Egypt. J. Chem. **Vol. 66,** No. SI 13, 963-972 (2023)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

LC-ESI-MS/MS profiling, GC/MS analysis, phytoconstituent isolation and evaluation of anticancer potential of *Juniperus phoenicea* L. aerial parts

Gehan F. Abdel Raoof [1*], Hala M. Mohamed Nagy[2]

[1]*Pharmacognosy Department, Pharmaceutical and Drug Industries Research Institute, National Research Centre, Dokki, Giza 12622, Egypt.*

[2]*Food Science Department, Faculty of agriculture, Cairo University*

Abstract

The current study intends to evaluate the cytotoxic activity of both 70% methanol extract and petroleum ether extract of *Juniperus phoenicea* L. aerial parts, as well as to analyze the chemical profile of both extracts. The result was affirmed that the hydroalcoholic extract possessed a very potent cytotoxicity against ovarian cancer cell line (SKOV-3) (IC_{50} =0.01 µg/ml) more than that of doxorubicin, a reference anticancer agent, (IC_{50} =0.95 µg/ml), and a potent cytotoxicity against prostate cancer cell line (PC-3) (IC_{50} =13.60 µg/mL). On the other hand, petroleum ether extract exhibited moderate cytotoxicity against SKOV-3 and PC-3 cell lines (IC_{50} = 29.6 and 73.63 µg/ml, respectively). Moreover, the results revealed the identification of sixteen phenolic compounds in hydroalcoholic extract using liquid chromatography–electrospray ionization–tandem mass spectrometry (LC-ESI-MS/MS). Catechin, 3.4-dihydroxybenzoic acid, naringenin and gallic acid are presented as the major compounds. In addition, myricetin, quercetin, catechin, gallic acid and p-Coumaric acid were isolated and identified by different spectrometric methods. GC/MS analysis revealed the identification of seventeen compounds of the unsaponifiable matter of *Juniperus phoenicea* L. Where, nonacosanol, 1-(2-Octyldecyl) octahydropentalene and gamma-sitosterol are presented as the major compounds. On the other hand, fourteen compounds were identified from the saponifable matter. Hexadecanoic acid, 9-octadecenoic acid, and docosanoic acid were found to be the major constituents. These findings suggest that *Juniperus phoenicea* aerial parts may be an effective drug for the manipulation of different types of cancer after applying clinical trials. These outcomes open new areas for the alternative use of natural products in the drug discovery.

Keywords: Cytotoxic; *Juniperus phoenicea* aerial parts; phytochemical profile; LC-ESI-MS/MS analysis; GC/MS analysis.

Introduction

Cancer is still a major risk factor for worldwide public health, and by 2030, there are expected to be 23.6 million new instances of cancer diagnosed annually **[1]**. Medicinal plants still provide hopeful alternatives and have been known for their medicinal uses with less side effects **[2]**. *Juniperus phoenicea* L. (Family: Cupressaceae), commonly known as Arar, is a shrub that can reach a height of eight meters. It is widely distributed in the northern hemisphere, and in Egypt it is growing in Sinai near the Red Sea **[3]**.

Juniperus phoenicea is used traditionally as a diuretic, to improve appetite, and to treat diabetes, rheumatism, diarrhea, and in bronchopulmonary, and urinary tract diseases **[4-6]**. In some cultures, it can be used as female contraceptive **[4]**. The fruits and leaves of *Juniperus phoenicea* have been reported to be rich in flavonoids, sterols and/or triterpenes, carbohydrates, and/or glycosides, and essential oil **[7]**. *Juniperus phoenicea* have been stated to display hepatoprotective, antibacterial and antioxidant properties **[8-10]**. Moreover, the plant exhibited significant anti-obesity, diuretic; hypoglycemic and lipoxygenase inhibitor activities **[11-13]**. Previous studies have reported that *Juniperus phoenicea* can suppress the spread of different cancer cells and exhibited a significant cytotoxic activity **[14-19]**.

In continuing to focus on *Juniperus phoenicea*, the current study aims to assess the anticancer potential of both hydroalcoholic and petroleum ether extracts of *Juniperus phoenicea* aerial parts on prostate cancer cell line (PC-3), and ovarian cancer cell line (SKOV-3), as well as to analyze the chemical profile of both extracts. To our knowledge, this study is the first report regarding the impact of *Juniperus phoenicea against* ovarian cancer cell line in Egyptian species.

*Corresponding author e-mail: gehankandeel9@yahoo.com; ahmedkhaled_1@hotmail.com
Receive Date: 08 March 2023, Revise Date: 28 April 2023, Accept Date: 30 April 2023
DOI: 10.21608/EJCHEM.2023.198778.7700
©2023 National Information and Documentation Center (NIDOC)

Materials and methods

General experimental procedures

Normal-phase polyamide (Sigma-Aldrich Chemicals) and Sephadex LH-20 (E Merck) were used as stationary phases for column chromatography. TLC silica gel F_{254} plates (200 mm layer thickness) (Merck; Darmstadt, Germany) used for thin layer chromatography (TLC). UV/VIS: Shimadzu UV were used to detect the ultraviolet spectra, chromatograms were visualized under visible recording spectrophotometer model-UV 240 (NRC, Egypt). For ^{1}H-NMR, JEOL EX-500 spectrometer (Tokyo, Japan) was used to measure the nuclear magnetic resonance (NMR) spectra with 500 MHz. Finnigan MAT SSQ 7000, 70 eV was used for mass spectrometric analysis.

Plant material

Fresh aerial parts of *Juniperus phoenicea* L. (family Cupressaceae) were collected from north Sinai, Magara Mountain, Egypt, in January 2022. The plant was kindly authenticated by Mrs. Therese Labib, consultant of plant taxonomy at the Ministry of Agriculture and Director of the Orman Botanical Garden, Giza, Egypt.

Preparation of the extracts

The dried powdered aerial parts of *J. phoenicea* (600g) were defatted with light petroleum (40-60°C) gave a yield of 11.5 g, then extracted with 70% methanol several times gave a yield of 90 g. The obtained two extracts were concentrated under reduced pressure at 45 °C using the rotary evaporate then kept in refrigerator for the further studies.

