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College of Pharmacy

GC mass anaylsis of Iraqi Orobanche aegyptiaca extract

A Thesis

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بسْمِ اللَّـهِ الرَّحْمَـٰنِ الرَّحِيمِ

﴿ وَيَسْأَلُونَكَ عَنِ الرُّوحِ قُلِ الرُّوحُ مِنْ أَمْرِ رَبِّي وَمَا أُوتِيتُمْ مِنَ الْعِلْمِ إِلَّا قَلِيلًا ﴾

سورة الإسراء (الآية ٨٥)

Dedication

-To my generous parents who did not deprive me of their prayers and optimism day and night whenever they saw me making an effort, who supported me throughout my life and encouraged me to complete my studies

- To the research supervisor, Dr. Noor Hamid, who overwhelmed us with appreciation, advice, instruction and guidance.

-To everyone who enlightened the mind of others with his knowledge and spared no effort in helping me .

Content

Introduction

Introduction

Orobanche L. belongs to the *Orobanchaceae* family, an entirely parasitic higher plant taxon, comprising around 133 species distributed all over the world[(1)].***Orabanche*** spp. Are holoparasites and only germinate in response to specific chemicals released by the host plant. Following germination, the seedlings attach to the host roots by the production of specialized feeding structures, described as haustoria that form a functional bridge into their hosts[(2)]. Egyptian broomrape attacks annual composites (members of the aster family, Asteraceae), although it occasionally attacks members of the legume family (Fabaceae) and other broad-leaved plants. Egyptian broomrape attacks a broad array of field crops and some ornamentals. Tomato, potato, tobacco, eggplant, peppers, peas, carrot, celery, mustard, spinach, and chrysanthemum are among the susceptible plants in areas around the Mediterranean, in central and eastern Europe, and in Asia[(3)].

Taxonomic Tree[4]

Domain
Eukaryota

Kingdom
Plantae

Phylum
Spermatophyta

Subphylum
Angiospermae

Class
Dicotyledonae

Order
Scrophulariales

Family
Orobanchaceae

Genus
Orobanche

Species
Orobanche aegyptiaca

Common Name

- Egyptian broomrape
- **Arabic Name**: halook

Description

O. aegyptiaca is closely comparable with ***O. ramosa*** but the plant is normally more robust, 20-30(-40) cm high, the flowers normally over 20mm long and anthers densely hairy[5]. The plant produces many slender, erect stems from a thick root. The yellowish stems grow 10 to 60 centimeters tall and are coated in glandular hairs. The broomrape is parasitic on other plants, draining nutrients from their roots, and it lacks leaves and chlorophyll. The inflorescence bears several flowers, each in a yellowish calyx of sepals and with a tubular white and blue to purple corolla.

Figure -1: ***Orobanche aegyptiaca***

Distribution

The distribution of *O. aegytpiaca* closely matches that of *O. ramosa* in Europe and the Middle East but there has been less dispersal to other continents than is the case with *O. ramosa*[6].

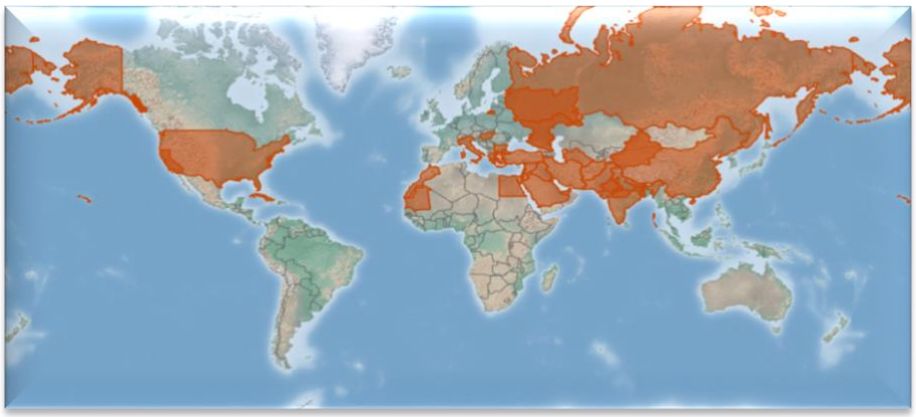

Figure-2: Distrubution of *Orobanche aegyptiaca*

Habitat

Most of the weedy *Orobanche* species are native to the Middle East and are adapted to soils of generally high pH. They occur to some extent in wild vegetation but the weedy species are mostly associated with the crops that they attack.

Phytochemistry

In recent years, several studies focused on the chemical constituents of *Orobanche spp.*[7]. More than 160 compounds have been isolated and identified from *Orobanche spp*. The most common compound isolated from *O.aegyptiaca* are listed in table 1

Table-1: Compounds isolated from *Orobanche aegyptiaca*

Classification	Compounds
Phenylethanoid glycosides	Poliumoside
	3′MeOpoliumoside
	3′MeO2acetylpoliumoside
	OrobancheosideC
	Orobancheoside D
	OrobancheosideE
	Orobancheoside G
	Orobancheoside F
	Orobancheoside H
	2-acetyl-poliumoside
	2-acetyl orobancheosideG
	Leucosceptoside
	Martynoside
	Acteoside

Pharmacological activities

The reported biological activities of *O. aegyptiaca* (7) are summarized in (table- 2).

