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Chemotaxonomical Study of the *Orobanche* L. and *Geranium* L. Species in Iraq

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Abstract

The soluble carbohydrate content of 10 species of *Orobanche* and 9 species of *Geranium* in Kurdistan Region-Iraq (were collected during Spring of 2014-2016 from different localities of districts of MAM, MRO, MSU, FAR, FKI and FPF) was determined for chemical classification, the results showed differences in carbohydrate content among the studied species, which extends between 19.618-87.84mg/gm in *Geranium* species and 33.506-174.479mg/gm in *Orobanche* species, accordingly, the *Geranium* species were divided into three groups: group with low carbohydrate content (less than 35 mg/gm) included *G. dissectum G. Lucidum, G. pusillum* and *G. purpurium*, medium content group (35-60 mg/gm) involved, *G. stepporum, G. kudicum* and *G. tuberosum* and high content group (more than 60 mg/gm) consist of *G. molle* and *G. rotundifolium* as well the *Orobanche* species were divided into three groups: low carbohydrate content group (less than 60 mg/gm) included *O. aegyptiaca, O. ovata, O. arenaria* and *O. ramose*, medium content group (60-90mg/gm) includes *O. mutilii, O. crenata* and *O. coelestis* and group with high content (more than 90mg/gm) involved *O. singarensis, O. kurdica* and *O. anatolica.*

Keywords: Plant Chemotaxonomy; *Orobanche* L. and *Geranium* L. ; Kurdistan Region-Iraq.

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1. Introduction

Plant chemotaxonomy is one of vary rapidly expanding areas of plant classification and how to use chemical information to support plant densification. The position of many taxa in the natural system of plants is still highly uncertain. Chemotaxonomic principles are considered and some examples are provided to show the importance of chemical evidence in taxonomic revision [7]. The potential value of plant secondary metabolites to taxonomy has been recognized for nearly 200 years [5 and 1].

The genus *Orobanche* L. belongs to holoparasitic members of Orobanchaceae Vent. and includes about 170 species [14]. It is mainly distributed throughout the subtropical and temperate regions of the northern hemisphere, and the Mediterranean region is one of the most important centers of its diversity [12]. *Orobanche* species are obligatory holoparasite, their chemical contents depends directly on their hosts, *Geranium* L. belongs to the family Geraniaceae [4] and comprises about 350 species distributed in temperate and tropical alpine regions in the world [10 and 2]. The deficiency of Chemotaxonomical studies of both genera in Iraq and due to the importance of plant chemical contents in field of plant classification thus this study make this study focus on estimating the soluble carbohydrate content of the studied species.

2. Materials and Methods:

Sample plants collection

Plant specimens were collected during the flowering period (March-Jun) of 2014-2016 from different localities in Kurdistan region districts of: Amadiya District (MAM), Rowanduz District (MRO), Sulaimani District (MSU), Arbil District (FAR), Kirkuk District (FKI), Persian District (FPF) (fig. 1, table 1, 2), after diagnosis the samples are dried at room temperature and grinded.

Determination of carbohydrate in plant samples by using Anthrone reagent [9].

Materials:

1- Anthrone*:* dissolving 200mg anthrone in 100ml of ice-cold 95% sulphuric acid
2- perchloric acid 52%
3- Ethanol 80%
4- Standard Glucose: Stock-100mg in 100ml water. Working Standard - 10ml of stock diluted to 100ml with water.

Samples Preparation:

1- 0.1 to 0.5gm of the plant sample in hot ethanol 80% was homogenized to remove sugars, was shacked for 5-10min. and centrifuged at 3000rpm for 10min., wash the residue repeatedly with hot 80% ethanol till the washing do not give color with anthrone reagent was retained and washed, the residue was dried.

2- 5.0ml of water and 6.5ml of 52% perchloric acid was added to the residue

3- The residue was cooled at 0C° for 20min. and was centrifuged then the supernatant was saved.

4- The extraction was repeated using fresh perchloric acid, and was centrifuged then the supernatant was pooled and make up to 100ml.

5- 0.1 or 0.2ml of the supernatant was pipetted out and make up the volume to 1ml with water.

6- The standards was prepared by taking 0.2, 0.4, 0.6, 0.8 and 1ml in each tube with water.

7- 4ml of anthrone reagent was added to each tube and heated for 8 minutes in a boiling water bath

8- The output was cooled quickly and the intensity of green to dark green was readed at 630 nm

9- The amount of glucose in 1gm of the powdery sample was calculate.

