8
11	18.8	19.5	22.6	22.4	23.2	22.4	116.4
12	34.6	36.0	30.3	30.3	27.7	27.5	153.1
13	37.4	39.0	147.0	146.9	145.0	145.3	133.8
14	129.3	128.2	139.0	138.8	122.5	122.3	126.8
15	149.0	147.8	115.5	115.1	35.0	34.8	27.4
16	110.5	113.2	113.2	113.0	21.0	20.8	23.0
17	26.2	29.9	106.8	106.8	21.5	21.4	23.1
18	16.8	17.6	23.8	23.6	29.1	26.7	17.6
19	185.3	185.7	182.1	182.0	184.0	64.7	29.3
20	15.3	15.2	12.4	12.1	12.9	14.7	25.8
C=O			170.3				
CH_3			21.2				

Table 3. Cytotoxic activities of different extracts of fruits of *Juniperus phoenicea* L against Erlich Ascites carcinoma.

No.	Extract	Concentrations in μg/ml	% inhibition of cell viability
1	Pet ether	25	15
		50	35
		100	80
2	Chloroform	25	< 30
		50	< 30
		100	< 30
3	Ethyl acetate	25	< 30
		50	< 30
		100	< 30
4	Methanol	25	< 30
		50	< 30
		100	< 30

Table 4. IC50 of petroleum ether extract of fruits of *Juniperus phoenicea* L against human tumor cell lines.

Cell line	U251	Hela	Hepg2	H460
IC50	4.72	10<	0.4	0.54

Sandaracopimaric acid is a diterpene acid of plant origin. It has been isolated from *J. phoenicea* L. grown in France (Comte *et al*., 1995) and from *J. excelsa* (Topcu *et al*., 1999).

Sandaracopimaric acid was proved to be lipoxygenase inhibitor, this is important because arachidonic acid metabolites are important mediators of inflammation, especially the products of the lipoxygenase pathway. lipoxygenase-dependent growth has been reported to various cancer cell lines such as neuroblastoma, mouse melanoma and MCF-7 human breast cancer cells (Comte *et al*., 1995). It was also found to exhibit significant activities against microbes and *Mycobcterium tuberculosis.*

Table 5. IC50 of isolated compounds from petroleum ether extract of fruits of *Juniperus phoenicea* L against human liver carcinoma.

Compound	I	II	III	IV	V	VI	VII	Cisplatin
IC50	7.39	>10	7.49	>10	5.72	5.52	>10	9.83

Pimaric acid was isolated from several plants e.g. *Cupressus lusitanica* (Kuiate *et al*., 2006), *Pinus pinaster* (Arrabal *et al*., 2005) and *Daemonorops draco* (Piozzi *et al*., 1974).

Juniperexcelsic acid was isolated from the berries of *J. excelsa* and was found to be moderately active against *M. tuberculosis*. Moreover, it exhibited cytotoxic response with KB cells (Topcu *et al*., 1999).

Abietane diterpenoids have been reported to possess several biological properties such as antimicrobial, antiulcer and cardiovascular activities (Tan *et al*., 2002), activity as antitumor promoters (Ohtsu *et al*., 2001), antileishmanial activity (Tan *et al*., 2002), antioxidant (Wang *et al*., 2002), tuberculostatic, antiplatelet aggregations activity, antiviral and several others (Cousins, 1994). Kotoda *et al*. (2002) described the remarkable antitumor activity of abietane derivatives in a patent. Also, Barrero *et al*. (2004) reported the activity of three abietane diterpenoids against human colon carcinoma.

4-epi-abietic acid, 4-epi-abietol and compound VII were isolated from the leaves of *J. excelsa* grown in Saudi Arabia and the first two compounds were reported to have antimicrobial activities (Mossa *et al*., 1992).

REFERENCES

AboulEla, M., ElShaer, N. and ElAzim, TA. 2005a. Chemical constituents and hypoglycemic effect of *Juniperus phoenicea*: Part I**.** Alexandria Journal of Pharmaceutical Sciences. 19(2):109-116.

AboulEla, M., ElShaer, N. and ElAzim, TA**.** 2005b. Chemical constituents and antihepatotoxic effect of the berries of *Juniperus Phoenicea* Part II . Natural Product Sciences. 11(4):240-247.

Adams, RP. 2004. Junipers of the World: The genus *Juniperus*. Trafford Publishing, Vancouver, BC, Canada.

Adams, RP. Nguyen, S. and Achak, N. 2006. Geographic variation in *Juniperus phoenicea* from the Canary islands, Morocco and Spain, based on RAPDs analysis. Phytologia. 88(3):270-278.

Angioni, A., Barra, A., Russo, MT., Coroneo, V., Dessi, S. and Cabras, P. 2003. Chemical composition of the essential oils of *Juniperus* from ripe and unripe berries and leaves and their antimicrobial activity. J Agric Food Chem. 51(10):3073-8.

Arrabal, C., Cortijo, M., Fernández de Simón, B., Vallejo, MCG. and Cadahía, E. 2005. Differentiation among five Spanish *Pinus pinaster* provenances based on its oleoresin terpenic composition. Biochemical Systematics and Ecology. 33(10):1007-1016.