Preliminary phytochemical screening tests

The phytochemical screening of *Juniperus phoenicea* L. extracts were conducted using standard procedures **[20].**

Analyses of petroleum ether extract

Saponification

By refluxing with 10% alcoholic potassium hydroxide, the petroleum ether extract (0.48 g) was saponified. The extract was diluted with water after removal of the solvent. Ether was used to extract the unsaponifiable material then the extract was concentrated, weighed, and saved for later analysis **[21].**

Sample derivatization

The unsaponifiable fraction extracted with ether and petroleum ether (1:1) was mixed with 50 µL of bis(trimethylsilyl)trifluoroacetamide (BSTFA)+ trimethylchloro-silane (TMCS) 99:1 silylation reagent and 50 µL pyridine for derivatization sample functional groups to trimethylsilyl groups (abbreviated TMS) prior to GC analysis.

Methylation of fatty acid

The procedure outlined by Liu [22] was followed in order to methylate free fatty acids. Where, 10% HCl was used to acidify the aqueous mother liquid, and ether was used to extract the released fatty acids. The dried extract was weighed and saved for investigation of the total fatty acids. Free fatty acids were methylated by refluxing with sulfuric acid and absolute methanol for two hours. Diethyl ether was used to extract the methylated fatty acids.

GC/MS analysis

The gas chromatography Agilent 7890B, with mass spectrometer detector (5977A) at 70 eV were used for analysis of the fatty matter in *Juniperus phoenicea* aerial parts. The identification was based on comparing the spectral fragmentation patterns with those of database libraries (NIST and Wiley), as well as with the published data **[23].**

Estimation of total phenolic and total flavonoid contents

The estimation of total phenolic (TP) and flavonoid (TFC) contents was done as previously described methods **[24, 25].** TP was expressed as mg gallic acid equivalents per g of the dry plant materials (mg GAE/g), while TFC was expressed as mg of rutin equivalents per g of the dry plant materials (mg QE/g).

Isolation and purification of compounds

70% methanol extract (50 g) was applied on polyamide column chromatography using gradient elution technique starting with water then mixture of water and methanol with decreasing polarity. After chromatographic analysis via paper chromatography, four major fractions were obtained**.** Fraction 1, eluted with H_20: MeOH (90:10), was purified on Sephadex LH-20 CC and eluted with methanol to afford compounds 1(**myricetin**). Fraction 2, one compound which was eluted with H_20: MeOH (80:20), was purified by applying on column of Sephadex using methanol as eluent to give compounds 2 (**quercetin**). Fraction 3, eluted with H_20: MeOH (70:30), was applied on Sephadex column to give compound 3 (**catechin**). Fraction 4, two compounds, eluted with H_20: MeOH (60:40), was applied on Sephadex column to separate compounds 4 and 5 (**gallic acid and p-Coumaric acid, respectively**).

LC/MS/MS

Instrument

Liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ESI-MS/MS) was used to analyse the plant material, with an Exion LC AC system for separation and a SCIEX Triple Quad

5500+ MS/MS system outfitted with an ESI for detection.

Positive and negative multiple reaction monitoring (MRM) mode chromatogram

The Sample was applied on ZORBAX SB-C18 Column (4.6×100 mm, 1.8 µm), the elution was carried out by two eluents (A: 0.1% formic acid in water; B: acetonitrile (LC grade)), with the injection volume 3 µl and flow rate 0.8 ml/min, using the following program for the mobile phase, 2% B from 0-1 min, 2-60% B from 1-21 min, 60% B from 21-25 min, 2% B from 25.01-28 min. Positive and negative ionization modes were used in the same run for multiple reaction ion monitoring (MRM analysis) of the chosen polyphenols, using these parameters: Ion source gas 1 & 2 were 55 pressure with a declustering potential of 50, collision energy spread of 10 and collision energy of 25; source temperature was 400°C; ion spray voltage was 4500.

Cytotoxic study

SKOV-3 and PC- 3 cancer cell lines were sourced from Nawah Scientific Inc. Cells were kept in humidified, 5% (v/v) CO_2 atmosphere at 37 °C in DMEM media supplemented with 10% heat-inactivated fetal bovine serum, 100 mg/mL streptomycin, and 100 units/mL penicillin. Tris EDTA buffer and Sulphorhodamine B stain were sourced from Sigma Co, Egypt, while a reference anticancer agent (Doxorubicin) was obtained from Pharmacia, Sweden. Cytotoxic assay was performed according to the methods previously reported **[26, 27].** Briefly**,** cell viability was assessed by SRB assay. Aliquots of 100µL cell suspension (5x10^3 cells) were in 96-well plates and incubated in complete media for 24 h. Cells were treated with another aliquot of 100µL media containing drugs at various concentrations ranging from (0.01,0.1,1,10,100 µg/ml). After 72 h of drug exposure, cells were fixed by replacing media with 150µL of 10% TCA and were incubated at 4 °C for 1 h. The TCA solution was removed, and the cells were washed 5 times with distilled water. Aliquots of 70µL SRB solution (0.4% w/v) were added and were incubated in a dark place at room temperature for 10 min. Plates were washed 3 times with 1% acetic acid and allowed to air-dry overnight. Then, 150µL of TRIS (10mM) were added to dissolve protein-bound SRB stain; the absorbance was measured at 540 nm using a BMG LABTECH®- FluoStar Omega microplate reader (Ortenberg, Germany).

Results and discussion

The percentage yield of *Juniperus phoenicea* L. extracts

The extraction of *J. phoenicea* aerial parts with petroleum ether gave a yield of 11.5 g, constituted 1.9% of the dried part. In addition, the unsaponifiable matter and the total fatty acid represented 59.5% and 35.6%, respectively. Moreover, the extraction with 70% methanol gave a yield of 90 g, constituting 15% of the dried part.