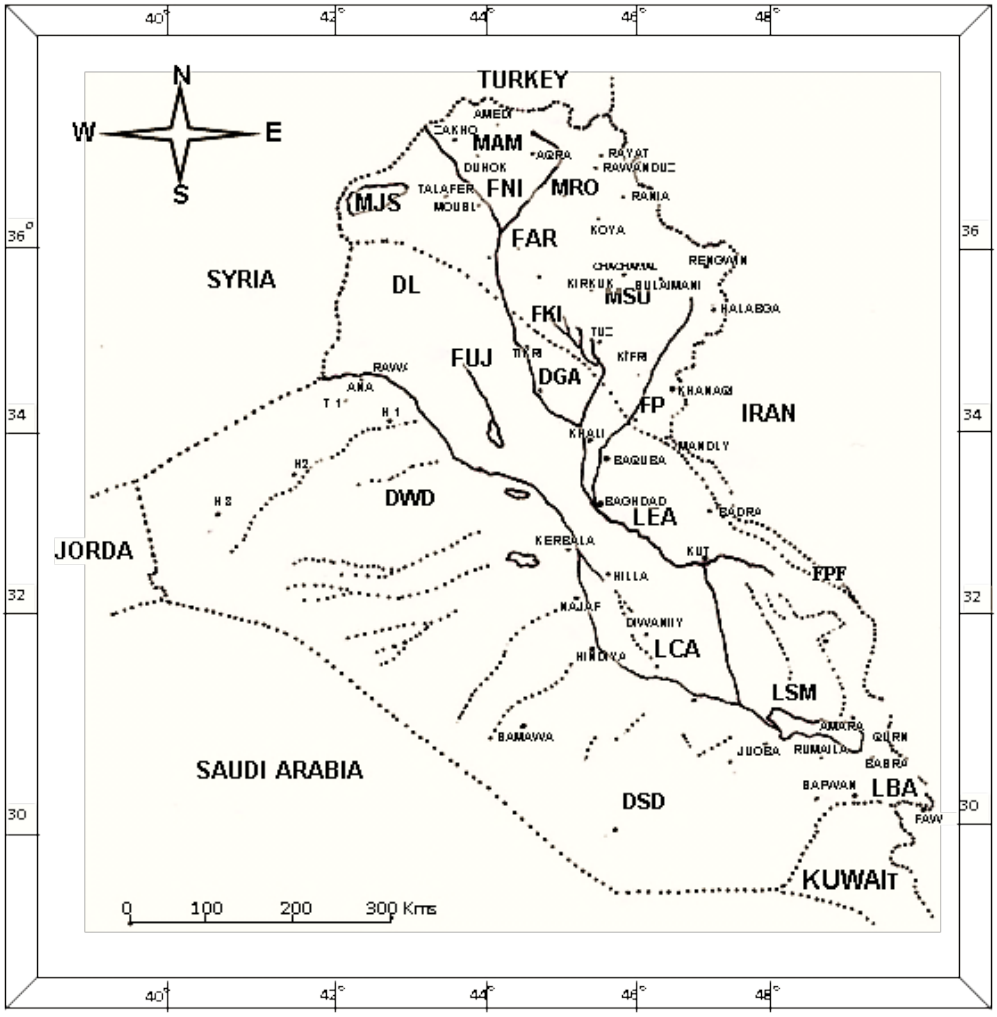

Figure 1: Physiographic regions and Districts map of Iraq

M - MOUNTAIN REGION	F - UPPER PLAINS AND FOOTHILLS REGION
MAM - Amadiya District	FUJ- Upper Jaziera District
MRO - Rowanduz District	FNI- Nieneveh District
MSU - Sulaimani District	FAR- Arbil District
MJS - Jabal Singar District	FKI- Kirkuk District
	FPF- Persian District
D - LOWER PLATEAU REGION	L - LOWER MESOPOTAMIAN REGIO
DLJ - Lower Jaziera District	LEA- Eastern Alluvial Plain District
DGA- Ghurfa - Adhaim District	LCA- Central Alluvial Plain District
DWD - Western Desert District	LSM- Southern Marsh District
DSD- Southern Desert District	LBA- Basra Estuarine District

