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Original Research Article

Antimicrobial and antioxidant activities of *Parkinsonia aculeata* and chemical composition of their essential oils

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Abstract

The essential oils of the air- dried aerial parts of Parkinsonia *aculeate* were studied by GC and GC/MS. In parallel the antimicrobial and antioxidant activities were evaluated. The result of GC/MS analysis revealed the presence of forty eight compounds of essential oils. The main components were identified asphenol, 2,4-bis(1-methyl-1-phenylethyl) (41, 13.46%), 2,4-bis(dimethylbenzyl) -6-t-butylphenol (42, 8.19%), dibutyl phthalate (27, 5.52%), nonadecane (38, 4.57%), phenol, 2,6-bis(1,1-dimethylethyl)-4-(1-methyl-1-phenylethyl) (29, 4.39%), ethyl iso-allocholate (31, 4.10%) and heptadecane (35, 4.03%). In addition, minor components were also detected in the essential oil as butylatedhydroxytoluene (14, 1.53%), Phenol,2-(1,1-dimethylethyl)-4-(1,1,3,3-tetramethylbutyl) (21, 1.0%), 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester (23, 0.9%), 1-Hexadecanol (24, 1.03%), Phenol,-(1,1-dimethylethyl)-4-(1-methyl-1-phenylethyl) (28, 2.89%), phytol (30, 2.55%) and eicosanoicacis (32, 1.65%). Essential oil showed moderate antimicrobial activity against bacteria and fungi. Furthermore, the essential oil of this plant deprived from antioxidant activity.

Keywords: Antioxidant, Antimicrobial activity, Essential oil, *Parkinsonia*

INTRODUCTION

Medicinal plants have been a major source in the maintenance of health, as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness. In response to the global need for scientific information on medicinal plants, the present work compiles some relevant information on the some biological and chemical aspects of *Parkinsonia aculeate* essential oil.

P.aculeata may be a spiny shrub or a small tree. It grows 2 to 8 m (6.6 to 26 ft) high, with a maximum height of 10 meters (33 ft). Palo verde may have single or multiple stems and many branches with pendulous leaves. The leaves and stems are hairless. The leaves are alternate and pennate (15 to 20 cm long). The flattened petiole is edged by two rows of 25–30 tiny oval leaflets; the leaflets are soon deciduous in dry weather (and during the winter in some areas) leaving the green petioles and branches to photosynthesize (Atiqur et al., 2004).

The branches grow double or triple sharp spines 7–12 mm (0.28–0.47 in) long at the axils of the leaves. The flowers are yellow- orange and fragrant, 20 mm (0.79 in) in diameter, growing from a long slender stalk in groups of eight to ten. They have five sepals and five petals, four of them clearer and rhomboid ovate, the fifth elongated, with a warmer yellow and purple spots at the base. The flowering period is the middle months of spring (March and April or September and October). The flowers are pollinated by bees. The fruit is a seedpod, leathery in appearance, light brown when mature (Atiqur et al., 2004).

The plant has been widely used in various traditional system of medicine. It is used since centuries as an antipyretic, diaphoretic and abortifacient. Resent

Figure 1. Aerial parts and flower of *P. aculeata*

research carried out indicated its other uses such as antibacterial, hepato-protective, antifertility and antidiabetic (Divya et al., 2011; Orwa et al., 2009). Leaves of the plant have been reported to contain C-glycosylflavones (Besson et al., 1980). The other constituents reported in leaves are C.glycosides, Epi-orientin, a C-glycoside of luteolin andParkinsonin-A (Bhatia et al., 1966; El-Sayed et al., 1991). Also extensive studies on the leaves reported that a new flavonone with epoxy-isopentyl moiety named as parkintin has been isolated from methanol soluble part of the leaves (Ali et al., 2005). *P. aculatea* seed oil contains a high amount of polyunsaturated fatty acids (Sharma et al., 2009). Aqueous and alkaline extraction of the milled endosperms of this plant yielded four galactomannan fractions (Tewari et al., 2009). Few rotenoids were identified and isolated from various parts of *P.aculeata* (Kamal and Mathur, 2007).

MATERIALS AND METHODS

Plant material

The aerial parts of *P.arkinsonia aculeate* (Figure 1) were collected from Riyadh, Saudi Arabia in March, 2012. The species was identified and confirmed by Prof. Dr. Jakob Thomas, College of science, KSU. A voucher specimen was prepared and deposited at the herbarium in department of pharmacognosy, College of Pharmacy, King Saud University. The aerial parts of *P. aculeate were* air dried and ground into coarse particles till use.

Experimental Apparatus

Isolation of the essential oil

The dried aerial parts (300 g) were subjected to Hydro distillation for extraction of volatile oil according to the method described before (Hassan et al., 2014). Essential oil obtained was yellow in colored, liquid with aromatic odour (0.03 v/dw). The oil was stored in sealed amber vial kept at 4^{o}C until analysis by GC/MS.

