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Chemical constituents and radical scavenging activity of *Cuscuta pedicellata* seed extracts

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Abstract : The seeds oil of *Cuscuta pedicellata* was extracted with pet. ether in a Soxhlet for two days and it's constituents were identified using GC/MS analysis. It was found that, the lipid constituents of pet. ether was found as oily residue which saponified to afford the unsaponifiable materials (saturated hydrocarbons, sterols and triterpenes) and 15 fatty acids which were identified by GC/MS analyses. The flavonoids were isolated from the ethyl acetate fraction and identified as: : Genkwanin, Astragalin, kaempferol and quercetin. The antioxidant activity of different extracts (pet. ether, unsap., fatty acids, 70% methanol, chloroform and ethyl acetate) were evaluated.Antioxidant properties were determined using the2,2-diphenyl-1-picryl-hydrazyl (DPPH) free radical. It was observed that methanol extract exhibited highest DPPH activity followed by ethyl acetate extract.
Key words : *Cuscutaceae, Cuscuta ,* lipid constituents, flavonoids and antioxidant activity.

Introduction

Cuscuta is a genus of about 100 species parasitic plants, formerly it belongs to family *Cuscutaceae* but now belonging to family *Convolvulaceae*, it represented in Egypt by seven species in which *C. pedicellata* is the most common parasite on *Trifolium alexandrinum*[1-2].The plants of this genus have many biological activities and different chemical constituents as reported by several investigators like Gupta in 2010 [(3)] who stated that, *C. reflexa* have medicinal propertie as purgative, used in flatulence, liver complaints, externally for itch, used in wash of sores, to be spasmolytic, plant extract is applied to get rid of dandruff and anti-bacterial. Many chemical constituents have been isolated such as: kaempferol, myricetin, quercetin, coumarin cuscutin, amarbelin, β-sitosterol, stigmasterol, dulcitol, and oleanolic acid [4-7].

Fozia et al[8] proved that, aqueous solvent of 80% methanol and 80% ethanol extracts of *Cuscuta* stems exhibited better antioxidant activities due to higher phenolic contents as well as reducing power. Antioxidant activity was found complimentary to the concentration of total phenolic content and reducing power of the extract. Guo et al in 1997[(9)] studied the flavonoidal constituents of *C. australis* seeds and they have been characterized three flavonoids as astragalin, kaempferol and quercetin, in addition to lacceroic acid, β-sitosterol and β-sitosterol-3-*O*-xyloside .

Five flavonoids known as: quercetin-3-*O*-apiosyl-(1→2)-galactoside, hyperoside, kaempferol-3-*O*-glucoside, kaempferol, quercetin, and chlorogenic acid, have been isolated by Shaobin et al[10] in 2014 from the seeds of *C.chinensis*. While, Mahmoud et al in 2015[(11)] proved the anticancer activity of flavonoidal extract from *C. kotschyana* using breast cancer cell line (MCF-7) and Jafarian et al[(12)] concluded that extracts of *C.chinensis* and *C. epithymum* exhibited cytotoxicactivity against Hela, HT-29 and MDA- MB-468 cells. Also, their chloroform extracts are more potent than hydroalcoholic extracts.

Isorhamentin 3-*O*-neohesperidoside was isolated with flavonol glycoside aromandendrin and taxifolin from *C. reflexa* by Dandapani et al., in 1989[13].

It was reported that, the level of phospholipid in the parasite *C. reflexa* was always higher than in phospholipid in the host, phosphatidyl inositol (20%) and phosphatidyl glycerol (12%). Phosphatidic acid constituted only 3% of the phospholipids[14].

Amna et al in 2014[15] reported that, the stem extracts of *C. pedicellata* exhibited antibacterial effect on *X. campestris*, *B. subtilis* and *St. aureus*, *E. coli* and *Ps. aeruginosa*, fungi (*P. citrium* and *As.niger*). Also it was found that, the extract of stem of *Cuscuta sp*. contain alkaloids, carbohydrates, some glycosides, flavonoids, tannins, phenolic compounds, and steroids[16].

