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Chemical composition and antimicrobial activity of the essential oil and lipid content of *Carduus pycnocephalus L.* growing in Saudi Arabia

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ABSTRACT

The essential oil of the air dried aerial parts of Carduus pycnocephalus L. F. Asteraceae was prepared by hydro-distillation. GC/MS analysis revealed the presence of nineteen components representing 100% of the total oil. Hexadecanoic acid (39.62%) was the prominent component of the oil. GC/MS analysis of the unsaponifiable matter of the petroleum ether extract of the aerial parts of the plant revealed the presence of nineteen compounds; sixteen compounds were identified constituting 72.80% of total unsaponifiable matter. olean-12-en-3-α-ol (20.39%), ursa-9(11), 12-dien-3-ol (17.74%) and hexadecanoic acid (17.62%) were the major components. GC/MS analysis of fatty acid methyl ester showed the presence of twenty fatty acids methyl esters (91.38%). The main known components were 1,2benzendicarboxylic acid dimethylester (31.08), palmitic acid methyl ester (20.08%) and azelaic acid dimethyl ester (7.60%). The antimicrobial, antispasmodic and anti-inflammatory effects were studied. The volatile oil and petroleum ether extract showed no antimicrobial activity, while the petroleum ether extract showed antispasmodic, anti-inflammatory effects. This is the first study on chemical composition and biological activities of the volatile oil and lipid contents of Carduus pycnocephalus L. endemic for Saudi Arabia.

Key words: chemical composition, antimicrobial activity, essential oil, lipid content, *carduus pycnocephalus l.* , growing in saudi arabia.

INTRODUCTION

One of the major plants growing in the Mediterranean region is that of aromatic plants which contain essential oils. These plants have the capacity to synthesize and emit volatiles that may act as aroma and flavour molecules. These low molecular weight substances derived from the fatty acids, amino acids and carbohydrates pools, constitute a heterogeneous group of molecules with saturated and unsaturated straight chain, branched chain and cyclic structures bearing various functional groups (e.g. alcohols, aldehydes, ketones, esters and ethers) and also sulfer and nitrogen [1]. Many of the essential oils are frequently used in aromatherapy and massage to obtain many clinical benefits, traditionally ascribed to their antibacterial, antifungal, carminative, anticonvulsant, sedative and antidepressant actions [2]. Genus *carduus* which belongs to the family Astraceae includes approximately 100 species worldwide [3] and is widely distributed around the Mediterranean is presented in Saudi Arabia by two species *C. Pycnocephalus* and *C. Arabicus*, they are widely spread in central, eastern and northern regions of Saudi Arabia [4]. In Chinese folk medicine, the plants of genus *Carduus* are used for the treatment of various human diseases such as cold, stomachache as well as rheumatism [5]. Genus *carduus* was found to possess a wide range of biological activities such as liver tonic, anti-inflammatory, antispasmodic, anticancer, antiviral and antibacterial [5-8]. Phytochemical studies on several *Carduus* species were carried out and revealed the presence of several classes of chemical constituents as lignans [7], polyacetylene [10], flavonoids [6,12-18], Coumarin [6,19], alkaloids [7, 20-21], sterols and triterpenes [18, 22]. The literature survey present on *C. Pycnocephalus* showed that only flavonoids were reported in the Egyptian plant [11-12, 16], in addition previous investigation of the essential oils of *C.*

pycnocephalus plant growing wildly in Iran showed the presence of twenty nine components representing 83.4% of the total oils with hexadecanoic acid (23.3%) as main constituent [5]. To our knowledge no biological or phytochemical studies were performed on Saudi plant which encouraged us to study in the present paper detailed analysis of its essential oil and lipid content as well as the biological activities.

EXPERIMENTAL SECTION

Collection of plant material

The fresh plant was collected from Al-Hada (Saudi Arabia) on March, 2008 and was kindly identified by Dr. Jakob Thomas, College of science, KSU. A voucher specimen (#15106) was deposited at the herbarium of department of pharmacognosy, College of Pharmacy, King Saud University.