Gas chromatography-mass spectrometry (GC-MS)

The essential oil of *P. Aculeate* was subjected to GC-MS analysis using Thermo Fisher Scientific U.S.A, TRACE DSQ Mass spectrometer, column type: - THERMO TR-5ms SQC, the injector temperature was 220, temp. program:- 60 ^{o}C (1min.) then 240 ^{o}C(5 min.) at 6 ^{o}C/min. Instrument Method: C:\Xcalibur\methods\GC.meth, START TIME:- 2.50 min. end time:- 36.00 min., sample type :- liquid, original data path: C:\XCALIBUR\DATA, current data path: C:\Documents and Settings\DATA, Run Time(min): 36 min. sample volume (µl): 1µl, ion source temp :- 200 ^{o}C.

Identification of the compounds

Identification of the essential oil components was based on GC retention time and matching with Wiley 275 L library as well as by comparison the fragmentation patterns of their mass spectra with those reported in the literatures (Adams, 1995; Mclafferty and Staffer, 1989).

Biological activities

Antimicrobial activity

Cup-plate method (Woods and Washington, 1995) was used to detect the preliminary antimicrobial activity of the essential oil. The sample was dissolved in dimethyl

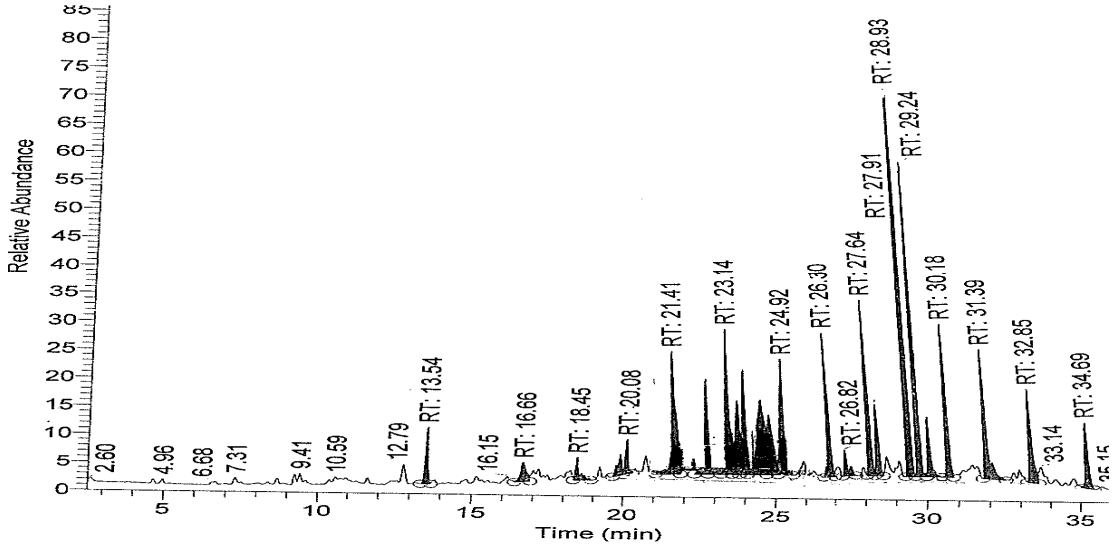


Figure 2. GC/MS chromatogram of the essential oil of *Parkinsonia aculeate* growing in Saudi Arabia

Table 1. Chemical composition of essential oil of *Parkinsonia aculeata* growing in Saudi Arabia

	Name	RT	%	M+
1	Diisoamylene	2.61	0.06	140
2	2-Furanmethanol-5-ethenyltetrahydro-a`,a`,-5-trimethyl cis	4.66	0.21	170
3	2-H-pyran-3-ol-6-ethenyltetrahydro-2,2,6-trimethyl	6.68	0.14	170
4	Geranyl vinyl ether	8.36	0.08	180
5	Cyclohexene-1-ethyl	9.24	0.27	110
6	Carvacrol	9.41	0.50	150
7	2-Cyclhexen-1-one-3-methyl-6-(1-methylethyl)	10.38	0.15	152
8	Geranic acid	10.59	0.26	168
9	2-Octyne,1,1 diethoxy	10.93	0.63	198
10	N-2,4-Dnp-L Aarginine	11.28	0.18	340
11	Cyclopenta[c]pyran-1(3H)-one , hexahydro-4,7-dimethyl-, (4à,4aà,7à,7aà)	11.64	0.24	168
12	2,5-Cyclohexadiene-1,4-dione , 2,6-bis(1,1-dimethylethyl)-	12.60	0.28	220
13	Menthol, 1'-(butyn-3-one-1-yl)-, (1S,2S,5R)-	12.79	0.68	222
14	ButylatedHydroxytoluene	13.54	1.53	220
15	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-tri methyl-, (R)	13.82	0.13	180
16	Caryophyllene oxide	14.82	0.19	220
17	3-Hydroxy-á-damascone	15.52	0.09	208
18	6-Acetamido-1,4-benzodioxan E	16.66	1.21	193
19	Phenol, o-(à,à-dimethylbenzyl)	17.01	0.38	212
20	Geranylisovalerate	18.23	0.53	238