Phytochemical screening

The results of phytochemical screening (Table 1) showed that carbohydrate and/or glycosides, flavonoids, and saponins are detected in hydroalcoholic extract of *Juniperus phoenicea*, while these constituents are absent in petroleum ether extract. In addition, fats and/ or oils, triterpenes and/or sterol are detected in both extracts. On the other hand, alkaloids and/or nitrogenous compounds, tannins, anthraquinones, and coumarins are absent in both extracts. Our result agreed with what was reported by El-Sawi who stated that the fruits and leaves of *Juniperus phoenicea* were rich in flavonoids, sterols and/or triterpenes, carbohydrates, and/or glycosides, and essential oil **[7].** Several biological activities were demonstrated for these phytoconstituents **[28-30].**

Table 1. Phytochemical screening of *Juniperus phoenicea* extracts

Chemical constituents	*Juniperus phoenicea*	
	Petroleum ether extract	Hydroalcoholic extract
Carbohydrate and/or glycosides	-	+
Triterpenes and/or sterol	+	+
Alkaloids	-	-
Tannins	-	-
Fats/oils	++	+
Flavonoids	-	+
Anthraquinones	-	-
Coumarins	-	-
Saponins	-	±

(+) Present (-) Absent (±) Traces

Total phenolic and flavonoids contents

According to the findings, total phenolics content of ***J. phoenicea aerial*** parts = 213.02 mg/ g extract, while the total flavonoids = 98.4 mg/ g extract. The findings indicated that hydroalcoholic extract of ***J. phoenicea*** aerial parts is a plentiful source of polyphenolic substances.

Polyphenolic profile by LC/MS/MS

Figure 1. Represents MRM ion chromatogram for polyphenols in hydroalcoholic extract of *Juniperus phoenicea* L. aerial parts in negative and positive ionization modes. The detected polyphenols were listed in Table 2. The results showed the identification of sixteen phenolic compounds in hydroalcoholic extract of *Juniperus phoenicea* L. aerial parts. Catechin, 3.4-dihydroxybenzoic acid, naringenin and gallic acid are presented as the major compounds with concentration 1853.54, 562.68, 377.89, and 185.78 µg/g, respectively. Polyphenolic compounds were reported to exhibit different biological activities including anticancer potential **[28; 30- 32].**

Characterization of the isolated compounds

Compound (1): It gave yellow green spot and by spraying with aluminum chloride, it showed a bright yellow color. EI-MS: *m/z* 318 and UV spectral investigations are in agreement with those reported for myricetin **[33].** The identification of compound 1 as myricetin is confirmed by co-chromatography with the authentic compound.

Compound (2): Yellow powder. With UV lamp, it gave yellow color which changed to yellow by ammonia vapor. EI-MS: *m/z* 302 and UV spectral investigations coincide with those of **[28].** It was recognized as quercetin after comparison with the reference sample.

Compound (3): Obtained as white crystals. By spraying with vanillin sulphuric acid reagent, gave strong pink color. The spectral data (^{1}H-NMR) are typical to the published one **[28; 34].** The identification of compound 3 was confirmed by co-chromatography with the authentic compound. So, it could be identified as catechin.

Compound (4): White amorphous powder. With UV lamp, it showed violet color. ^{1}H-NMR spectral data are in accordance with those of **[28].** By co-chromatography with authentic compound, it could be recognized as gallic acid.

Compound 5: Obtained as faint yellow powder. ^{1}H NMR spectral data were confirmed with the reported data **[35]** and based on comparison with the authentic compound, it was recognized as p-Coumaric acid.

GC/MS analysis of *Juniperus phoenicea* L.

GC/MS analysis revealed the identification of seventeen compounds of the unsaponifiable matter of *Juniperus phoenicea* L. Where, nonacosanol, 1-(2-Octyldecyl) octahydropentalene and gamma-sitosterol are presented as the major compounds represented 35.6% and 29.64%, and 20.16%, respectively of the total identified compounds. Moreover, the unoxygenated compounds consisted 31.22% of the total identified compounds, while oxygenated compounds represented 68.78% of the total identified compounds (Table 3). On the other hand, fourteen compounds were identified from the saponifable matter of *Juniperus phoenicea*. aerial parts. Hexadecanoic acid, 9-octadecenoic acid, and docosanoic acid were found to be the major constituents which represented 37.11%, 19.03%, and 13.63%, respectively of the total identified compounds. The result showed that the saturated fatty acids represented the vast majority (72.85%), while the unsaturated fatty acids constituted 27.15% % of the total identified compounds (Table 4). Figures 2 &3 represent EI scan and chemical structure of major identified compounds in unsaponifiable and saponifiable matters, respectively. Our results were found to be different from the previously reported data **[12, 36, 37].**

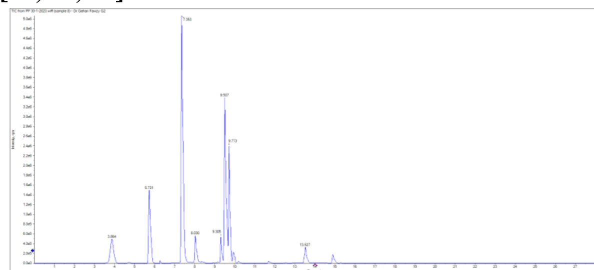


Figure 1: MRM ion chromatogram for polyphenols in hydroalcoholic extract of *Juniperus phoenicea* L. aerial parts

Table 2: Identified polyphenols in hydroalcoholic extract of *Juniperus phoenicea* L. aerial parts by LC-ESI-MS/MS (Positive and negative MRM mode)