Isolation of essential oil

The air dried aerial parts of *C. Pycnocephalus* (500 g) were subjected to hydro distillation in glass Clevenger – type apparatus for 4 h to obtain the yellowish oil (0.03% w/w). The volatile oil was collected and dried over anhydrous sodium sulphate and stored in amber – coloured vial at 4 ^{0}C until analysis.

Preparation of lipoidal matter:

500 grams of the air dried aerial parts of *C. pycnocephalus* were exhaustively extracted with petroleum ether in continuous extraction apparatus, the extract was evaporated under vacuum to yield 8 g of dry extract.

Preparation of unsaponifiable matter [23-24]:

One gm of the residue of petroleum ether fraction of *Carduus pycnocephalus* was saponified by reflux with 100 ml of alcoholic KOH for 5 hrs. The alcohol was distilled off almost to dryness. The obtained residue was suspended in 100 ml water and extracted with chloroform till complete extraction of unsaponifiable matters. The combined chloroformic extract was washed with distilled water to remove any alkalinity, dried over anhydrous sodium sulphate and evaporated to yield (0.4 g).

Preparation of the fatty acids methyl esters [25]:

The alkaline aqueous layer remained after extraction of unsaponifiable matters was acidified with dilute hydrochloric acid. The liberated fatty acids were extracted with successive portions of ether. The combined ethereal extract was washed with distilled water, dried over anhydrous sodium sulphate, evaporated to dryness and subjected to methylation using methanol and dry concentrated sulphoric acid to yield (0.52 gm).

GC/MS analysis conditions

GC/MS analysis was performed in Kuwait University. The analysis of volatile constituents was run on high resolution gas chromatography mass spectrophotometer-double focusing sector (GC/MS DFS), on TR-5 (Thremo)-capillary column (30m, 0.25 ID, film thickness of 0.25 µm), material packing was poly silxane. Helium was used as a carrier gas at flow rate 0.8ml/min. Injector temperature was 300 ^{0}C (split less mode), injection volume was 1µl. Column Ramp: initial temp - 40 (Hold time 5 min), Ramp 1: 10 /min till 210 (hold time 8min), Ramp 2: 10 C till 300 (hold time 15 min), total time was 54 min, transfer line temperature was 270 ^{0}C. The mass spectrophotometer was electron impact mode, electron energy was -70.1 eV, and emission current was 1 mA, source temperature: 175 ^{0}C, filament current: 2.80 A, scan range: 50-900Da, scan range: 1.1, mass analyser was Magnetic sector, mass detector was electron multiplier.

Identification of the compounds

Identification of the essential oil components was based on GC retention indices relative to *–n-* alkanes and computer matching with Wiley 275 L library as well as by comparison the fragmentation patterns of their mass spectra with those reported in the literatures [26-27]. The relative percentage of the oil constituents was calculated from the GC peak areas. Identification of the components of hydrocarbons, sterols and fatty acids methyl esters was carried out by comparing their relative retention times and mass spectra with the available reference compounds. The percentage was based on peak area integration and internal normalization.

Biological activity

Antimicrobial activity test [28-29]

The antimicrobial activity of the essential oil was tested according to the National committee of Clinical Laboratory standards (NCCLS) using American Type of Culture Collection (ATCC) against various microorganisms namely; *Escherichia coli* (ATCC 25922), *Staphylococcus aereus* (ATCC 25923), *Bacillus subtillus* (ATCC 6633), *Candida albicans* (ATCC 10231) and Pseudomonus aeruginosa (ATCC 27853) strains [28-29]. The positive antimicrobial

and antifungal activity were established by the presence of measurable zone of inhibition after 28 hrs incubation period.

Anti-inflammatory activity [30]

In order to evaluate the anti-inflammatory activities, the formaldehyde-induced paw oedema is tested on male Westar rats weighing 180-230 g formaldehyde will be injected in to two groups (two animals each). Group (a) serves as negative control saline, group (b) petroleum ether extract group. The extract was suspended with 0.25% sodium carboxy methyl cellulose and injected at a dose of 500 mg/kg intra-peritoneal one hour before formaldehyde injection. Edema is induced by injecting formaldehyde solution into the rat hind paws.