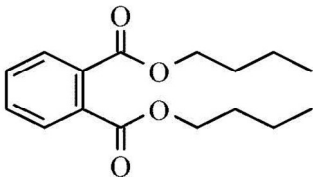
Table 1. Continue

21	Phenol, 2-(1,1-dimethylethyl)-4-(1,1,3, 3-tetramethylbutyl)-	18.45	1.00	262
22	Benzene, 1,1'-(3,3-dimethyl-1-butenylid ene)bis	19.21	0.35	236
23	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	19.89	0.91	278
24	1-Hexadecanol	20.01	1.03	242
25	Octadecane, 3-ethyl-5-(2-ethylbutyl)-	20.37	0.13	366
26	7,9-Di-tert-butyl-1-oxaspiro(4, 5)deca-6,9-diene-2,8-dione	20.70	0.44	276
27	**Dibutyl phthalate**	**21.41**	**5.52**	**278**
28	Phenol, 2-(1,1-dimethylethyl)-4-(1-met hyl-1-phenylethyl)	22.47	2.89	268
29	**Phenol, 2,6-bis(1,1-dimethylethyl)-4-(1 -methyl-1-phenylethyl)-**	**23.14**	**4.39**	**324**
30	Phytol	23.68	2.55	296
31	**Ethyl iso-allocholate**	**24.23**	**4.10**	**436**
32	Eicosanoic acid	24.50	1.65	312
34	Nonadecane	24.92	2.81	268
35	**Heptadecane**	**26.30**	**4.03**	**240**
36	1-Phenanthrenecarboxylic acid, 1,2,3,4,4a,9,10,10a-octahydro -1,4a-dimethyl-7-(1-methyleth yl)-, methyl ester, [1R-(1à,4aá,10aà)]-	26.82	1.04	314
37	Dimethyldithiocarbamic acid, S-(2-cyano-2-methyl-1-phenyl) vinyl ester	27.41	0.24	262
38	**Nonadecane**	**27.64**	**4.57**	**268**
39	Phenol, 2,2'-methylenebis[6-(1,1-dimet hylethyl)-4-methyl-	27.91	2.14	340
40	-1Phenanthrenecarboxylic acid, 1,2,3,4,4 a,9,10,10a-octahydro-1,4a-dimethyl-7-(1-methylethy l)-, [1R-(1à,4aá,10aà)]-	28.18	0.83	300
41	**Phenol, 2,4-bis(1-methyl-1-phenylethy l)-**	**28.93**	**13.46**	**330**
42	**2,4-Bis(dimethylbenzyl)-6-t-b Utylphenol**	**29.24**	**8.19**	**386**
43	1,2-Benzenedicarboxylic acid, diisooctyleste	29.56	1.84	278

Table 1. Continue

44	Nonadecane	30.18	4.07	268
45	Octadecane, 2-methy	31.39	4.44	268
46	Octadecane, 3-ethyl-5-(2-ethylbutyl)	32.24	0.33	366
47	Phenol, 2,6-bis(1,1-dimethylethyl)-4-e Thyl	32.43	0.55	234
48	4-Methoxyphenoxyformamide	33.14	0.87	302
total	,		82.11%	

Rt=retention time M+= molecular ion peak



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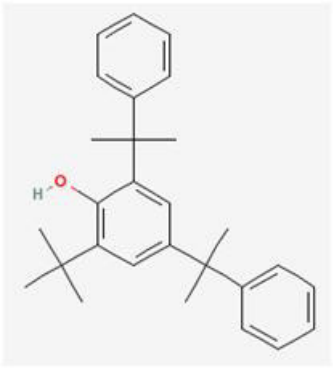

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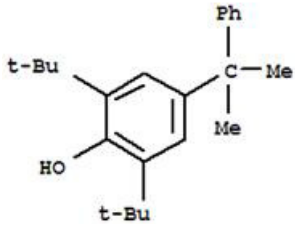

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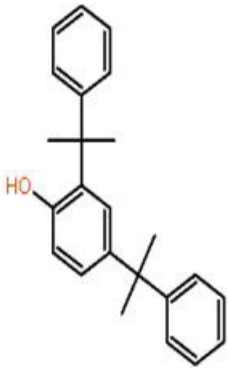

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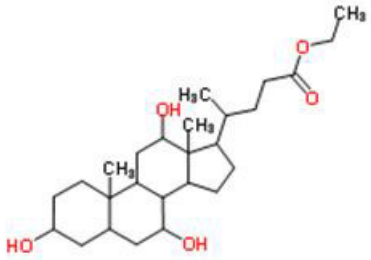

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(38)

Figure 3. Major compounds isolated from *Parkinsonia aculeate* growing in Saudi Arabia.

