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Preliminary phytochemical analysis of *Orobanche crenata*

A Thesis
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وما أوتيتم من العلم إلا قليلًا

Dedication

I dedicate this project to my esteemed supervisor **Faten Essam Hussein** and my esteemed college **College of Pharmacy**.

To my supervisor, **Faten Essam Hussein**,
I am deeply grateful for your profound expertise, invaluable insights, and tireless dedication. Your mentorship has been a beacon of light throughout this journey. Thank you for your constant guidance and for imparting your wisdom, which will undoubtedly leave a lasting impact on my academic and professional pursuits.

To my college, I extend my heartfelt appreciation for providing an environment conducive to learning and personal development. The institution's commitment to academic excellence and the vibrant community of scholars have transformed my educational experience.

This project stands as a testament to the collective efforts, unwavering support, and invaluable contributions of my supervisor and my college. Without their guidance and the nurturing environment they have provided, this endeavor would not have been possible. I am truly humbled and honored to have had the privilege of working under their tutelage and being a part of this esteemed academic institution.

With deepest gratitude,

NOUR AWUAD HASSAN

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List of Contents

Introduction

Orobanche, commonly known as broomrape, is a parasitic plant that belongs to the Orobanchaceae family. It is a root parasite that attaches itself to the roots of other plants and derives its nutrients from them. Orobanche plants lack chlorophyll, which is the pigment that gives plants their green color and enables them to perform photosynthesis. This means that Orobanche is unable to produce its own food, and must obtain all of its nutrients from its host plant[(1)]. In order to do this, Orobanche attaches itself to the roots of its host plant and forms a structure called a haustorium. The haustorium penetrates the host plant's root and absorbs nutrients directly from the host plant. There are around 200 species of Orobanche found worldwide, and they grow in a variety of habitats ranging from deserts to forests. The plant has a unique and fascinating life cycle, which makes it an interesting subject for researchers and botanists[(2)].

Taxonomic Tree[(3)]