Statistical analysis

The volume of the rat paws is measured prior formaldehyde injection and then two hours afterwards using a – Hydro-Plethysmograph. The rats held firmly and the right paw will be immersed into the pool of mercury up to the tibiotarsic articulation. The pressure increase due to the slight rise in mercury level is transmitted to the pressure transducer. After amplification the transduced signal is increased as a deflection in the pen on the chart. The % inhibition of edema are expressed as the difference between the final and the initial right hind paw volume (V_i- V_f) two hours after formaldehyde injection in comparison with the control group**.**

Antispasmodic effect [31-32]

White male New Zealand rabbits (2.5 Kg) were killed and pieces of the jejunum (2.5 cm long) were cut and suspended in oxygenated Kerb's solution at 37 ^{o}C as described by. To test the effect of the different extract on the spontaneously contracting jejunum, separate different volumes of suspended extract were added individually to the tissue and allowed to contact it for 4 minutes to record any effect. Then the extract was washed out and the tissue was allowed rest for 10 min when another dose was tested. Each tissue was used to test one extract only.

RESULTS AND DISCUSSION

Hydro distillation of the air dried aerial parts of *C. Pycnocephalus* afforded nineteen constituents representing 100% of the oil (Table 1) The main components were hexadecanoic acid (39.62%), 9,12- linoleic acid (19.46%), 1,2 benzendicarboxylicaciddiisooctylester (7.11%) and phytol isomer (6.31%). The chemical profile of the studied volatile oil (Table 1) was compared with the previously reported one (Table 2). Although the main component was similar, the result of GC/MS analysis indicated that the chemical composition of the two volatile oils were differed quantitatively and qualitatively. The reported oil showed 29 constituents corresponding to 83.4% of the total essential oil. While Saudi plant volatile oil contains 19 components (100 %). The major component of both was hexadecanoic acid (23.3 % Iran) and (39.62% Saudi Arabia), while compounds such as 9, 12- linoleic acid (19.46%), 1,2benzendicarboxylicaciddiisooctylester (7.11%), linolenic acid ethyl ester (4.84%), hexadecanoic acid ethyl ester (4.81%), Musk xylene (1.31%), 2- pentadecanone, 6, 10, 14-trimethyl (0.8%) and hexadecanoic acid methyl ester (0.64%), were present in volatile oil of Saudi plant only. It is well known that the cultivation environments, weather condition, region, harvesting season and extraction method are from the reasons that lead to variation in the fragrance composition of the plants. And at least the first three reasons are involved in our case which led to this variation. GC/MS analysis of the unsaponifiable matter, Table 3, of the petroleum ether extract of the aerial parts of the plant revealed the presence of nineteen compounds; sixteen compounds were identified constituting 72.80 % of total unsaponifiable matter. Olean-12-en-3-ol (20.39%), n-hexadecanoic acid (17.62%) and ursa-9 (11),12-dien-3-ol (17.74%) were the major components. By GC/MS analysis of the fatty acid methyl ester, twenty fatty acids were identified in the extract, which represented about 91.38% of the total fatty acid composition (Table 4). The fatty acids were consisted of five unsaturated fatty acids (52.23%) and fifteen saturated fatty acids (39.13%). 1,2-benzenedicarboxylic acid, dimethyl ester (31.08%), Palmetic acid (20.08%), 1,2- benzenedicarboxylic acid, diisononyl ester (18.46%) and azelaic acid (7.60%) were the major components. Our *in vitro* experiments revealed that the petroleum ether extract showed dose dependent antispasmodic effect. At a dose of 500 mg/kg the petroleum ether extract induced 30% edema reduction after two hours from formaldehyde injection. The essential oil showed antimicrobial effect against *Bacillus subtillus*, *Staphylococcus aereus* and *Mucobacterium smegmatus* with minimum inhibitory concentrations 1, 5 and 5mg/ml respectively at concentration of 5mg/disk .