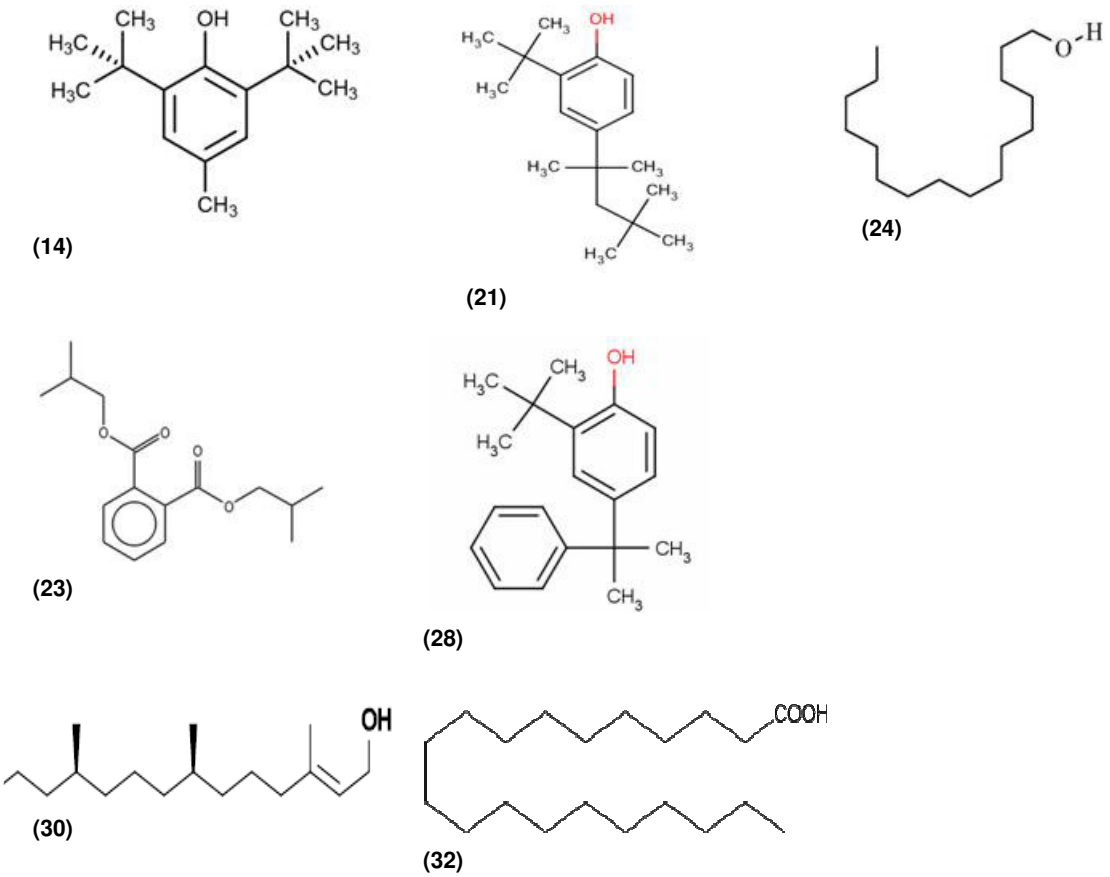
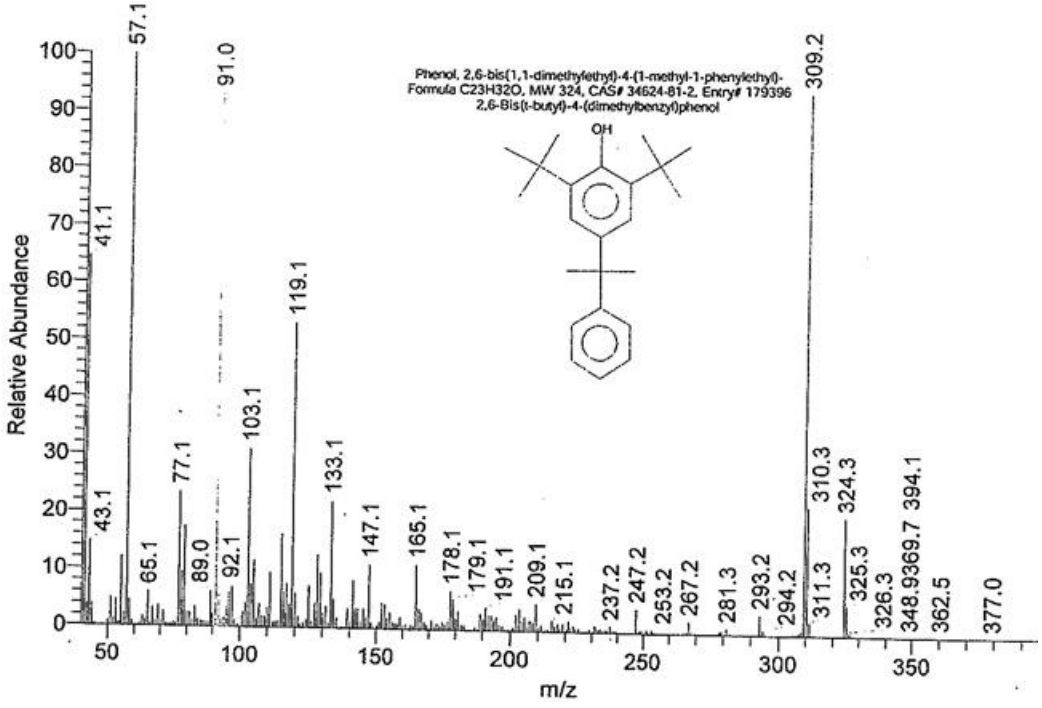