Table-2: Biological activities of *Orobanche aegyptiaca*

Effects	Extract(s) or compounds	Dose	Results	Reference
Antibacterial and antiviral activities	Extracts of *O.aegyptiaca*	1526.5,µg/ mL	These findings indicated that *O.aegyptiaca* have strong resistance to bacterial infection.	Ismail et al.(2008)
Antihypertensive	Alkaloidal fraction	10 mg	The blood pressure was significantly reduced, and the effect lasted for more than 3 h. A possible mechanism may be that the residual alkaloidal fraction is not eliminated by ganglion blockage or peripheral adrenergic blockage, and directly acts on the arterial wall, resulting in a decrease in blood pressure.	Sharaf, and Youssef (1971)
Effects on smooth muscle	Water extract	200 mg/50 mL bath	The heart and intestine of frog were stimulated and uterine contraction was inhibited after injection of a water extract.	Sharaf, and Youssef (1971)
Cardiovascular Effects	Water extract	-	The extract stimulated the contractions of frog hearts	Sharaf and Youssef (1971)

Toxicity

Orobanche spp. are non-toxic in a wide concentration range.The alkaloids fraction of *O.aegyptiaca* were injected intraperitoneally into mice. The coagulation time of haemorrhagic animals was measured, the highest dose of drugs was estimated, and histopathological examination was performed. Miller-Taylor method was used to evaluate the blood coagulation time of haemorrhagic animals. The results showed that the LD50 of the residual *O.aegyptiaca* alkaloid was 610 mg/kg. Interestingly, at a sublethal dose, no pathological changes to organs, clotting time, or bodyweight were observed[8].

Materials and Methods

Method:

- Preparing materials

  After plant collection, cleaned the plants and dried it under natural open air, then grinded and placed in plastic bags and labeled.

- Extraction

Extraction process carried out in laboratory at pharmacognosy department of pharmacy collage. About 5 g of powdered leaves of *O.aegyptiaca* plant mixed with 150 ml of 70% ethanol and boiled under a reflux condenser at 70 C° for 45 min[9]. The ethanolic extract was then filtered, put in glass container and stored at -4 C° until they are used for the further prliminary anaylasis study.

- Preliminary identification study of *Orobanche aegyptiaca*[10]

1. Dragendoff'stest

1 ml of Dragendoff's reagent was added drop by drop. Formation of a reddish-brown precipitate indicates the presence of alkaloids.

2. Salkowski test

1 ml of reagent was added drop by drop. Formation of a red color indicates the presence of steroids.

3. Molish test

Take 1ml of sample in dry test tube, add 2-3 drops of Molisch's reagent (α-naphthol and sulfuric acid). Formation of a purpleor violet -ring indicates the presence of the carbohydrates.

4. $FeCl_3$ test

1 ml of $FeCl_3$ was added drop by drop. Formation of a reddish-precipitate indicates the presence of phenols.

Gas Chromatography–Mass Spectrometry (GC-MS) Analysis

A screening of ethanolic extracts carried by using a mass spectrometer Agilent. Gas chromatograph equipped and coupled to a mass detector Agilent 5977A spectrometer with an HP- 5MS (5% Phenyl methyl siloxen) (130).

GCMS Condition:

Column type: 30m ×0.25mm × 0.25 mm ID of capillary column.

Temperature of the injector: 290°C

Oven temperature: starting at 40°C and maintained for 5 min then raised to 300 °C.

Mobile phase: The helium gas 99.9%

Flow rate: 1ml\min

Injection volume of 1 µl.

The mass spectra: at 70 eV.

The solvent delay: 4min The total GC-MS running time : 45min.

The samples were injected in split mode (50:1).

The mass spectral scan range: 45 to 650 (m/z)

Results and Discussions

Results and Discussions

The qualitative estimation for various constituents revealed that the plant extracts contained alkaloids, phenolic compounds, carbohydrate and steroids. The results of preliminary phytochemical analysis were given in Table-1 below .

Table-3: Preliminary phytochemical identification of O. aegyptiaca extract.

Extract of	Phenol	Alkaloids	Carbohydrate	Steroids
O. aegyptiaca	++	++	++	+

Gas Chromatography–Mass Spectrometry (GC-MS Analysis)

GC-MS has been regarded as a "gold standard" for forensic substance identification because it is used to perform a 100% specific test, which positively identifies the presence of a particular substance. A nonspecific test merely indicates that any of several in a category of substances is presen.The qualitative analysis of *O.aegyptiaca* crude extracts was performed by GC/MS chromatography as shown in (figure-4). Identification of the compound was performed by comparing its mass spectra.