In conclusion, our study indicates that the volatile oil and fatty acid methyl ester constituents isolated from *C. pycnocephalus* contain large amount of different phethalate compounds such as diisobutylphethalate (0.67%), Dibutylphethalate (1.56%) and 1,2-benzendicarboxylic acid diisooctylester (7.11%) from volatile oil as well as 1,2-benzenedicarboxylic acid, dimethyl ester (31.08%), 1,2-benzenedicarboxylic acid, 1-ethyl 2-methyl ester (0.83%) and 1,2- benzenedicarboxylic acid diisononyl ester (18.46%) from fatty acid methyl ester.

Table 1: Chemical composition of essential oil of *Carduus pycnocephalus L.* growing in Saudi Arabia

No.	Name of compound	RI	Percentage
1	Tetradecanoic acid	1042	2.74
2	Musk xylene	1043	1.31
3	6,10,14 trimethyl -2-pentadecanone	1044	0.8
4	diisobutylphethalate	1044	0.67
5	1-Hexadecanol	1046	0.62
6	Hexadecanoic acid methyl ester	1048	0.64
7	Dibutylphethalate	1049	1.56
8	Hexadecanoic acid ethyl ester	1051	4.81
9	Hexadecanoic acid	1055	39.62
10	2-9-Octadecanoic acid	1058	2.02
11	Methyl linolenate	1058	1.2
12	Phytol isomer	1060	6.31
13	Ethyl linolate	1064	1.47
14	Linolenic acid ethyl ester	1065	4.84
15	9,12-linoleic acid	1069	19.46
16	Pentacosane	1095	0.62
17	1,2benzendicarboxylic aciddiisooctylester	1096	7.11
18	Heptacosane	1100	2.34
19	Nonacosane	1104	1.86
	Total		**100**

RI = retention index

Table 2: Chemical composition of essential oil of *Carduus pycnocephalus L.* Growing in Iran [5]

No.	Compound	RI	Percentage
1	(E)-β-lonone	1485	1.0
2	δ-cadenen	1524	0.4
3	1-butyl hexylbenzene	1533	0.7
4	1-propyl heptyl benzene	1542	0.5
5	Elemol	1549	2.1
6	1-ethyl octylbenzene	1560	0.4
7	Dodecanoic acid	1570	3.0
8	Caryophyllene oxide	1581	1.6
9	Hexadecane	1600	0.9
10	1-pentyl hexyl benzene	1629	1.1
11	1-butyl hexylbenzene	1630	2.6
12	1-propyl octylbenzene	1640	2.1
13	B-eudesmol	1649	2.7
14	α- cardinal	1653	1.7
15	Heptadecane	1700	0.6
16	1-methyl decylbenzene	1704	1.9
17	1-pentyl heptylbenzene	1730	3.2
18	1-butyl octylbenzene	1736	2.9
19	1-propyl nonyl benzene	1747	2.1
20	Tetradecanoic acid	1770	4.3
21	Octadecane	1800	0.5
22	1-pentyl octylbenzene	1830	3.7
23	1-butyl nonylbenzene	1835	2.3
24	6,10,14 trimethyl -2-pentadecanone	1846	7.4
25	1-propyl decylbenzene	1849	1.5
26	Nonadecane	1900	0.4
27	Dibutyl,1,2-benzene dicarboxylate	1967	8.2
28	Hexadecanoic acid	1973	23.3
29	Eicosane	2000	0.3
	Total		**83.4**

RI = retention index

Since plants have a rich source of these bioactive chemicals, they can be an alternative source of control agents for mosquito larvae. Recently, plants belonging to the family Asteraceae have been reported as potential sources for mosquito control [33-34]. Also these compounds had previously been isolated from marine brown algae [35-36], as well as from plants *Euphorbia pulcherrima* [37] and *Pterocarpus angolensis* [38] and it has been reported to exhibit antibacterial activity [36].