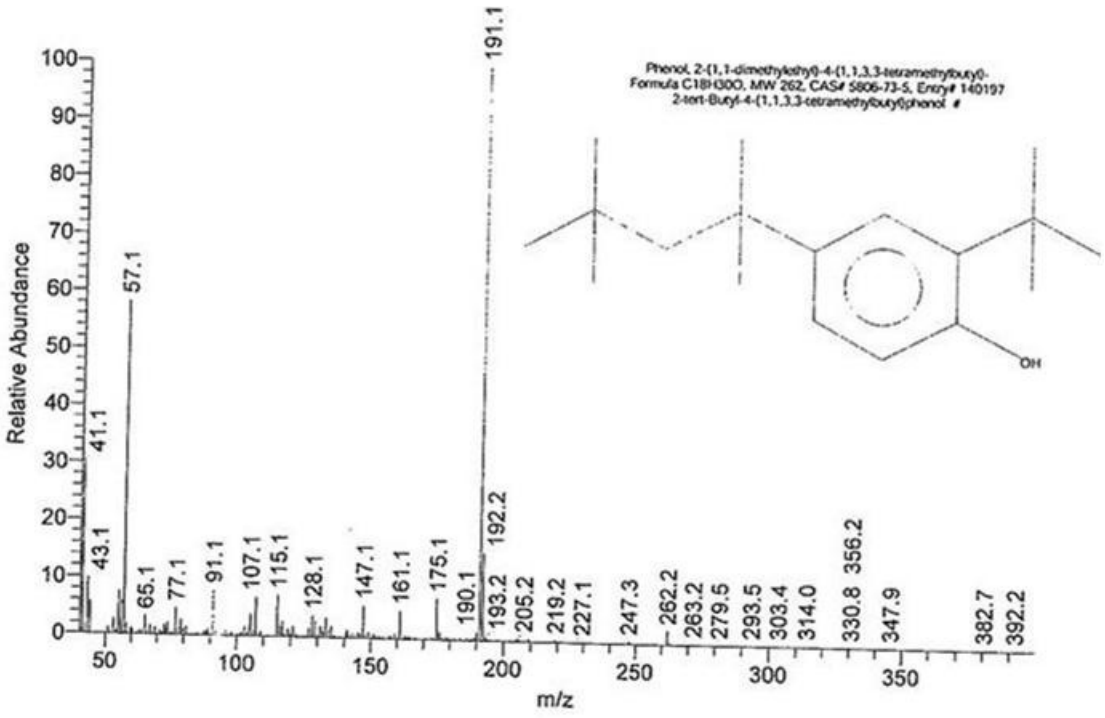
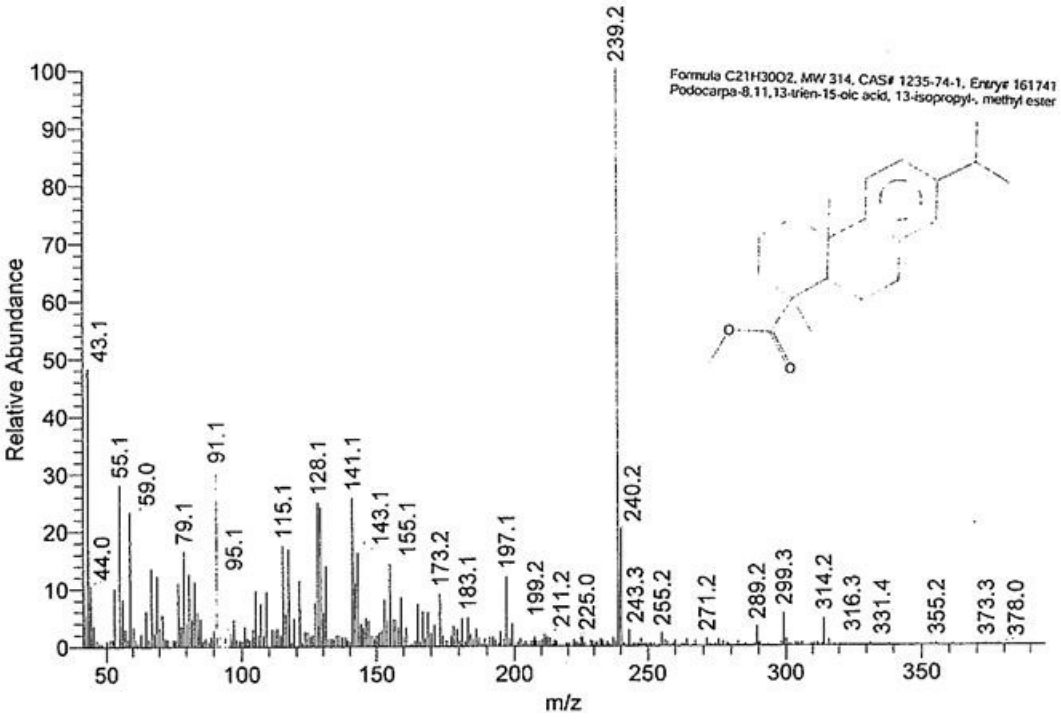