Polyphenols	Main fragments	R_t	Formula	Concentration (µg/g)
Chlorogenic acid	355.1/163	7.36	$C_{16}H_{18}O_9$	10.04
Daidzein	255.1/199	ND	$C_{15}H_{10}O_4$	ND
Gallic acid	**168.9/124.9**	**3.87**	$C_7H_6O_5$	**185.78**
Caffeic acid	178/135	8.04	$C_9H_8O_4$	9.73
Rutin	609/299.9	9.74	$C_{27}H_{30}O_{16}$	24.88
Coumaric acid	162.9/119	9.52	$C_9H_8O_3$	45.04
Vanillin	151/136	ND	$C_8H_8O_3$	ND
Naringenin	**271/119**	**14.91**	$C_{15}H_{12}O_5$	**377.89**
Querectin	301/151	13.54	$C_{15}H_{10}O_7$	8.15
Ellagic acid	301/145	9.95	$C_{14}H_6O_8$	3.50
3.4-Dihydroxybenzoic acid	**152.9/109**	**5.74**	$C_7H_6O_4$	**562.68**
Hesperetin	301/136	ND	$C_{16}H_{14}O_6$	ND
Myricetin	317/137	11.7	$C_{15}H_{10}O_8$	51.37
Cinnamic acid	146.9/102.6	ND	$C_9H_8O_2$	ND
Methyl gallate	183/124	7.43	$C_8H_8O_5$	5.19
Kaempferol	284.7/93	15.29	$C_{15}H_{10}O_6$	0.61
Ferulic acid	192.8/133.9	10.2	$C_{10}H_{10}O_4$	8.81
Syringic acid	196.8/181.9	8.37	$C_9H_{10}O_5$	10.61
Apigenin	269/151	ND	$C_{15}H_{10}O_5$	ND
Catechin	**288.8/244.9**	**7.36**	$C_{15}H_{14}O_6$	**1853.54**
Luteolin	284.7/132.9	13.48	$C_{15}H_{10}O_6$	6.42

Table 3: GC⁄MS of the unsaponifiable matter of *Juniperus phoenicea* L.

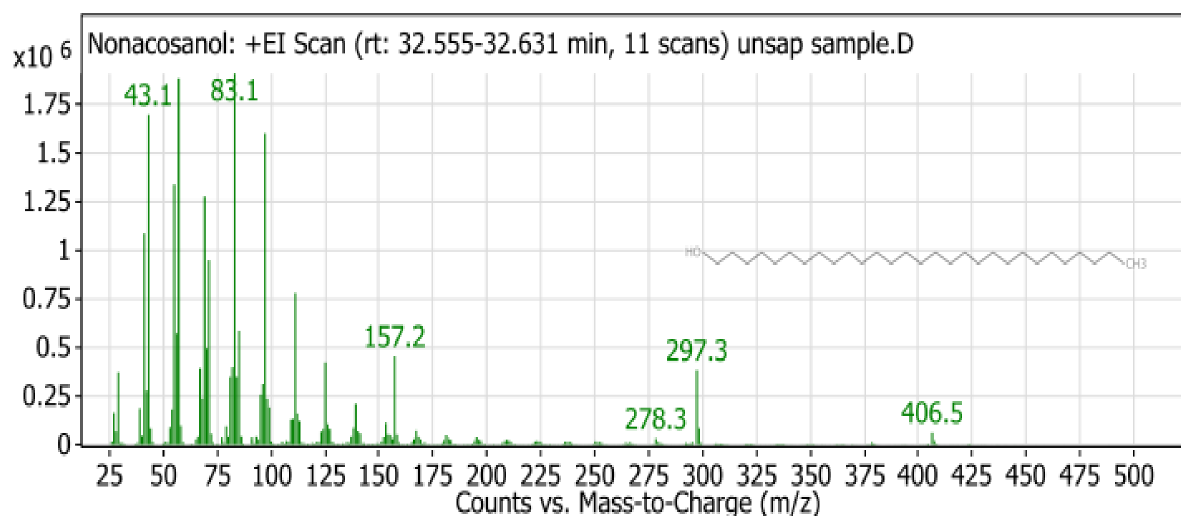
Peak	R_t	Name	Formula	Area Sum %
1	25.18	1b,5,5,6a-Tetramethyl-octahydro-1-oxa-cyclopropa[a]inden-6-one	$C_{13}H_{20}O_2$	0.7
2	25.76	Phytol, TMS derivative	$C_{23}H_{48}OSi$	0.86
3	28.71	1,2-Benzenedicarboxylic acid, bis(2-ethylhexyl) ester	$C_{24}H_{38}O_4$	0.39
4	28.77	Docosanol, TMS derivative	$C_{25}H_{54}OSi$	0.95
5	32.37	Nonacosan-10-ol, O-TMS	$C_{32}H_{68}OSi$	1.39
6	**32.60**	**Nonacosanol**	$\mathbf{C_{29}H_{60}O}$	**35.6**
7	33.24	Campesterol	$C_{28}H_{48}O$	0.53
8	33.62	1-Heneicosanol, TMS derivative	$C_{24}H_{52}OSi$	1.48
9	**33.76**	**Gamma-Sitosterol**	$\mathbf{C_{29}H_{50}O}$	**20.16**
10	**33.92**	**1-(2-Octyldecyl)octahydropentalene**	$\mathbf{C_{26}H_{50}}$	**29.64**
11	33.99	Stigmast-5-ene, 3.beta.-(trimethylsiloxy)-, (24S)-	$C_{32}H_{58}OSi$	4.41
12	34.20	1-Heptatriacotanol	$C_{37}H_{76}O$	0.63
13	34.31	Cholesta-8,24-dien-3-ol, 4-methyl-, (3.beta.,4.alpha.)-	$C_{28}H_{46}O$	0.43
14	34.42	9,19-Cyclolanostan-3-ol, acetate, (3.beta.)-	$C_{32}H_{54}O_2$	0.5
15	34.49	Dimethyl[bis(tridecyloxy)]silane	$C_{28}H_{60}O_2Si$	0.36
16	34.87	Dotriacontane	$C_{32}H_{66}$	1.58
17	35.05	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	0.38
Unoxygenated compounds			**31.22 %**	
Oxygenated compounds			**68.78 %**	

R_t: Retention time in minute

Table 4: GC⁄MS of the saponifiable matter of *Juniperus phoenicea* L.

