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GC-MS analysis and prliminary phytochemical identification for a wild specimen of the *Orobanche minor*

A Thesis

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بسْم اللَّـهِ الرَّحْمَـٰن الرَّحِيم

يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ
دَرَجَاتٍ ۚ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ

صدق الله العظيم

سورة المجادلة (١١)

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Introduction

Introduction

Orobanche L. belongs to the Orobanchaceae family, an entirely parasitic higher plant taxon, comprises 102 genera and over 2100 species distributed all over the world[(1)]. *Orobanche* species (the broomrapes) are parasitic angiosperms whose host range includes members of the Apiaceae, Asteraceae, Fabaceae and Solanaceae plant families[(2)]. Holoparasitic plants are characterized by the production of large quantities of 'dust seeds', the smallest kind of seed from flora, which are less than 1 mm in size. Seed storage materials have numerous biological functions, including seed germination, protection, and development[(3)]. Holoparasites are only germinate in response to specific chemicals released by the host plant[(4)]. *Orobanche minor Sm.* is a root holoparasitic angiosperm that has lost photosynthetic function and depends entirely on host plants for its supply of water and inorganic and organic resources[(5)].

Life cycle of broomrape:

The pre-conditioning period requires moist and warm (59°F [15°C] to 68°F [20°C]) environmental conditions from 5 to 21 days.

Conditions remain conducive, multiple flushes of germination can occur within a single season as in figure-1.

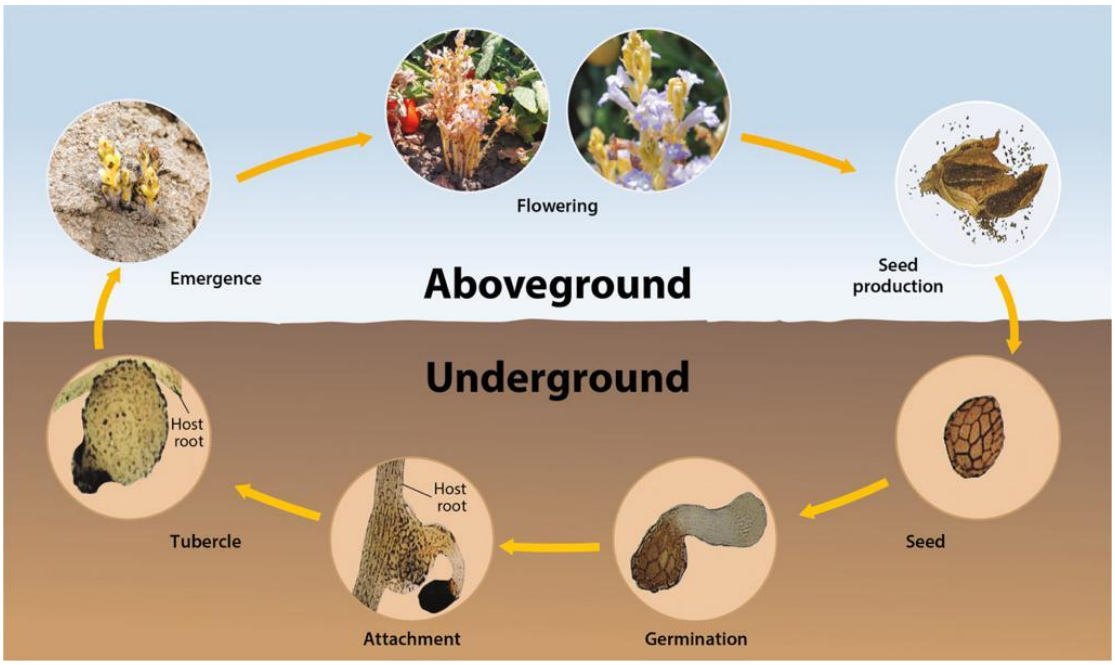

Figure-1: ***Life cycle of Orobanch minor***

Taxonomic Tree[6]

Domain
Eukaryota
Kingdom
Plantae
Phylum
Spermatophyta
Subphylum
Angiospermae
Class
Dicotyledonae

Order
Scrophulariales
Family
Orobanchaceae
Genus
Orobanche
Species
minor

Description

Orobanche minor is a fleshy, herbaceous, annual, parasitic plant that grows up to 22 in. (55.9 cm) tall. It attaches to the roots of broad leaf hosts (especially Trifolium spp.). The fleshy stem is yellow to straw colored and sticky. Leaves are greatly reduced (vestigial), alternate and triangular shaped. Flowers, borne on terminal clusters, are snapdragon-like, with 0.5 in. (1.3 cm) long, purple-tinged petals. Flowering occurs in the winter and spring. Fruit, This plant is highly prolific. Seeds are very small (dust-size) and remain viable for 10 or more years[7].

Figure-2*: Orobanche minor Sm.*

Distribution

O. Minor distributed mainly in Mediterranean countries, Western Asia and East Africa. Further distrubution in USA, S. America, Australia, New Zealand and southern Africa[8].