Figure 4. Some of the Minor compounds isolated from *Parkinsonia aculea*ta growing in Saudi Arabia.




Phenol, 2-(1,1-dimethylethyl)-4-(1,1,3,3-tetramethylbutyl)-
Formula C18H30O, MW 262, CAS# 5906-73-5, Entry# 140197
2-tert-Butyl-4-(1,1,3,3-tetramethylbutyl)phenol #
OH
Relative Abundance
m/z
191.1
57.1
41.1
43.1
192.2
65.1
77.1
91.1
107.1
115.1
128.1
147.1
161.1
175.1
190.1
193.2
205.2
219.2
227.1
247.3
262.2
263.2
279.5
293.5
303.4
314.0
330.8
356.2
347.9
382.7
392.2


Formula C21H30O2, MW 314, CAS# 1235-74-1, Entry# 161741
Podocarpa-8,11,13-trien-15-oic acid, 13-isopropyl-, methyl ester
O
O
Relative Abundance
m/z
239.2
43.1
55.1
59.0
91.1
79.1
44.0
95.1
115.1
128.1
141.1
143.1
155.1
173.2
183.1
197.1
199.2
211.2
225.0
240.2
243.3
255.2
271.2
289.2
299.3
314.2
316.3
331.4
355.2
373.3
378.0