In this study we will investigate the oil, lipid constituents, flavonoids and antioxidant activity of different extracts from *Cuscuta pedicellata* seeds.

Materials and methods

Plant material*:*

The seeds of *Cuscuta pedicellata* were collected in May 2015 after harvesting of *Trifolium alexandrinum* from Al-Sharqia governorate, dried in shade and grinding to fine powder. The seeds were kindly, identified at Nat. Cent. of Agric. Res. and the voucher specimen was deposited at NRC herbarium.

Extraction of the oil and lipid constituents*:*

About 2.20 kg of dried powdered seeds were extracted with petroleum ether (b.r. 40-60 °C) in a Soxhlet apparatus. The pet. ether extract was passed through fuller's earth, filtered, dried over anhydrous sodium sulphate and evaporated in *vacuo* at 40 °C till dryness to give an yellow oily residue (2.8% v/w) and kept in refrigerator to GC/MS analysis. About 5 g of the oily residue were subjected to saponification process to afford the unsaponifiable materials and fatty acid methyl esters.

Extraction and isolation of flavonoidal constituents:

About 2.0 kg of defatted powdered seeds were extracted with methylene chloride in a Soxhlet for 24 hrs. followed by maceration with methanol (70%, 3x2.5L). The combined methanol extract was evaporated *in vacuo* at 50˚ C till free from methanol and diluted with hot distilled water (600 ml). The aqueous methanolic was partitioned with ethyl acetate (400 ml x 3), the combined solvents was dried over anhydrous sodium sulfate and evaporated till dryness**.**

The ethyl acetate fraction was found to contain four flavonoidal spots (CI-CIV), three of them are aglycones and the fourth is a glycoside in nature (through their behavior on paper chromatography with different solvents, 15% AcOH and BAW 4:1:5). They were isolated by passing over Sephadex LH-20 column (3 x 60), eluted with 80% aqueous methanol and the fractions of 100 ml each were collected. The fractions containing these compounds in semipure form were polled together, after then were rechromatographed on many smaller Sephadex columns to afford compound **C-I** and **C-II** in pure form as yellowish powder (15 and 10mg respectively). The fraction containing compounds **C-III** and **C-IV** was subjected to preparative thick layer chromatography (PTLC) with cellulose plates developed with 15% acetic acid. Two bands were localized, scraped off, eluted with 95% methanol and further purified over Sephadex LH-20 column eluted with 90% methanol to afford compounds **C-III** and **C-IV** in pure form (12 mg and 14 mg respectively).

GC/MS analysis of oil*:*

The obtained oily residue was subjected to The GC-Ms analysis of the essential oil samples was carried out using gas chromatography-mass spectrometry instrument stands at the Laboratory of medicinal and aromatic plants, National Research Center, Egypt with the following specifications. Instrument: a TRACE GC Ultra Gas Chromatographs (THERMO Scientific Corp., USA), coupled with a THERMO mass spectrometer detector (ISQ Single Quadrupole Mass Spectrometer). The GC-MS system was equipped with a TR-5MS column (30 m x 0.32 mm i.d., 0.25 μm film thickness). Analyses were carried out using helium as carrier gas at

a flow rate of 1.3 mL/min at a split ratio of 1:10 and the following temperature program: 80 ºC for 1 min; rising at 4 ºC/min to 300 ºC and held for 1min. The injector and detector were held at 220 and 200 ºC, respectively.

Mass spectra were obtained by electron ionization (EI) at 70 eV, using a spectral range of *m/z* 40-450. Most of the compounds were identified using mass spectra (authentic chemicals, Wiley spectral library collection and NSIT library). The separated components of the essential oil were identified by matching with the National Institute of Standards and Technology (NIST) published as shown in Table 1.

GLC analysis of unsaponifiable matters and GC/MS of fatty acid methyl esters:

The GLC analysis was carried out using the following conditions; Instrument: Varian model 3700 GC. Column for unsap.: 10% OV-101 on chromsorb W/HP, 80/100, (2m stainless steel, 0.25mm i.d.), Column for fatty acid methyl esters:15% DEGS on chromsorb W/AW, 80/100, (2m stainless steel, 0.25mm i.d.), Temp.

for unsap:column:70°C up to 270°C, 4°C/min., injector: 280°C., Detector (FID):290°C. Temp. for fatty acid methyl esters: column:70°C up to190°C, 4°C/min., injector: 240°C, Detector :280°C, Flow Rates for both of them :N_2 and H_2: 30 ml / min., Air: 300 ml/min. The data were tabulated in Table 2&3.