Domain: Eukaryota

Kingdom: Plantae

Phylum: Spermatophyta

Subphylum: Angiospermae

Class: Dicotyledonae

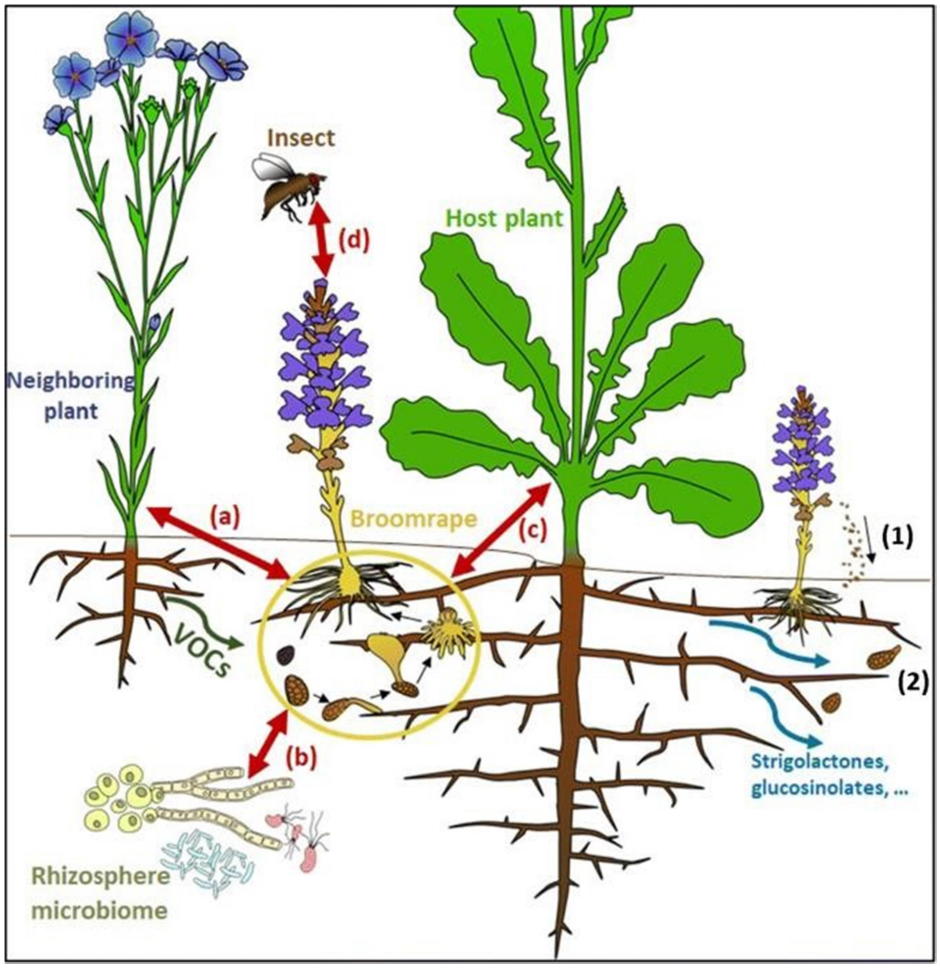
Order: Scrophulariales

Family: Orobanchaceae

Genus: Orobanche

Species: *Orobanche crenata*

Morphology, description and Physiology:-



Description

O. crenata produces leafless flowering stems, up to 100 cm high, usually un-branched, bearing alternate scales, less than 2 cm long. The plant is pale, completely lacking any chlorophyll. The base of the stem, below ground, is normally swollen and tuberous. The inflorescence, occupying up to half the length of the stems carries many acropetally developing flowers, arranged in spikes or racemes, each subtended by a bract 15-25 mm long . The calyx has four free segments, more-or-less bidentate, 10-20 mm long. The white corolla tube, 20-30 mm long, is campanulate, with wide, divergent lips up to

15 mm across, usually with distinct lilac veins. Flowers are distinctly fragrant. [4]

Physiology:

The physiology of Orobanche plants is closely linked to their unique parasitic lifestyle. Since they lack chlorophyll, they are unable to perform photosynthesis and must obtain all of their nutrients from the host plant. Studies have shown that Orobanche plants produce a range of specialized compounds that allow them to establish and maintain their parasitic connection with the host plant[5]. These compounds include enzymes that break down the host plant's cell walls, as well as plant hormones that regulate the development of the haustorium

Distribution

O. crenata is commonest in countries adjacent to the Mediterranean. It extends sporadically eastwards as far as Pakistan and India, and northward into northern Europe but is rarely a significant problem away from the immediate Mediterranean region[6]. The suspected occurrence in Ethiopia is an alarming development in a country where fava bean is a major crop.

Chemical Compound Identified in *O crenata*

The phytochemical screening revealed the presence of different classes of chemical compounds (Table 1) [7]

Table 1. Chemical Compounds Isolated from O. crenata.

Whole plant	Water, chloroform	UV, IR, MS, CI-MS, ^{1}H and ^{13}C NMR	Orobanone	[10]	Fruchier et al. (1981)
Whole plant	Chloroform, 70% ethanol, ethyl acetate	TLC, NMR	Two phenylpropanoid glycosides (not characterised)	–	El-Shabrawy et al. (1989)
Aerial parts	90% ethanol	UV, IR, TLC, ^{1}H and ^{13}C NMR, ^{13}C NMR-DEPT, FAB-MS	Orobanchoside Verbascoside	[1] [2]	Afifi et al. (1993)
Aerial parts	Petroleum ether, chloroform, methanol	UV, IR, ^{1}H and ^{13}C NMR	Orobanchoside Verbascoside Poliumoside Vanillin Isovanillin Syringaldehyde p-hydroxybenzaldehyde p-hydroxyacetophenone	[1] [2] [4] [11] [12] [13] [14] [15]	Dini et al. (1995)
Seeds	Garces and Mancha (1993) extraction method	GLC	Palmitic acid (16:0) Stearic acid (18:0) Oleic acid (18:1) Linoleic acid (18:2)	[16] [17] [18] [19]	Velasco et al. (2000)
	Iso-octane	HPLC	δ-tocopherol γ-tocotrienol δ-tocotrienol	[20] [21] [22]	
Flowering plants	Methanol	^{1}H and ^{13}C NMR, HPLC	Orobanchoside Verbascoside Poliumoside	[1] [2] [4]	Serafini et al. (2005)
Stems	80% methanol under reflux	UV, HPLC-DAD	Verbascoside Isoverbascoside	[2] [3]	Gatto et al. (2013)
Aerial parts	Methanol, water	–	Phenols, tannins (not characterised)	–	Abbes et al. (2014)
Whole plant	80% ethanol	GC-MS	Glycitein Hexestrol 2,4-di-tert-butylphenyl benzoate 6-Monohydroxyflavone Actinobolin	[5] [6] [7] [8] [9]	Nada and El-Chaghaby (2015)
Leaves	Acetone	TLC	Phenols, flavonoids (not characterised)	–	Genovese et al. (2019)