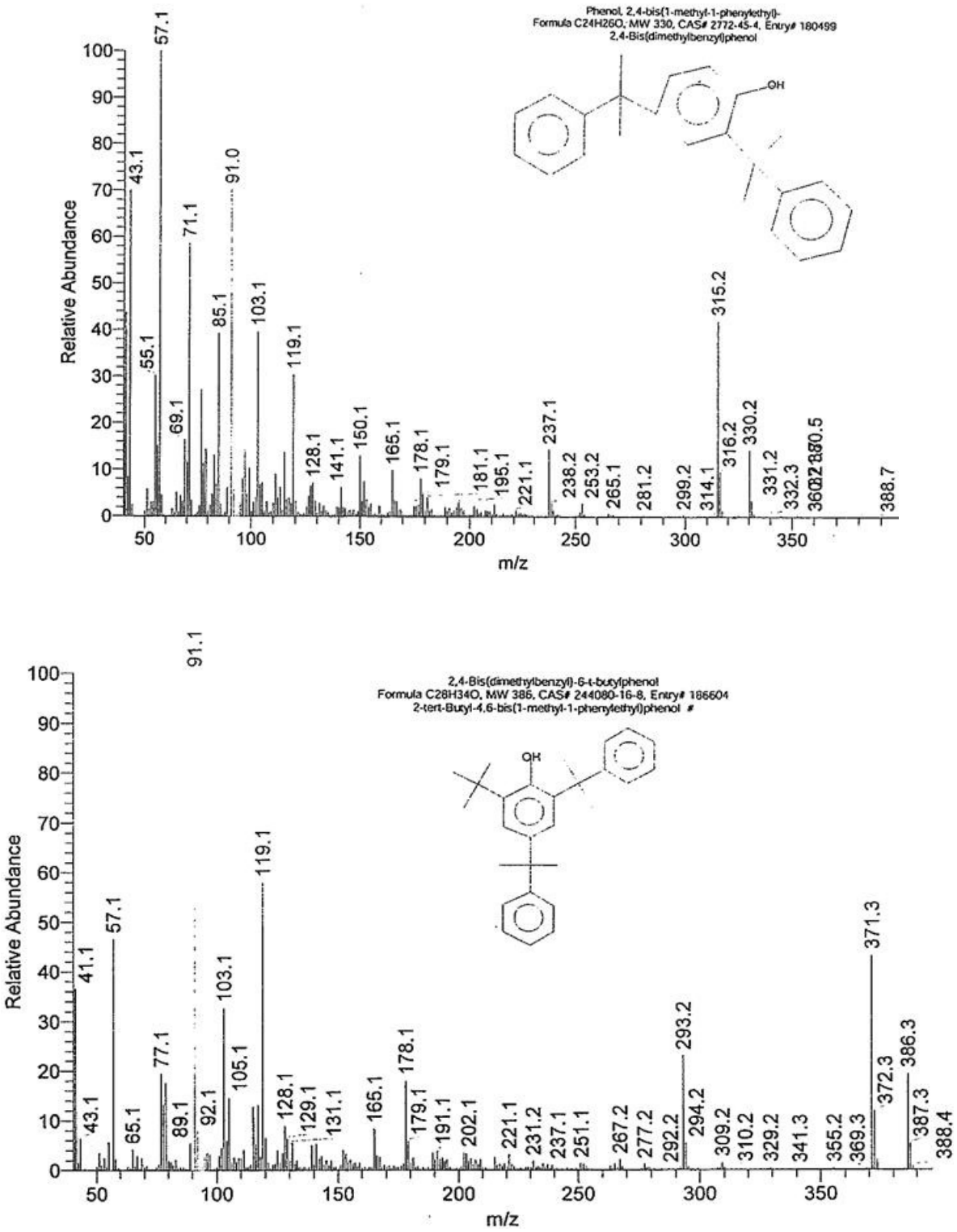


Figure 5. Mass spectra of some essential oil components of *Parkinsoniaaculeata*growing in Saudi Arabia

formamide (DMF) at concentration of 100 mg/ml. The nutrient agar and Saburaud's agar were seeded by about 10^6 microbial cells. Gram +ve bacteria (*Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus* ATCC 12228 and *Staphylococcus epidermidis* ATCC 12228) and Gram –ve bacteria (*Pseudomonas aeruginosa* ATCC 27853, *Escherichia. coli* ATCC 10536 and *Escherichia. coli* ATCC25922) as well as fungi (*Aspragillus niger*ATCC

Table 2. Antimicrobial activity of essential oil of *P. aculeate*

Microorganism	Inhibition zone diameter [mm] of	
	Total alcoholic extract of *P. aculeata* (50µg)	Standard(50µg)
Fungi:		***Amphotricin B***
Asperagillus fumigates	19.3± 0.63*	23.7 ± 0.1
Syncephalastrumracemosum	17.3± 0.58*	19.7 ± 0.2
Geotricumcandidum	21.6± 1.2	28.7 ± 0.2
Candida albicans	NA	25.4 ± 0.1
Gram positive bacteria:		***Ampicillin***
Streptococcus pneumonia	20.8 ± 0.58*	23.8 ± 0.2
Bacillus subtillis	23.3± 0.58	32.4 ± 0.3
Gram negative bacteria:		***Gentamicin***
Pseudomonusaeruginosa	16.2 ± 0.58	17.3 ± 0.1
Escherichiaa coli	19.3± 0.72*	19.9 ± 0.3

NA= no activity
*p<0.05

16404 and *Candida albicans* ATCC 10231) are standard strains obtained from the Department of Microbiology, Faculty of Pharmacy, Zagazig University and used as tested microorganisms. Each cup was filled by about 50 µl from extract (50 mg/ml). Ampicillin, Gentamicin and Amphotricin B (50 mg/ml) were used as standard antibacterial and antifungal, respectively. The plates were incubated overnight at 37˚C for bacteria and at 30˚C for fungi for days. Zones of inhibition were measured (mm) and recorded in Table (2).

Anti-oxidant activity (DPPH free radical scavenging activity)

1,1-diphenyl-2-picrylhydrazyl is a stable deep purple radical due to its unpaired electron. In the presence of an antioxidant radical scavenger, which can donate an electron to DPPH, the deep violet color decolorize to the pale yellow non-radical form. The change in colorization and the subsequent fall in absorbance are monitored spectrophotometrically at $\lambda_{max.}$517 nm (Ratty and J Das, 1988). One hundred µL of extracts (10 mg extract/10 mL methanol) was added to 3 mL of 0.1 mM DPPH dissolved in methanol according to the solvent used for extraction. After incubation period of 30 min. at room temperature, the absorbance was determined against a control at 517 nm. Ascorbic acid was used as a positive control. All the determinations were performed in four replicates and averaged. Percentage of antioxidant activity of free radical DPPH was calculated as follow:

DPPH scavenging activity % = $100-\{[(A_{\circ}- A_1)/A_{\circ}] \times 100\}$

Where $A_{\circ}$ is the absorbance of the control reaction and A_1 is the absorbance in the presence of plant extract +DPPH.