Peak	R_t	Name	Formula	Area Sum %
1	7.98	Tetradecanoic acid, methyl ester	$C_{15}H_{30}O_2$	2.99
2	8.58	13,16-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	1.53
3	**9.11**	**Hexadecanoic acid, methyl ester**	$\mathbf{C_{17}H_{34}O_2}$	**37.11**
4	9.43	12,15-Octadecadiynoic acid, methyl ester	$C_{19}H_{30}O_2$	0.54
5	9.86	Cyclopentaneundecanoic acid, methyl ester	$C_{17}H_{32}O_2$	0.4
6	10.21	7-Methyl-Z-tetradecen-1-ol acetate	$C_{17}H_{32}O_2$	1.18
7	10.51	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	4.33
8	10.86	Methyl stearate	$C_{19}H_{38}O_2$	4.72
9	13.03	6,9,12-Octadecatrienoic acid, methyl ester	$C_{19}H_{32}O_2$	0.56
10	14.13	Eicosanoic acid, methyl ester	$C_{21}H_{42}O_2$	4.7
11	**16.02**	**9-Octadecenoic acid (Z)-, methyl ester**	$\mathbf{C_{19}H_{36}O_2}$	**19.03**
12	**17.90**	**Docosanoic acid, methyl ester**	$\mathbf{C_{23}H_{46}O_2}$	**13.63**
13	21.46	Tetracosanoic acid, methyl ester	$C_{25}H_{50}O_2$	8.9
14	26.98	Hexacosanoic acid, methyl ester	$C_{27}H_{54}O_2$	0.4
Saturated fatty acids			**72.85%**	
Unsaturated fatty acids			**27.15%**	

R_t: Retention time in minute



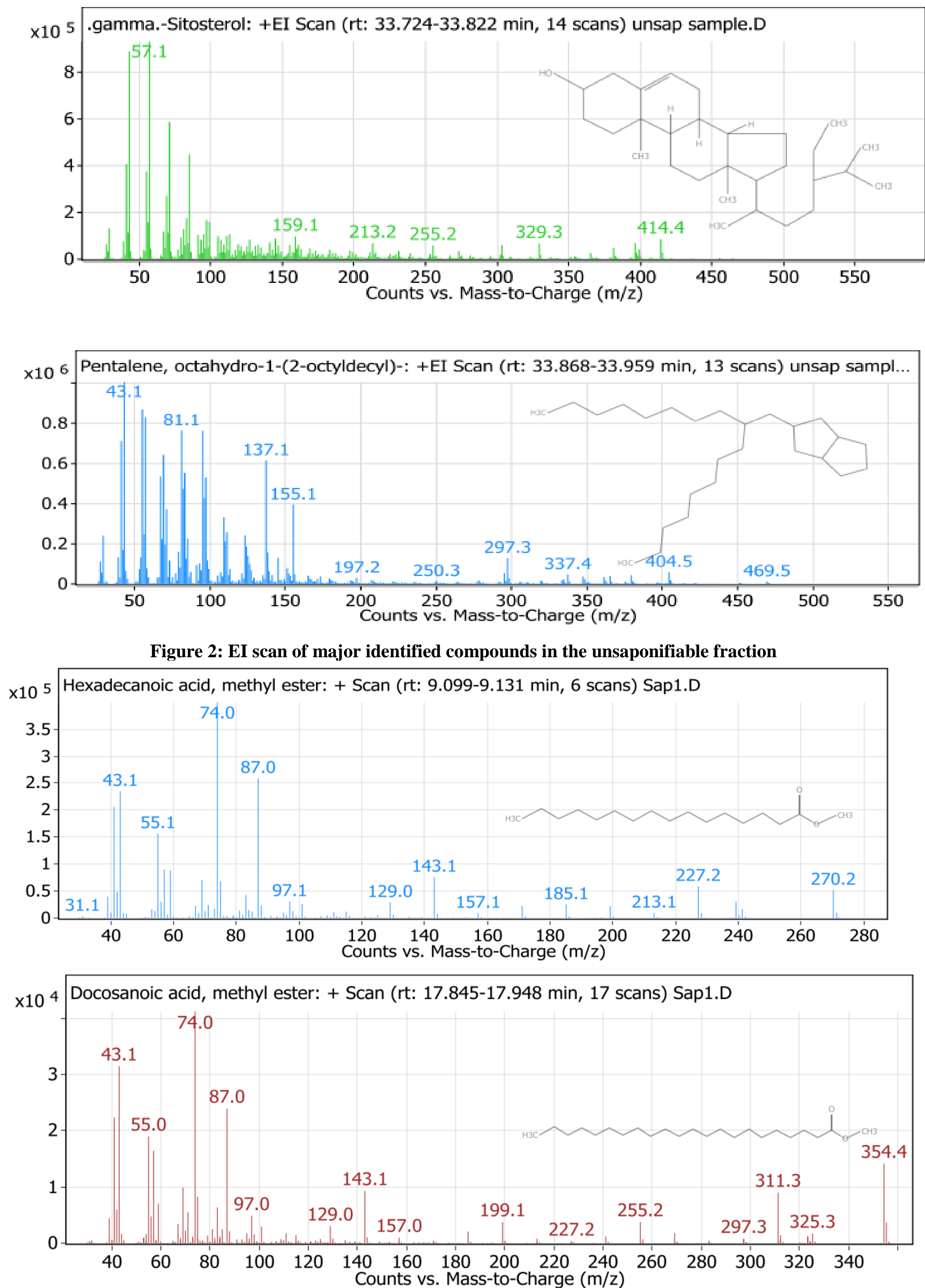


Figure 2: EI scan of major identified compounds in the unsaponifiable fraction

Figure 3: EI scan of major identified compounds in the fatty acid methyl ester fraction