DPPH Assay

The antioxidant capacity of the *cuscuta pedicellata* extracts was measured using a DPPH method described by Sun *et al.,*[17] using the free radical 2,2-diphyenyl-picrylhydrazyl (DPPH), with some minor revisions. Aliquots (0.1 mL) of diluted extracts in DMSO were added to 1 mL of DPPH solution and the absorbance of the DPPH solution was determined at 520 nm after 30 min of incubation at room temperature[18]. Appropriate blanks (DMSO) and standard (Trolox solution in DMSO) were used to compare the antioxidant capacity of *cuscuta pedicellata* extracts. All measurements were done in triplicate.The radical-scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the equation :

$$\%\,\mathrm{RSA} = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \mathrm{X100}$$

Results and Discussion:

Table 1. GC/MS data of *C. pedicellata* oil

Peak No.	R_t (min.)	%	Mass data			compounds
			MW	B.P.	fragments	
1	5.37	7.39	188	70	155(15),97(21),57(92)	2-methyl-2-undecanthiol
2	5.54	5.92	168	70	150(10), 83(72), 57(95)	(Z)-2-decenal
3	6.06	6.69	152	81	95(10), 67(20), 41 (40)	(E,E)-2,4-decadienal
4	6.34	14.39	296	57	211(5), 197(5), 127(100)	Heneicosane
5	6.48	4.12	182	41	138(5), 97(19), 83(58)	(E)-2-dodecenal
6	7.09	3.71	172	74	141(18), 129(17), 87(55)	9-oxo-nonanoic acid methyl ester
7	7.61	15.19	503	281	415(32),327(42), 73(81)	Cycloheptasiloxane tetradeca methyl
8	7.82	6.40	220	205	177(15), 145(29), 91(34)	Butylated hydroxytoulene(BHT)
9	11.18	5.15	268	58	250(14),109(32), 71(45)	6,10,14-trimethyl-2-pentadecanone
10	12.13	15.64	270	74	227(19), 143(35), 87(81)	Methyl palmitate
11	12.50	4.82	256	43	213(15), 171(10), 60(90)	Palmitic acid
12	14.15	6.14	296	55	264(10), 96(32), 74(69)	Methyl olieate
13	14.34	5.44	298	74	255(15), 199(10), 87(70)	Methyl stearate
14	18.19	5.69	390	149	279(21), 167(33), 57(42)	Diisooctyl phthalate

Table 2. GC /MS data of FAME fraction of *C. pedicellata.*

Peak No.	R_t (min.)	Rel. %	Mol. formula	Mol. Wt.	compounds
1	23.38	0.70	$C_{15}H_{30}O_2$	242	Methyl myrestate , n-C 14(0)
2	26.04	0.40	$C_{16}H_{32}O_2$	256	Methyl pentadecanoate , n-C 15(0)
3	28.04	3.88	$C_{17}H_{32}O_2$	268	Methyl palmitoleate , n-C 16(1)
4	28.67	24.79	$C_{17}H_{34}O_2$	270	Methyl palmitate , n-C 16(0)
5	31.01	0.58	$C_{18}H_{36}O_2$	284	Methyl margarate , n-C 17(0)
6	32.64	19.52	$C_{19}H_{34}O_2$	294	Methyl linoleate , n-C 18(2)
7	33.12	14.05	$C_{19}H_{36}O_2$	296	Methyl oliate, n-C 18(1)
8	33.47	13.57	$C_{19}H_{38}O_2$	298	Methyl stearate, n-C 18(0)
9	35.55	0.39	$C_{20}H_{40}O_2$	300	Methyl nonadecanoate, n-C 19(0)
10	36.36	1.52	$C_{19}H_{32}O_2$	292	Methyl octadecatrienoate , n-C 18(3)
11	37.14	7.60	$C_{21}H_{40}O_2$	312	Methyl gadoleate, n-C 20(1)
12	39.13	0.77	$C_{22}H_{36}O_2$	346	Methyl docostetrenoate, n-C 22(4)
13	41.81	9.82	$C_{23}H_{46}O_2$	354	Methyl behenate, n-C 22(0)
14	43.67	0.92	$C_{24}H_{48}O_2$	368	Methyl tricosanoate, n-C 23(0)
15	45.52	2.08	$C_{25}H_{50}O_2$	382	Methyl lignocerate , n-C 24(0)