Data analysis

Values are presented as arithmetic mean ± standard error of mean. Data were statistically analyzed by using one-way ANOVA test. The P value <0.05 was taken as a statistically significant difference.

RESULTS AND DISCUSSION

Hydrodistillation of the air dried aerial parts of *P. aculeate* using Clevenger-type apparatus yielded 0.03% (v/w). The essential oil showed a yellow coloration with an aromatic fragrance. Analysis of the oil by GC and GC-MS resulted in forty eight compounds representing 82.11%. The results are viewed in Table 1 and Figures 2,3, 4and 5 show the main components, Figure 3, of volatile oil were phenol, 2,4-bis(1-methyl-1-phenylethyl) (41, 13.46%), 2,4-bis(dimethylbenzyl) -6-t-butylphenol (42, 8.19%), dibutyl phthalate (27, 5.52%), nonadecane (38, 4.57%), phenol, 2,6-bis(1,1-dimethylethyl)-4-(1-methyl-1-pheny lethyl) (29, 4.39%), ethyl iso-allocholate (31, 4.10%) and heptadecane (35, 4.03%).Some minor components, Figure 4, of volatile oil were isolated, such as butylatedhydroxytoluene (14, 1.53%), Phenol,2-(1,1-dimethylethyl)-4-(1,1,3,3-tetramethylbutyl) (21, 1.0%), 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester (23, 0.9%), 1-Hexadecanol (24, 1.03%), Phenol,-(1,1-dimethylethyl)-4-(1-methyl-1-phenylethyl) (28, 2.89%), phytol (30, 2.55%) and eicosanoicacis (32, 1.65%). There is no previous work on the volatile oil of this plant in Saudi Arabia. So, it is the first time to isolate components of volatile oil and report from *Parkinsonia aculeate*.

Results and discussion of biological activity

Antimicrobial activity

Antimicrobial screening for the essential oil of *P.aculeata* was assessed against Gram +ve bacteria

(*Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus* ATCC 12228 and *Staphylococcus epidermidis* ATCC 12228) and Gram –ve bacteria (*Pseudomonas aeruginosa* ATCC 27853, *Escherichia. coli* ATCC 10536 and *Escherichia. coli* ATCC25922) as well as fungi (*Aspragillus niger*ATCC 16404 and *Candida albicans* ATCC 10231) at a dose of 50mg/ml tested by determination of the zone of inhibition, Table 2. It was found to be strong activity against *E.coli*, *S.pneumonia*, *A.fumigates* and *S. racemosum* while the others showed no activity when compare to control standard. Fortunately, the crude extract of *P.aculatea* leaves showed maximum antibacterial activity against *E.coli* with zone of inhibition 23±0.02mm at concentration of 500mg/ml and *P.aeruginosa* with zone of inhibition 21.12±0.033mm at 10mg/disc concentration (ShrivastaveR et al., 2011). Hexane and methanol extracts of *P. aculeata*was found to be active against various bacteria at concentration of 400 ug/ml (Ali et al., 1999). In the other study, crude ethyl alcohol, pet. Ether and chloroform extracts of the leaves of *P. aculatea* were found to inhibit many bacteria. MIC of the crude extracts were determined ranged between 35-50 mg/ml (Kamba and Hassan, 2010). Earlier reports suggested that plants containing phenolic compounds like tannins, therefore, the principle active compounds detected may be responsible for antibacterial activity of the tested organisms (Kamba and Hassan, 2010), which that confirm our results and this may be due to the presence of phenolic components in the essential oil of this plant.

Anti-oxidant activity (DPPH free radical scavenging activity)

The aim of the present study was to examine the antioxidant capacity of the essential oil of *P. aculeate*and the oil showed no DPPH scavenging activity. However, the antioxidant activity of the 70% hydroalcoholic extract of *P.aculeata* was evaluated *in vitro* by various experimental parameters such as DPPH radical scavenging activity, nitric oxide scavenging, β-carotene-linoleic acid module system, hydroxyl radical scavenging activity and lipid peroxidation. The extract successfully reduced ferric ions and its total phenolic content was determined (Mruthunjaya and Hukkeri, 2008).

CONCLUSION

The plant *P. aculeata* has been widely used in various traditional system of medicine. The plant is also an important source of various types of compounds with diverse chemical structures. However, very little study has been done on the chemical constituents of the plant specially in Saudi Arabia. So, there is a wide scope for investigation of more activities from the compounds isolated from the plant. Also since it was reported that the species can grow in presence of heavy metals, *P. aculeata* can be cultivated in polluted areas where it can resist heavy metals toxicities (Shaukat et al., 1999).

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