Barrero, AF., Quilez del Moral, JF., Herrador, MM., Akssira, M., Bennamara, A., Akkad, S. and Aitigri, M. 2004. Oxygenated diterpenes and other constituents from Moroccan *Juniperus phoenicea* and *Juniperus thurifera* var. africana. Phytochemistry. 65(17):2507-15.

Bellakhder, J. 1997. La Pharmacopee Marocaine Traditionelle. Ibis Press, Paris. 271-272.

Boulos, L. 1999. Flora of Egypt, vol 1 (p 10). Alhadara Publishing, Cairo, Egypt.

Cairnes, DA., Ekundayo, O. and Kingston, DGI. 1980. Plant anticancer agents. X. Lignans from *Juniperus phoenicea*. J. Nat. Prod. 43:495-497.

Comte, G., Allais, DP., Chulia, AJ., Vercauteren, J. and Delage, C. 1996. Two furanone glucoside drevatives from *Juniperus phoenicea*. Phytochemistry. 41(5): 1329-1332.

Comte, G., Allais, DP., Chulia, AJ., Simon, A., Es-Saady, D. and Delage, C. 1995. Crystal structure of sandaracopimaric acid, a lipoxygenase inhibitor from *Juniperus phoenicea*. J. Nat. Products. 58(2): 239-243.

Comte, G., Vercauteren, J., Chulia, AJ., Allais, DP. and Delage, C. 1997. Phenylpropanoids from leaves of *Juniperus phoenicea*. Phytochemistry. 45(8): 1679-1682.

Cosentino, S., Barra, A., Pisano, B., Cabizza, M., Pirisi, FM. and Palmas, F. 2003. Composition and antimicrobial properties of Sardinian *Juniperus* essential oils against foodborne pathogens and spoilage microorganisms. J. Food Prot. 66(7):1288-91.

Cousins, DJ. 1994. Medicinal essential oil, culinary herb and pesticidal plant of the Labietae, parts I and II. CAB International, Wallingford.

El-Hossary, GA., Fathy, MM., Kassem, HA., Kandil, ZA., Abd El-Latif, HA. and Shehabb, GG. 2000. Cytotoxic triterpens from the leaves of *Eriobotrya japonica* L. growing in Egypt and the effect of the leaves on renal failure. Bull. Fac. Pharm. Cairo Univ. 36 (1):87-97.

El-Sawi, S.A., Motawae, HM. and Ali, AM. 2007. Chemical composition, cytotoxic activity and antimicrobial activity of essential oils of leaves and berries of *Juniperus phoenicea* L. grown in Egypt. Afr. J. Trad. CAM. 4 (4):417-426.

Kotoda, H., Suzuki, K. and Uchid, S. 2002. Antitumor abietane diterpene quinone and its production from conifer bark. JP 2002193869 A2 20020710.

Kuiate, J, Bessière, J., Zollo, PH. and Kuate, SP. 2006. Chemical composition and antidermatophytic properties of volatile fractions of hexanic extract from leaves of *Cupressus lusitanica* Mill from Cameroon. Journal of Ethnopharmacology. 103 (2):160-165.

Meloni, M., Perini, D., Filigheddu, R. and Binelli, G. 2006. Genetic variation in five Mediterranean populations of *Juniperus phoenicea* as revealed by Inter-Simple Sequence Repeat (ISSR) Markers. Annals of Botany. 97(2):299-304.

Mossa, JS., Muhammad, I., El-Feraly, FS. and Hufford, CD. 1992. 3 β, 12 dihrdroxyabieta-8, 11,13- triene-1-one and other constituents from *Juniperus excelsa* leaves. Phytochemistry. 31(8):2789-2792.

Ohtsu, H., Tanaka, R: In Matsunaga,Y., Tokuda, S. and Nishino, H. 2001. Abietane diterpenoids from the cones of *Larix kaempferi* and their inhibitory effects on Epstein Barr virus activation. Planta Med. 67:55-60.

Piozzi, F., Passannanti, S., Paternostro, MP. and Nasini, G. 1974. Diterpenoid resin acids of *Daemonorops draco* . Phytochemistry. 13 (10):2231-2233.

Skehan, P. and Storeng, R. 1990. New colorimetric cytotoxicity assay for anticancer drug screening. Journal National cancer Institute. 82:1107-1112.

Tabacik, C. and Laporthe, Y. 1971. Diterpenes de *Juniperus phoenica* — constituants majeurs. Phyto-chemistry. 10 (9):2147-2153.

Tabacik, C. and Poisson, C. 1971. Diterpenes de *Juniperus phoenica* — constituants mineurs. Phyto-chemistry. 10 (7):1639-1645.

Tan, N., Kaloga, M., Radtke, OA., Kiderlen, AF., Oksuz, S., Ulubelen, A. and Kolodziej, H. 2002. Abietane diterpenoids and triterpenoic acids from *Salvia cilicica* and their antileishmanial activities. Phytochemistry. 61: 881-884.

Topcu, G., Erenler, R., Cakmak, O., Johansson, CB., Celik, C., Chai, H. and Pezzuto, JM. 1999. Diterpenes from the berries of *Juniperus excelsa*. Phytochemistry. 50: 1195-1199.

Wang, S., Wu, J., Shyur, L., Kuo, Y. and Chang, S. 2002. Antioxidant activity of abietane-type diterpenes from heartwood of *Taiwania cryptomerioides* Hayata. Holzforschung. 56:487-492.