Materials and Methods

Materials and Methods

Methode:

- 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.minor*. 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 minor* [(9)]**

1. Dragendoff's test

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

2. Liebermann-Burchard test

5 drops of acetic anhydride was added to the extract then mix, after that on side of test tube add 2 drops of conc. sulfuric acid (H2SO4). A positive result is observed when the solution becomes a red color then bluish –green color.

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 purple or 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 was given in Table-1 below .

Table-1: Preliminary phytochemical identification of *O. minor extract.*

Extract of	Phenols	Alkaloids	Carbohydrate	Steroids
O. minor	++	+	+	+++

In libermans test, the steroid portion is react as a typical alcohol with a strong concentrated acids and the product are colored substances. Acetic anhydride are used as solvent and dehydrating agents, and the sulfuric acid is used as dehydrating and oxidizing agent.

(GC-MS) Analysis:

Lipids are the main storage material in Orobanchaceae seeds and are likely to play a crucial role in seed germination at low and high temperatures, and act as an energy source for the growing embryo. The analysis of the seed fatty acid composition and tocochromanols in a wider range allows for the characterization of chemotaxonomic relationships in angiosperm families, such as, Fabaceae, and Malvaceae [10,11]. Previous studies have clearly demonstrated that the determination of the fatty acid content and composition is a promising tool with taxonomic potential for some plant groups.The results of analysis of *Orobanche minor* were given in (figure-2) and table-2 , reveled that the presence of phenols and lipid contents such as fumaric acid, oxalic acid, and n-deconic acid at Rt of 27.867, 14.934, and 19.977 respectively.

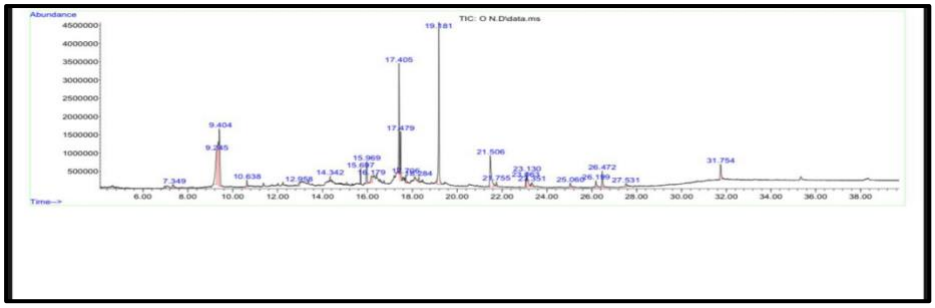

Figure-3: The GC-MS chromatograms of *Orobanche minor* extract.

Table-2: The results of GCMS analysis :

RT	Area	%	Base Peak	Formula	Name
25.989	8046	0.2425907 46	57	C H3 N5	5H-Tetrazol-5-amine
27.867	6903	0.2081287 5	57	C14 H24 O4	Fumaric acid, isobutyl 2-methylpent-3-yl ester
29.907	11916	0.3592730 96	57	C13 H28	Heptane, 4-ethyl-2,2,6,6-tetramethyl-
34.522	124888	3.7654328 99	57	C2 H3 N O	Methane, isocyanato-
35.666	199955	6.0287388 33	57	C4 H5 N3 O2	2,4(1H,3H)-Pyrimidinedione, 5-amino-
23.483	31776	0.9580615 9	59	C9 H16 O3	Cyclobutanecarboxylic acid, 2-ethoxyethyl ester
21.784	10822	0.3262884 73	60	C9 H18 N2	N,N'-di-tert-Butylcarbodiimide
21.555	95221	2.8709586 68	67	C8 H14	1,1'-Bicyclobutyl
39.06	15894	0.4792116 98	69	C8 H12 O2	Crotonic acid, 1-buten-4-yl ester
21.615	12760	0.3847201	70	C4 H7 N O	Acetone cyanohydrin
14.486	66475	2.0042530 26	73	C10 H26 Si2	Silane, 1,4-butanediylbis[trimethyl-
14.934	10125	0.3052735 9	73	C8 H18 O4 Si2	Oxalic acid, 2TMS derivative
19.977	231026	6.9655443 35	73	C10 H20 O2	n-Decanoic acid
14.439	13491	0.4067600 99	77	C8 H7 N3	1-Phenyl-1,2,3-triazole
34.548	50956	1.5363477 58	83	C H Cl2 N O2	Methane, dichloronitro-
21.586	26609	0.8022740 7	84	C13 H22 O2	3(2H)-Furanone, 5-methyl-2-octyl-
19.946	56887	1.7151702 43	85	C16 H16 O3	6-Isopropyl-3-phenoxytropolone
39.049	90420	2.7262062 23	85	C11 H20 O2	2-(1-Methylcyclopentyloxy)-tetrahydropyran

Future approach:

1. Study to analyse and evaluate the fatty acid content and compositions in the seeds of holoparasitic *Orobanche minor* .
2. Investigate the relative effects of taxonomic affiliations, hosts, and environmental properties on the interspecific variation in fatty acid composition
3. Analyse the fatty acid composition, in terms of the nutritional value of the Orobanchaceae seeds as a food additive.

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