Traditional uses of *Orobanche crenata*

Orobanche plants have potential uses in various industries, including pharmaceuticals, cosmetics, and agriculture. Research has shown that Orobanche plants contain various compounds with pharmacological properties, making them useful in the development of new drugs. Orobanche plants are also used in the cosmetics industry due to their skin nourishing properties.[8]

Therapeutic uses of *Orobanche crenata*

1- **Analgesic effect**:

Potent analgesic effect of phenylpropanoid containing fraction of *O crenata* extract (oral application 0 were observed in mice using the hot plate method for test.

The result implicate a clear analgesic activity of *O. crenata*, other [9] studies on pharmacological effect also reveal analgesic effect the mechanism proposed due to direct effect on COX1 and COX2 enzyme

2- **Anti -microbial Activities**

O crenata extract has been showed moderate antibacterial activity in vitro studies against three-gram positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis* and *Streptococcus faecalis*) and three-gram negative bacteria (*Escherichia coli, Pseudomonas aeruginosa and Neisseria gonorrhoeae).* [10]

3- **Anti oxidant effect**

O crenata ethanolic extract total activity was tested using DPPH method , ascorbic acid as standard . the anti oxidant activity was expressed as ascorbic acid equivalent (AE), *O crenata* showed good total antioxidant activity that related to its ability to inhibit lipid peroxidase enzyme and reactive oxygen species scavenging activity[11].

4- **Diuretic effect**

Oral application of phenylpropanoid containing fraction of *O crenata* extract to rats show strong diuretic activity. [12]

5- Inhibition of Amyloid β aggregation

O crenata was tested for its inhibitory effects on aggregation of human 42 mer amyloid B protein (Aβ- 42) (which play important role in Alzheimer disease) show moderate inhibition in the former protein. [12]

6- Memory enhancing effect

O crenata show memory enhancement effect and increase significantly the expression of nerve growth factors (NGF) and tropomycin receptor kinase and protein in the hippocampus in mice which are closely related to Alzheimer disease. [12]

7- Anticancer activity

The effect of O crenata ethanolic extract was tested on viability of human breast cancer MCF7 cell line , the result showed reduction in the viability of MCF7 cell in concentration dependent manner.[13]

Material and Methods

Material and methods

Collection and identification of plants

plants were obtained and identified by Al-Asadi, which collected them during her PhD research 2017. Authentication of the plant was conducted at Biology Department, College of Science, University of Basrah. Voucher specimens are deposited in department herbarium.

Sample's preparation

The collected samples were cut into small parts, wash many times with water. It was then air-dried for 14 days. The dried samples were ground mechanically into a fine powder to easily extraction and kept in paper bags for more extraction processes

Method of Extraction and Chromatographic Analysis

Reflux Extraction

Sample of the crude extract was prepared by mixing 5 g of whole plant of *Orobanche crenata* plant with 150 ml of 70% ethanol and boiled under a reflux condenser at 70 C° for 45 min [14]. The ethanolic extract was then filtered, put in glass container and stored at -4 C° until they are used for the identification.

Fig (1) Reflux extraction process

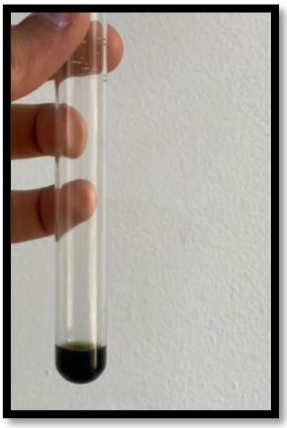
Preliminary Detections Study of Orobanche crenata Leaves

The extract of Orobanche crenata leaves was screened according to standard procedures of qualitative investigations to identify the major classes of natural secondary metabolites

Fig (2) O crenata crude extract

Test for Phenols

Alcoholic extract (2 ml) was treated with 3 to 4 drops of ferric- chloride 5%. The color was noted and the results were recorded.