Cytotoxic activity

Cytotoxic activity of hydroalcoholic and petroleum ether extracts of *Juniperus phoenicea* aerial parts were investigated *in vitro* against prostate cancer cell line (PC-3) and ovarian cancer cell line (SKOV-3) using doxorubicin as anticancer drug. The findings revealed that both extracts reduced the viability % of cancer cells in a dose-dependent manner. This screening analysis disclosed that both extracts of *Juniperus phoenicea* aerial parts revealed a significant cytotoxic activity on the examined cell lines. The results are shown in figures (4,5). Our findings are in accordance with what numerous researchers reported, confirming the cytotoxic activity of *Juniperus phoenicea against* various cancer cell lines **[38; 15-19].** Moreover, hydroalcoholic extract of *Juniperus phoenicea* aerial parts exhibited cytotoxic effect on PC-3 and SKOV-3 cell lines with IC_{50} of 13.60 and 0.01 (µg/ml), respectively, while petroleum ether extract exhibited cytotoxic effect on PC-3 and SKOV-3 cell lines with IC_{50} of 73.63 and 29.6 (µg/ml), respectively, by comparing these results with that of doxorubicin IC_{50} (5.37 and 0.97 µg/ ml, respectively) (Table 5), it was affirmed that the 70% methanol extract of *Juniperus phoenicea* aerial parts exhibited a very potent effect on SKOV-3 that is greater than doxorubicin, a reference anticancer agent, and a potent effect on PC-3. On the other hand, petroleum ether extract exhibited moderate cytotoxicity against SKOV-3 (IC_{50} = 29.6) and a weak effect against PC-3 cell lines (73.63 µg/ml). (Table 5). The current findings revealed that hydroalcoholic extract of *Juniperus phoenicea* aerial parts exhibited more potent cytotoxic activity than that of petroleum ether extract on the examined cell lines. In addition, the results showed that hydroalcoholic extract of *Juniperus phoenicea* aerial parts is rich with polyphenolic components which have been stated to exhibit different biological activities including anticancer potential **[28;30-32].** Although the result confirmed the cytotoxic activity of hydroalcoholic extract on the examined cell lines, previous research reported that the isolated compound (Cupressuflavone) was safe on the normal cell line. Moreover, a previous study did not record any adverse effects or alterations in the behaviour of the treated animals with hydroalcoholic extract of *Juniperus phoenicea* up to 14 days at doses up to 400 mg/kg BW, and no deaths were noted which confirmed the safety of *Juniperus phoenicea* **[38, 39].**

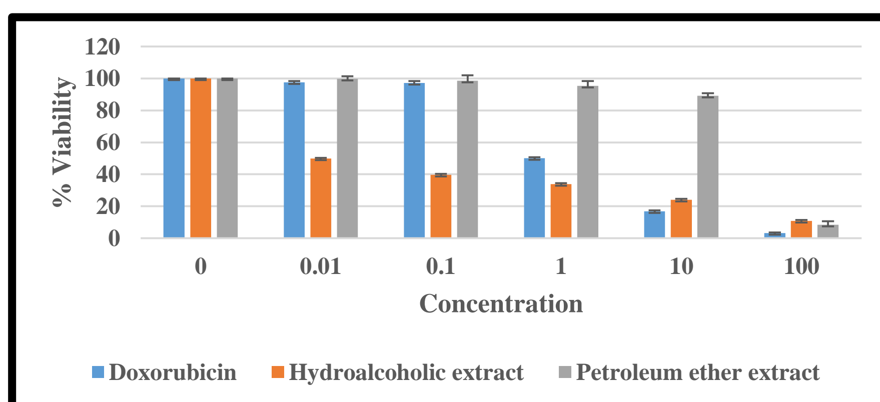


Figure 4: ***In vitro*** **cytotoxic activity of hydroalcoholic and petroleum ether extracts of** ***Juniperus phoenicea*** **aerial parts against ovarian cancer cell line (SKOV-3)**

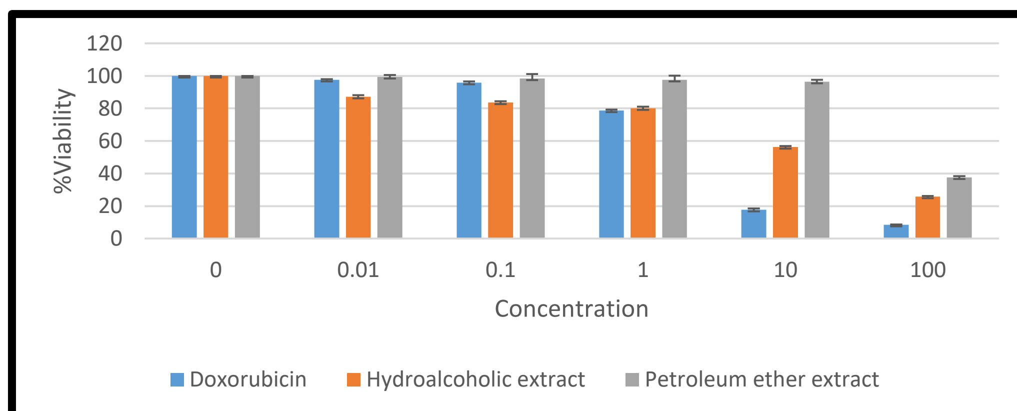


Figure 5: ***In vitro*** **cytotoxic activity of hydroalcoholic and petroleum ether extracts of** ***Juniperus phoenicea*** **aerial parts against prostate cancer cell line (PC-3)**

Table 5. IC_{50} values of hydroalcoholic and petroleum ether extracts of *Juniperus phoenicea* aerial parts on ovarian and prostate cancer cell line

cell line	IC_{50} µg/ml		
	Hydroalcoholic extract	Petroleum ether extract	Doxorubicin
SKOV-3	0.01	29.6	0.97
PC-3	13.60	73.63	5.37

Conclusion

The current findings revealed that hydroalcoholic extract of *Juniperus phoenicea* aerial parts possessed a very potent cytotoxicity against ovarian cancer cell line (SKOV-3) and a potent cytotoxicity against prostate cancer cell line (PC-3). On the other hand, petroleum ether extract exhibited moderate cytotoxicity against SKOV-3 and a weak effect against PC-3 cell lines (73.63 µg/ml). In addition, the results showed that hydroalcoholic and petroleum ether extracts of *Juniperus phoenicea* aerial parts are rich with different phytoconstituents which showed a significant influence on the bioactivities of plants. After applying clinical studies, *Juniperus phoenicea* aerial parts might be a useful drug for treating different forms of cancer. These results provide new opportunities for the alternative use of natural ingredients in the development of novel drugs.