Physiographic regions and districts of Iraq

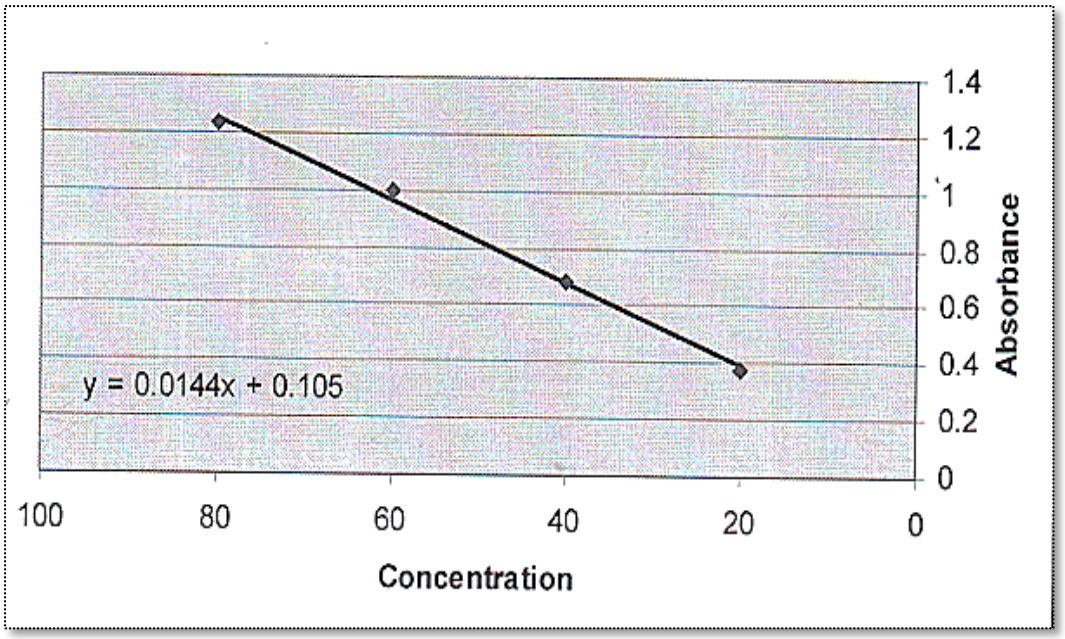

Figure 2: Glucose standard curve

Table 1: Distribution and altitudes of *Geranium* species

Species	Districts							Altitude (m)
	MAM	MRO	MSU	MJS	FNI	FKI	FPF	
Geranium dissectum	*	*	*	*	*	*	*	200-1550
G. kurdicum		*						1300-1550
G. lucidum	*	*	*	*		*	*	400-1550
G. molle	*	*	*	*			*	450-1300
G. purpureum	*	*	*				*	750-1400
G. pusillum	*	*	*					700-1400
G. rotundifolium	*	*	*	*		*	*	250-1400
G. stepporum	*	*	*				*	800-1600
G. tuberosum	*	*	*					800-1600

Table 2: Distribution and altitudes of *Orobanche* species

Spp.	Districts							Altitude (m)
	MAM	MRO	MSU	MJS	FNI	FKI	FPF	
Orobanche aegyptiaca	*	*	*	*	*	*	*	250-1200
O. anatolica				*				1100-1600
O. arenaria				*				1400-1650
O. coelestis		*	*					600-1400
O. crenata	*		*					1400-1900
O. kurdica	*		*					1000-1600
O. mutilii							*	250-550
O. ovata		*	*					1400-1800
O. ramosa	*				*			500-850
O. singarensis	*	*					*	400-1300

3. Results and Discussion

The results (table 3 and 4) showed a differences in soluble carbohydrate content of all samples included species, which extends between 19.618-87.84mg/gm in *Geranium* species and 33.506-174.479mg/gm in *Orobanche* species, the lowest carbohydrate content was recorded in *G. purpureum* (19.61 mg/gm) and the highest in *G. rotundifolium* (87.84 mg/gm) of *Geranium* species as well in *Orobanche* the lowest amount was determined in species *O. aegyptiaca* (33.50mg/gm) and the highest in *O. kurdica* (174.47 mg/gm), based on the differences in carbohydrate estimation, the *Geranium* species were divided into three groups: group with low carbohydrate

content (less than 35mg/gm) included *G. dissectum G. Lucidum, G. pusillum* and *G. purpurium*, medium content group (35-60mg/gm) involved, *G. stepporum, G. kudicum* and *G. tuberosum* and high content group (more than 60mg/gm) they are *G. molle* and *G. rotundifolium* as well the *Orobanche* species were divided also into three groups: low carbohydrate content group (less than 60mg/gm) included *O. aegyptiaca, O. ovata, O. arenaria* and *O. ramose*, medium content group (60-90mg/gm) includes *O. mutilii, O. crenata* and *O. coelestis* and group with high content (more than 90mg/gm) involved *O. singarensis, O. kurdica* and *O. anatolica.*