The data in table 2 showed that the mixture of fatty acid methyl esters consists of fifteen acids in which palmitic acid C-16(0) is the main acid (24.79%), also it was found that the saturated fatty acids constitute 52.95% while the unsaturated acids are 46.95% in which monounsaturated are 25.43%, diunsaturated are 19.32% and the poly unsaturated are 2.29%.

Table 3. GC /MS data of the unsap. fraction of *C. pedicellata.*

Peak No.	R_t (min.)	Rel. %	Compounds
1	25.17	1.38	Eicosane, n-C 20
2	41.10	17.40	phytol
3	45.23	0.62	heneicosane , n-C 21
4	47.30	1.01	tetracosane, n-C 24
5	48.29	2.18	pentacosane, n-C 25
6	51.21	2.15	Hexacosane, n-C 26
7	53.67	3.63	Heptacosane, n-C 27
8	54.37	0.24	isononacosane, C 29
9	54.86	1.80	octacosane, n-C 28
10	56.59	4.68	nonacosane, n-C 29
11	58.27	1.02	tricontane , n-C 30
12	59.89	1.19	hentricontane, n-C 31
13	60.16	0.46	Cholesterol
14	60.80	2.14	ergosterol
15	61.86	7.64	campasterol
16	62.24	8.81	Stigmasterol
17	63.22	40.14	β- sitosterol
18	63.81	2.23	α- amyrine
19	64.58	1.01	betulin
20	66.24	0.27	Lupeyl acetate

The GC /MS analysis of the unsap. fraction (table 3) revealed the presence of a mixture of n-hydrocarbons (37.3%)in which phytol is the most abundant (17.4%), sterol fraction (59.19 %) contain five compounds with β-sitosterol as a main sterol (40.14%) and a triterpene fraction (3.51%) with α- amyrine (2.23%) as a major one.

Four flavonoidal compounds were isolated from the ethyl acetate fraction of aqueous methanol extract which were identified as follow:

Compound C-I, *Genkwanin*:

This compound was obtained as a pale yellow amorphous powder and have low R_f value in 15% AcOH which confirm it's aglycone nature. The UV absorption spectra showed λ_{max} in methanol at 336 and 268 nm confirming the flavone type structure. The other shifts reagents with $AlCl_3$, NaOAc and H_3BO_3 proved the absence of an orthodihyroxy system and absence of a free OH group at C-7. The EI-MS displayed a molecular ion peak M^+ at *m/z* =284 which related to the molecular formula $C_{16}H_{12}O_5$. The other data of NMR measurements confirmed the presence of a methoxy group at C-7, so, compound C-I could be identified as Genkwanin[19-20].

Compound C-II, *Astraglin* :

The compound was isolated in pure form as amorphous deep yellow colour, which is glycosidic in nature through it's behavior on paper chromatography and a flavonol type structure, where it's spectra in UV displayed λ_{max}(MeOH) at 356.5, 269 nm, in addition to the presence of a free OH group at C-7 and occupation at C-3 through the other shift reagent. The EI-MS gave M^+ at *m/z*= 448 which calculated to the molecular formula $C_{21}H_{20}O_{11}$. The ^{1}H-NMR in DMSOd6 displayed δ at: 6.25(1H,d,*J*=2.1,H-6), 6.49(1H,d,*J*=2.1,H-8), 6.92(2H,d,*J*=8.4,H-3', H-5'), 8.07(2H,d,*J*=8.4, H-2',H-6') and the anomeric proton of the sugar moiety was assigned for glucose at 5.2 ppm, the other data of NMR were shown in table 4.