Conflicts of Interest

There are no apparent conflicts of interest for the authors to disclose.

Acknowledgements

The authors would like to appreciate National Research Centre and Faculty of Agriculture, Cairo University for providing technical knowledge, assistance, and facilities for this study.

References

[1]. Bray F., Ferlay J., Soerjomataram I., Siegel R.L., Torre L.A., Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. **68** (6), 394–424 (2018).

[2]. Choudhari A.S., Mandave P.C., Deshpande M., Ranjekar P., Prakash O. Phytochemicals in cancer treatment: from preclinical studies to clinical practice. *Front pharmacol*. 10:1614 (2020)

[3]. Bolous L. Flora of Egypt, Cairo. Al Hadara Publishing, Vol. 1. (1999)

[4]. Aljaiyash A., Gonaid M., Islam M., Chaouch A. Antibacterial and cytotoxic activities of some Libyan medicinal plants. *Journal of Natural Product and Plant Resources*. **4**(2), 43-51(2014).

[5]. Allali H., Benmehdi H., Dib M., Tabti B., Ghalem S., Benabadji N. Phytotherapy of diabetes in west Algeria. *Asian J Chem*.**20** (4), 2701 (2008).

[6]. Amer M., Wasif M., Abo-Aytta A., Gabr F. Chemical and biological evaluation of *Juniperus phoenicea* as a hypoglycaemic agent. *Zagazig J Agric Res*.**21**(4),1077–91(1995).

[7]. El-Sawi S.A., Motawae H.M., Sleem M.A., El-Shabrawy A.O., Sleem A., Ismail M.A. Phytochemical screening, investigation of carbohydrate contents, and antiviral activity of *Juniperus phoenicea L.* growing in Egypt. *J. Herbs Spices Med. Plants*, 20, 83–91(2014)

[8]. Laouar A., Klibet F., Bourogaa E., Benamara A., Boumendjel A., Chefrour A., Messarah M. Potential antioxidant properties and hepatoprotective effffects of *Juniperus phoenicea* berries against CCl4 induced hepatic damage in rats. *Asian Pac J Trop med.* **10**(3),263–9 (2017).

[9]. Elmhdwi F., Attitalla H., Khan A. Evaluation of antibacterial activity and antioxidant potential of difffferent extracts from the leaves of *Juniperus phoenicea*. *J Plant Pathol Microb*. **6** (9),300 (2015).

[10]. El-Sawi S., Motawae H., Sleem M., El-Shabrawy A., Sleem, M., Sleem A., Maamoun M. Ethnopharmacological In *Vivo* Studies On *Juniperus Phoenicea*: Evaluation Of Hepatoprotective Role And The Anti-Inflammatory Action, *Journal Of Basic And Applied Research International*. **5** (3), 157-165 (2015).

[11]. Kim S-J., Jung T.Y., Kim H.W., Park T. Anti-obesity effects of Juniperus chinensis extract are associated with increased AMP-activated protein kinase expression and phosphory – Lation in the visceral adipose tissue of rats. *Boils pharm Bull*. **31**(7), 1415-1421 (2008).

[12]. El-Sawi S., Motawae H., Sleem M. El-Shabrawy A., Sleem A., Ali A., Ismail MA. Investigation of lipoidal matter, antimicrobial and diuretic activities of leaves and berries of *Juniperus phoenicea* L growing in Egypt. *Aust J Med Herbal.* **23**(4),174- 179 (2011).

[13]. Seca AML., and Silva AMS. The chemical composition of the Juniperus genus (1970-2004) In book; Recent progress in Medicinal plants, Editors: J.N. Govil V.K. Sing, R. Bhardrnaj, Vol 16: Phytomedicines, chapter: publisher: stadium press (India) PVT. Ltd., PP. 401-522 (2007).

[14]. Al Groshi A., Evans A.R., Ismail F.M., Nahar L., Sarker SD. Cytotoxicity of libyan Juniperus phoenicea against human cancer cell lines A549, EJ138, HepG2 and MCF7. *Pharm Sci*. **24**(1),3–7(2018)

[15]. Barrero A.F., Quí lez del Moral J.F., Herrador M.M., Akssira M., Bennamara A., Akkad S., et al. Oxygenated diterpenes and other constituents from moroccan *Juniperus phoenicea* and *Juniperus thurifera* var. africana. *Phytochemistry*. **65**(17), 2507–15 (2004).

[16]. El-Sawi S.A., Motawae H.M., Ali A.M. Chemical composition, cytotoxic activity and antimicrobial activity of essential oils of leaves and berries of *Juniperus phoenicea* L. grown in Egypt. *AJTCAM*.**4** (4),417–26 (2007).

[17]. Maamoun M., El-sawi S.A., Motawae H.M., Sleem M.A., El-shabrawy A.O., Usama H.W., et al. Antiproliferative effffect of extracts and flflavonoids of *Juniperus phoenicea* L. growing in Egypt. *Mintage J Pharm Med Sci*.**5**(2),1–7 2016)

[18]. Barnawi I. O., Nasr F. A., Noman O. M., Alqahtani A. S., Al-zharani M., Alotaibi A. A., Daradka H. M., Al-Mishari A. A., Alobaid W. A., Alqahtani,A., & Herqash R. N. Induction of apoptosis and cell cycle arrest by chloroform fraction of juniperus phoenicea and chemical constituents analysis. *De Gruyter*. 19, 119–127 (2021)

[19]. Al Masoudi L.M., Alqurashi A.S., Abu Zaid A., Hamdi H. Characterization and Biological Studies of Synthesized Titanium Dioxide Nanoparticles from Leaf Extract of *Juniperus phoenicea* (L.) Growing in Taif Region, Saudi Arabia. *Processes*. 11, 272 (2023).