Table 3: Chemical composition of unsaponifiable matters of *Carduus pycnocephalus L.* growing in Saudi Arabia

No.	Name of compound	R_t	Relative R_t	%	B.P	M^+
1	1-hexadecene	17.79	0.15	0.22	55	224
2	1- Octadecene	20.03	0.1	0.48	57	252
3	2-pentadecanone, 6, 10, 14-rimethyl	20.57	0.13	1.73	58	268
4	2-nonadecanone	21.26	0.13	0.3	71	282
5	n-Hexadecanoic acid	22.26	0.74	17.62	73	256
6	1-octadecene	23.16	0.1	0.27	57	252
7	Phytol isomer	23.46	0.15	0.98	71	296
8	Octadec-9-enoic acid	24.1	0.3	1.08	55	282
9	Octadecanoic acid	24.51	0.23	1.07	57	284
10	Unknown	25.13	0.26	0.47	-	-
11	Tricosane	26.68	0.17	0.34	57	324
12	Pentacosane	31.78	0.28	1.49	57	352
13	Unknown	32.67	0.69	25.98	-	-
14	Heptacosane	34.93	0.41	4.58	57	380
15	Octacosane	36	0.28	0.56	57	394
16	Nonacosane	37.18	0.36	3.95	57	408
17	Unknown	38.12	0.2	0.74	-	-
18	Ursa-9(11), 12-dien-3-α-ol	40.27	2.38	17.74	424	424
19	Olean-12-en-3-α-ol	42.25	8	20.39	218	426

Rt = retention time *B.P = base peak* *M^+ = molecular ion peak*

Table 4: Chemical composition of fatty acids methyl esters of *Carduus pycnocephalus L.* growing in Saudi Arabia

Peak No.	Name of compounds	R_t	%	B.P	M^+
1	Butanedioic acid, dimethyl ester	8.11	1.39	119	146
2	Benzoic acid methyl ester	9.36	0.42	136	136
3	Pentanedioic, dimethyl ester	10.51	0.25	100	160
4	Adipic acid dimethyl ester	13.01	1.53	59	174
5	Pimelic acid dimethyl ester	15.25	0.38	55	188
6	1,2-benzenedicarboxylic acid, dimethyl ester	17.8	31.08	163	194
7	1,2-benzenedicarboxylic acid, 1-ethyl 2-methyl ester	18.97	0.83	163	208
8	Azelaic acid dimethyl ester	19.64	7.60	55	216
9	Decanedioic acid, dimethyl ester	21.47	0.95	55	230
10	Tetradecanoic acid methyl ester	22.9	1.09	74	242
11	Undecanedioc acid dimethyl ester	23.33	0.81	74	244
12	Tetradecanoic acid, 12 methyl- methyl ester	24.17	1.11	74	256
13	Pentadecanoic acid methyl ester	24.68	0.42	74	256
14	Dodecanedioic acid dimethyl ester	25.09	0.21	98	258
15	Palmitic acid methyl ester	26.52	20.08	74	270
16	9-octadecenoic acid methyl ester	29.15	1.44	55	296
17	Isostearic acid methyl ester	29.61	2.63	74	298
18	Arachidic acid methyl ester	32.51	0.49	74	326
19	Adipic acid di(2-ethylhexyl)ester	33.35	0.21	129	370
20	1,2- benzenedicarboxylic acid diisononyl ester	39.09	18.46	149	418

Rt= retention time *B.P = base peak* *M^+ = molecular ion peak*

Table 5: anti-inflammatory effect of petroleum ether extract of *C.pycnocephalus*

Extract mg/kg*	Paw volume ml 2hrs	Edema reduction % After 2 hrs
control	**1.13**	**-----**
Pet.ether 500mg/kg	**0.78**	**30**

**rats number n=3*

Table 6: antispasmodic effect of pet.ether extract of *C.pycnocephalus*

	Dose mg/ml of tissue bath fluid (Tyroid's solution)	
Pet.ether extract	**10**	**15**
%inhibition of spontaneously contraction rabbit jejunum	**11.9**	**23.8**

Table 7: antimicrobial activity of the essential oil of *C.pycnocephalus*

Micro-organisms	MIC of volatile oil (5mg/ml)
Bacillus subtillus	**1**
Staphylococcus aereus	**5**
Escherichia coli	**na**
Pseudomonus aeruginosa	**na**
Mucobacterium smegmatus	**5**
Candida albicans	**na**

n.a. =no activity

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