The acid hydrolysis proved that, the aglycone is kaempferol and the sugar is glucose using PC and authentic samples and the presence of the glucose at C-3 through the UV spectra of the aglycone. So the compound **C-II** was identified as kaempferol 3-*O*-glucoside= Astragalin [21-22].

Compound C-III, *kaempferol* :

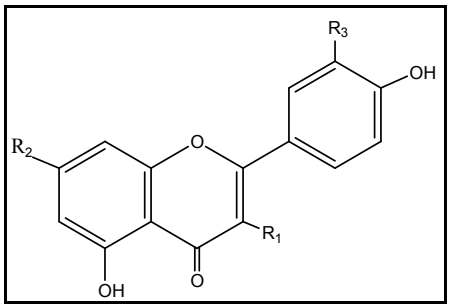
It was obtained as a yellow powder of a flavonol aglycone in nature where it displayed in UV spectra band –I at λ_{max}=367 nm, in addition to free OH groups at C-3 and C-7. The EI-MS gave M^+ at *m/z*= 286 269(M^+-OH), 241(M^+-[CO+OH]) and 153(A^{+1}). The NMR data were in accidence with that reported in the literature [21] and the compound **C-III** identified as kaempferol.

Compound C-IV, *quercetin*:

The compound was isolated as a yellow powder, UV (λ_{max} MeOH): band-I at 370 nm and the other shift reagent confirmed the presence of an orthodihydroxy system in ring B, in addition to free OH groups at C-3 and C-7. The EI-MS , M^+ at *m/z*= 302 correspond to the molecular formula $C_{15}H_{12}O_6$. The NMR data were in accidence with that reported in the literature[21,23] and the compound **C-IV** identified as quercetin.

Table 4. ^{13}C-NMR data for Compounds C-I to C-IV.

Carbon No.	δ in ppm			
	Compound C-I	**Compound C-II**	**Compound C-III**	**Compound C-IV**
2	164.0	161.1	148.0	156.2
3	103.0	133.1	137.2	133.0
4	181.9	177.3	177.3	178.2
5	157.2	156.1	162.5	161.5
6	97.9	98.7	99.2	93.6
7	165.1	164.4	165.6	164.2
8	92.6	93.6	94.4	98.4
9	161.2	156.4	158.2	157.0
10	104.6	103.8	104.5	108.7
1'	121.0	120.8	124.2	122.5
2'	128.3	130.8	160.0	129.5
3'	115.9	115.0	146.2	115.3
4'	161.3	159.7	148.8	159.3
5'	115.9	114.9	116.2	115.5
6'	128.5	130.6	121.7	129.5
7-OCH_3	56.0	-	-	-
1"		100.5		
2"		73.9		
3"		76.1		
4"		69.5		
5"		77.3		
6"		60.5		



1- R_1=H, R_2=OCH_3, R_3=H (Genkwanin)
2- R_1=o-glucose, R_2=OH, R_3=H (Astraglin)
3- R_1, R_2=OH, R_3=H (Kaempferol)
4- R_1, R_2, R_3=OH (Quercetin)

Table 5. Radical scavenging activity of *Cuscuta pedicelata* seeds extracts.

Samples	Mean absorption	* % DPPH Inhibition ± S.D.
Trolox	0.013	97.3±0.45
Un sap.	0.358	23.15±0.45
Fatty acid	0.207	55.50± 0.53
Petroleum ether extract	0.160	65.66±0.83
Chloroform extract	0.143	69.30±0.77
Ethyl acetate extract	0.113	75.74±0.64
Methanol extract	0.076	83.57±0.84

Data are mean ± SD values.

*Expressed as % of neutralised DPPH free radicals

As the absorption at 520 nm decreased, the radical scavenging activity increased. The antioxidant activity of different extracts table 5 showed that, the highest antioxidant extract was methanol extract which may due it contain phenolic and flavonoidal compounds[24], while the oil (pet ether ext.) exhibited moderate activity which attributed to the presence of BHT. Finally, the lowest activity was associated with the unsaponifiable fraction.

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