[20]. Shaikh J.R., Patil M.K. Qualitative tests for preliminary phytochemical screening: An overview. *International Journal of Chemical Studies*. **8**(2),603-608 (2020). https://doi.org/10.22271/chemi.2020.v8.i2i.8834

[21]. Tsuda K., Sakai K., Tanabe K., Kishida Y. Isolation of 22-dehydrocholesterol from *Hypnea japonica*. *Chem Pharm Bull.* **7**(6),747 (1959). doi: 10.1248/cpb.7.747.

[22]. Liu K.S. Preparation of fatty acid methyl esters for gas-chromatographic analysis of lipids in biological materials. *J Am Oil Chem Soc.* **71**(11),1179-871 (994) doi: 10.1007/ bf02540534.

[23]. Adams R.P. Identification of Essential Oils by Ion Trap Mass Spectroscopy. New York: Academic Press; 1995. doi: 10.1002/ffj.2730050215.

[24]. Attard E., A rapid microtiter plate folin-ciocalteu method for the assessment of polyphenols. *Central European Journal of Biology*. **8**(1), 48-53 (2013).

[25]. Herald T. J., Gadgil P., Tilley M., High-throughput micro plate assays for screening flavonoid content and DPPH-scavenging activity in sorghum bran and flour. *Journal of the Science of Food and Agricultur*e., **92**, 2326-2331(2012).

[26]. Skehan P., Storeng R., Scudiero D., Monks A., Mahon J.M., Vistica D., New colorimetric cytotoxicity assay for anticancer-drug screening. *Journal of National Cancer Institute*., **82**(13), 1107-1112 (1990).

[27]. Allam R. M., Al-Abd A. M., Khedr A., Sharaf O. A., Fingolimod interrupts the cross talk between estrogen metabolism and sphingolipid metabolism within prostate cancer cells. *Toxicology Letter*., **11**(291),77-85 (2018).

[28]. Abdel Raoof G.F., Said A.A., Mohamed K.Y., Gomaa H.A. Phytoconstituents and bioactivities of the bark of *Pleiogynium timorense* (DC.) Leenh (Anacardiaceae). *J Herbmed Pharmacol*. **9**(1),20-27 (2020) doi: 10.15171/jhp.2020.03.

[29]. El-anssary A.A., Abdel Raoof G.F., Saleh D.O., El-Masry H.M. Bioactivities, physicochemical parameters and GC/MS profiling of the fixed oil of *Cucumis melo* L seeds: A focus on anti-inflammatory, immunomodulatory, and antimicrobial activities. *J Herbmed Pharmacol*.10(4),476-485 (2021) doi: 10.34172/jhp.2021.55.

[30]. Abdel Raoof, G. F., & Abdelfatah Elsayed, M. (2020). Cytotoxic activity, molecular docking study and phytochemical investigation on *Cichorium Intybus* herb. *Egyptian Journal of Chemistry*. https://doi.org/10.21608/ejchem.2020.44299.2896

[31]. Teniente S. L., Flores-Gallegos A. C., Esparza-González S. C., Campos-Múzquiz L. G., Nery-Flores S. D., & Rodríguez-Herrera R. Anticancer effect of Pomegranate Peel polyphenols against cervical cancer. *Antioxidants*. **12**(1), 127 (2023). https://doi.org/10.3390/antiox12010127

[32]. Gancedo N.C., Isolani R., de Oliveira N.C., Nakamura C.V., de Medeiros Araújo D.C., Sanches A.C.C., Tonin F.S., Fernandez-Llimos F., Chierrito D., de Mello J.C.P. Chemical Constituents, Anticancer and Anti-Proliferative Potential of Limonium Species: A Systematic Review. *Pharmaceuticals*., 16, 293(2023). https://doi.org/10.3390/ ph16020293

[33]. Anwer S.T, Mobashir M., Fantoukh O.I., Khan B., Imtiyaz K., Naqvi IH;Rizvi MMA. Synthesis of Silver Nano Particles Using Myricetin and the *in-Vitro* Assessment of Anti-Colorectal Cancer Activity: In-Silico Integration. *Int. J. Mol. Sci.*, 23, 11024 (2022).

[34]. Hye A., Taher M.A., Ali M.Y., Ali M.U. and Zaman S., Isolation of (+)-catechin from *Acacia*

catechu (Cutch Tree) by a convenient method., *J Sci Res.*,**1**(2), 300-5(2009).

[35]. Jo B. S., & Cho Y. J. Inhibitory activity against helicobacter pylori of isolated compounds from *Pinus koraiensis* siebold et Zucc leaves. *Journal of Applied Biological Chemistry*. 59, 19–23 (2016). https://doi.org/10. 3839/jabc.2016.005

[36]. Keskes H., Belhadj S., Jlail L., El Feki A., Damak M., Sayadi S., Allouche N. LC-MS-MS and GC-MS Analyses of Biologically Active Extracts and Fractions from Tunisian *Juniperus Phoenice* Leaves. *Pharmaceutical Biology*. **55**(1),88-95 (2017).

[37]. Aboul-Ela M., El-Shaer N., Abd El-Azim T. Chemical constituents and hypoglycemic effect of the berries of *Juniperus phoenicea* Part I. *Alex J Pharm Sci* 19;109 (2005).

[38]. Al Groshi A., Jasim H.A., Evans A.R., et al. Growth inhibitory activity of biflavonoids and diterpenoids from the leaves of the Libyan *Juniperus phoenicea* against human cancer cells. *Phytotherapy Research*.33,2075–2082 (2019) https://doi.org/10.1002/ptr.6397

[39]. Zouari Bouassida K., Makni S., Tounsi A., Jlaiel L., Trigui M., Tounsi S. Effects of *Juniperus phoenicea* Hydroalcoholic Extract on Inflammatory Mediators and Oxidative Stress Markers in Carrageenan-Induced Paw Oedema in Mice. *Biomed Res Int*. 2018, 3785487 (2018). doi:10.1155/2018/3785487.