Fig(3) : test for phenols

Test for Alkaloids

Dragendroff's Test

The alcoholic extract (2 ml) and (0.2 ml) of the dilute hydrochloric acid were put in a test tube. Then (1 ml) of Dragendroff's reagent was added and the results were recorded.

Fig (4) : **Dragendroff's Test**

Test for Terpenoids (Salkowski Test)

The alcoholic extract (5ml) was mixed with chloroform (2ml), and carefully added concentrated sulphuric acid (3ml) and results were recorded.

Fig (5): Salkowski Test)

Test for Carbohydrates (Molisch's Test)

Alcoholic extract (1ml) was mixed with Molisch's reagent (a solution of a-naphthol in ethanol) and the subsequent addition of a few drops of concentrated H2SO4 (sulphuric acid) to the mixture on the side of test tube

Fig (7) Molisch's test

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 Discussion

Results

Preliminary Phytochemical Identification of Active Constituents

After obtaining the crude extracts or active fractions from plant materials phytochemical screening was performed with appropriate tests to give the following results as shown in table (1)

Table (1) Preliminary Phytochemical tests Results

	Test for Phenols	Dragendroff's Test	Salkowski Test	Molisch's Test
O crenata	**+ ve**	**+ ve**	**+ ve**	**+ ve**

In phenol test , phenols form a complex with ferric ions, this complex has an intense color.

While in Dragendroff's Test the precipitate comes from complex compound of potassium-alkaloid in the presence of Bi3+ ion from bismuth nitrate that react to potassium iodide producing dark reddish precipitate of Bismuth (III) iodide and dissolved in the excess of potassium iodide producing potassium tetra iodo bismuthate which react with N in alkaloid to form covalent coordination bond with K+ ion .

And for Salkowski Test color is due to formation of bi-sulphuric acid in which concentrate sulphuric acid removes two molecules of water from two molecules of cholesterol and bi-cholestadien is formed. [16]

Gas Chromatography–Mass Spectrometry (GC-MS Analysis)

The qualitative and analysis of *O crenata* crude extracts was performed by GC/MS chromatogram as shown in figures (8) Identification of the compound was performed by comparing its mass spectra

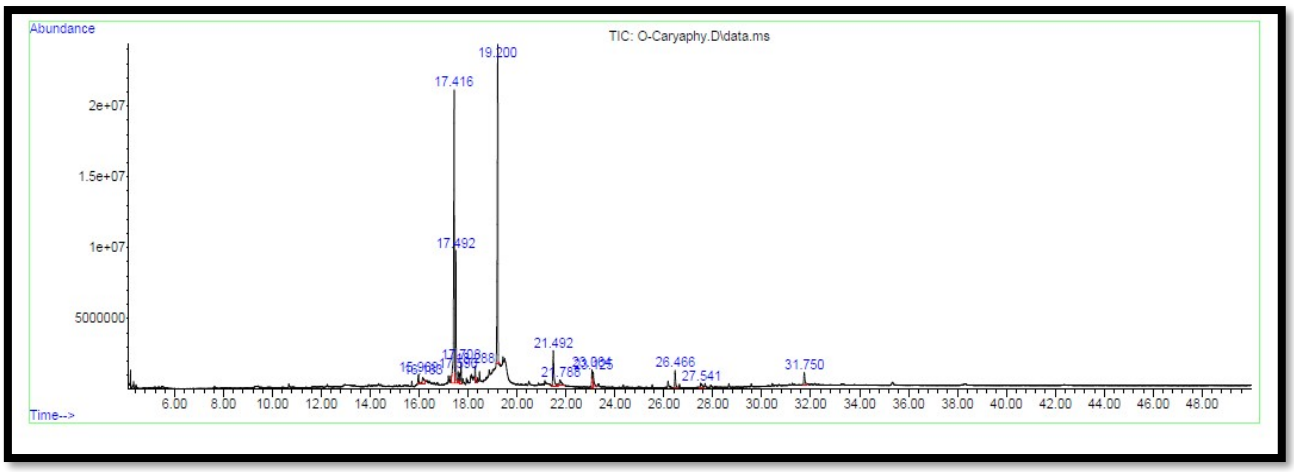


Fig (8) :The GC-MS chromatograms of Orobanche crenata crude extract.