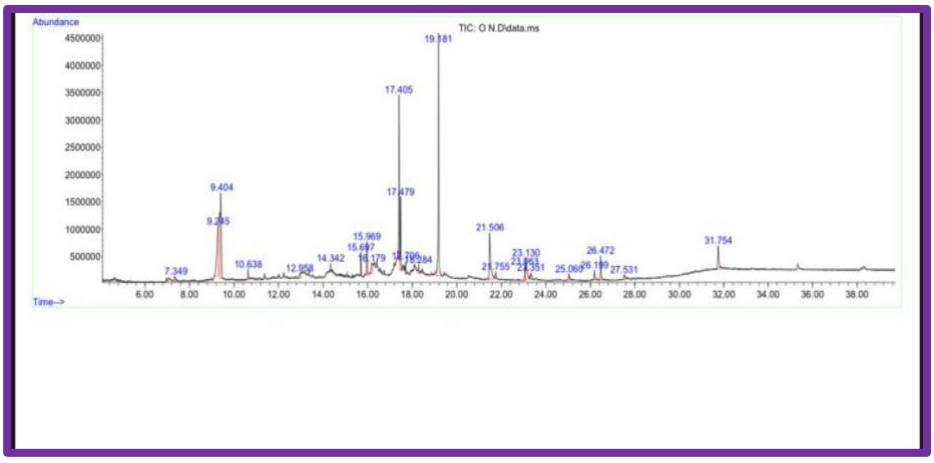

Figure-4: The GC-MS chromatograms of *Orobanche aegyptiaca* extract.

Table-4: The results of GCMS analysis

RT	Area	%	Base Peak	Formula	m/z	Name
4.48	23410	0.32860983	59	C7 H16 O	59	Ethanol, pentamethyl-
11.052	94051	1.320208592	86	C5 H10 O	86	3-Pentanone
12.044	164323	2.306627643	91	C3 H9 N S	91	Ethanamine, 2-(methylthio)-
12.097	255305	3.583756203	65	C2 H3 Cl O2	65	Methyl chloroformate
12.151	235434	3.304823869	110	C8 H9 N O3	110	1,2-Benzenediol, mono(methylcarbamate)
15.118	13662	0.19177563	61	C5 H10 O2	61	3-Methoxy-2,2-dimethyloxirane
19.57	551842	7.746292436	73	C10 H18 O9	73	Ribo-ribo disaccharide
19.945	424573	5.959797584	60	C16 H32 O2	60	n-Hexadecanoic acid
19.952	504787	7.085774044	73	C16 H32 O2	73	n-Hexadecanoic acid
19.962	130117	1.826472673	83	C18 H34 O6	83	Succinic acid, di(5-methoxy-3-methylpent-2-yl) ester
21.579	142903	2.005951754	55	C8 H16 N2	55	2-Pyrazoline, 1-butyl-5-methyl-
21.784	26810	0.376336162	73	C10 H20 O2	73	n-Decanoic acid
24.876	680916	9.558124355	149	C24 H38 O4	149	Phthalic acid, di(2-propylpentyl) ester
25.967	25690	0.360614547	67	C8 H13 N	67	8-Azabicyclo[3.2.1]oct-2-ene, 8-methyl-
26.53	14763	0.207230539	57	C19 H36 O5	57	1,3-Dioctanoin
29.104	76141	1.068803122	150	C11 H15 N O3	150	3-Amino-3-(4-ethoxy-phenyl)-propionic acid
29.128	104222	1.462980509	183	C18 H27 N O4	183	4-Nitrophenyl laurate
30.338	40730	0.571733378	97	C4 H6 N2 O	97	1H-Imidazole-4-methanol
30.906	1298240	18.22359786	283	C10 H9 N3 O7	283	6-(2-Acetoxyethoxy)-4-nitrobenzofuroxan
31.402	30312	0.425494283	57	C6 H9 F3 O2	57	Acetic acid, trifluoro-, 1,1-dimethylethyl ester
33.147	38658	0.54264839	207	C11 H13 N5 O2 S	207	1H-Purine-2,6-dione, 7-ethyl-3,7-dihydro-8-(isothiocyanatomethyl)-1,3-dimethyl-
33.167	403330	5.661606272	183	C28 H60 O Si	183	Docosanol, TBDMS derivative
35.634	74013	1.038932053	55	C5 H6 O2	55	Furan, 2-methoxy-
35.646	226213	3.17538725	183	C14 H19 F O2	183	Octanoic acid, 2-fluorophenyl ester
38.968	54493	0.764926761	257	C7 H4 F10 O3	257	1,2,2-Trifluoro-2-[1,2,2,2-tetrafluoro-1-(trifluoromethyl)ethoxy]ethyl acetate
44.072	22401	0.314446339	97	C18 H23 Cl O5	97	Succinic acid, cyclohexylmethyl 4-chloro-2-methoxyphenyl ester

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