The identified compounds is shown in table (3)revealed variety of chemical constituent in O crenata crude extract appear at different retention times with high concentration compound present at Rt19.2 and Rt17.418

Table (3) compounds list from preliminary GCMS analysis of crude extract *Orobanche crenata.*

RT	Area	%	Base Peak	Formula	Name
19.96	199112	3.640785975	55	C7 H12 O2	2-Propenoic acid, butyl ester
21.57	85747	1.567893823	55	C7 H12 O	1-Hepten-3-one
30.05	150861	2.758510853	55	C29 H50 O	Stigmast-7-en-3-ol, (3.beta.,5.alpha.,24S)-
30.06	166041	3.036078911	55	C15 H24 O	cis-.alpha.-Copaene-8-ol
30.34	34912	0.638369962	57	C15 H25 F3 O4	Glutaric acid, 1,1,1-trifluoroprop-2-yl 2,4-dimethylpent-3-yl ester
17.11	81782	1.495393339	58	C10 H19 N O4	Glycine, N-ethyl-n-propoxycarbonyl-, ethyl ester
4.323	48665	0.88984516	59	C6 H12 O2	2-Pentanone, 4-hydroxy-4-methyl-
19.95	242158	4.427887069	60	C11 H22 O2	3,5,5-Trimethylhexyl acetate
11.96	99496	1.81929588	64	C4 H12 N O P S	S-Methyl isopropylphosphonamidothioate
21.52	69149	1.264397472	67	C14 H42 O5 Si6	Hexasiloxane, tetradecamethyl-
21.56	80954	1.480253263	69	C7 H7 F	Benzene, 1-fluoro-3-methyl-
7.931	48759	0.891563961	81	C10 H18 O	Eucalyptol
16.98	1934616	35.37467756	87	C7 H14 O6	Myo-Inositol, 4-C-methyl-
32.61	21231	0.388211293	95	C11 H9 N O2	2-Furanilide
37.73	19414	0.354987238	96	C3 H3 N3 O	1H-1,2,3-Triazole-4-carboxaldehyde
17.01	325689	5.955261076	102	C21 H41 N O4	Glycine, N-ethyl-N-methoxycarbonyl-, pentadecyl ester
24.89	29097	0.532042014	113	C20 H36 O2 Si	1-Tributylsilyloxymethyl-4-methoxybenzene
35.66	145336	2.657485588	127	C6 H15 O4 P	Butyl dimethyl phosphate
20	38278	0.699917662	129	C10 H25 N3	N,N,N',N',N'',N''-Hexamethyl-2-aminomethyl-1,3-propane-diamine
37	21773	0.398121826	133	C15 H10 O6	1,2-Benzenediol, O-(2-furoyl)-O'-propargyloxycarbonyl-
24.88	352788	6.450769429	149	C24 H38 O4	Bis(2-ethylhexyl) phthalate
29.13	49596	0.906868603	183	C13 H21 F3 O4	Succinic acid, 2-methylhex-3-yl 2,2,2-trifluoroethyl ester
33.16	265457	4.853911982	183	C12 H27 F2 P	Tri(n-butyl)difluorophosphorane
43.98	32391	0.592273185	183	C18 H27 N O4	4-Nitrophenyl laurate
30.5	201414	3.682878311	223	C19 H23 N3 O2 S	Benzenamine, N-(4-dimethylaminobenzylidene)-4-(1-pyrrolidinylsulfonyl)-
30.84	466366	8.527556309	253	C14 H11 N3 O2	6-Nitro-2-p-tolyl-2H-indazole
30.47	42196	0.771558746	281	C16 H14 O3	Isoparvifuran
30.07	41885	0.765872075	314	C22 H28 O4	Estra-1,3,5(10)-triene-3,17-diol, 3,17-diacetate
33.17	138667	2.535542151	383	C21 H26 O4 Si2	Chrysophanol, 2TMS derivative

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