Table 3: Carbohydrate estimation of *Geranium* species

Species	Absorb	Dil. 1	Dil. 2	Y-0.105	(Y-0.105)/0.0144	Dil. 1xDil. 2	mg. soluble carbohydrate/gm. dry weight	µg/gm
Geranium rotundifolium	1.37	500	2	1.265	87.8472222	87847.2222	87.84722222	878.4722
G. dissectum	0.77	500	1	0.665	46.18055556	23090.2778	23.09027778	230.9028
G. stepporum	1.14	500	1	1.035	71.875	35937.5	35.9375	359.375
G. purpurium	0.67	500	1	0.565	39.2361111	19618.0556	19.61805556	196.1806
G. lucidum	0.79	500	1	0.685	47.56944444	23784.7222	23.784772222	237.8472
G. pusillum	0.83	500	1	0.725	50.3472222	25173.6111	25.17361111	251.7361
G. tuberosum	0.94	500	2	0.835	57.9861111	57986.1111	57.98611111	579.8611
G. molle	1.04	500	2	0.935	64.9305556	64930.5556	64.93055556	649.3056
G. kudicum	1.39	500	1	1.285	89.2361111	44618.0556	44.61805556	446.1806

The variation in carbohydrate quantity indicates to that these species differ in their metabolic activity that in turn is controlled by genetic factors as well as environmental conditions (soil, weather and altitude), which needs further researches and investigation, this study is the first one submitted a description for the carbohydrate content of the included *Orobanche* species, especially in *O. aegyptiaca* and *O. ramosa* which can be used in separation them and to solution of their complex (Species Complex) [12], and between *O. aegyptiaca* and *O. mutilii* [11], on the other hand the taxonomic problem between *O. mutilii* and *O. ramosa*, besides they presence in two different groups which indicates their differences taxonomically nevertheless there are those who see the species *O. mutilii* is subspecies of *O. ramosa* [11], *O. aegyptiaca* show many

morphological variations (plant size, flower color, and flowering) due to growth conditions, which makes the morphological characters in such cases are a difficult criterion to deal with in this field. The phytochemistry of *Geranium* species is reasonably well known today [3], according to Hegnauers dictionary of plant chemistry [6 and 7] at least 55 species have been investigated chemically, most of chemical studies of the *Geranium* are specific for flavonoids, phenols and other compounds, so this study also may be the first one in Iraq which interested in quantitative carbohydrate content of *Geranium*, although there is a significant difference morphologically between *G. dissectum* and *G. lucidum* in their leaf outline, stem branch and size, however they are in the same group as low carbohydrate content (less than 35mg / gm), as well as the two species *G. pusillum* and *G. purpurium* which are different in morphology but they fall into the same group, then it can't rely mostly on morphological characters in classification process, the phytochemical studies a good alternative for taxonomic studies.

Table 4: Carbohydrate estimation of *Orobanch* species

Species	Absorb	Dil. 1	Dil. 2	Y-0.105	(Y0.105)/0.0144	Dil. 1 x Dil.2	mg. soluble carbohydrate/gm. dry weight	µg/gm
Orobanch ovata	1.2	500	1	1.095	76.04166667	38020.8333	38.02083333	380.2083
O. singarensis	0.79	500	4	0.685	47.56944444	95138.8889	95.13888889	951.3889
O. anatolica	1.27	500	4	1.165	80.90277778	161805.556	161.8055556	1618.056
O. aegyptiaca	1.07	500	1	0.965	67.01388889	33506.9444	33.50694444	335.0694
O. kurdica	1.11	500	5	1.005	69.79166667	174479.167	174.47911667	1744.791
O. arenaria	1.2	500	1	1.095	76.04166667	38020.8333	38.02083333	380.2083
O. crenata	1.01	500	2	0.905	62.84722222	62847.2222	62.84722222	628.4722
O. ramose	1.56	500	1	1.455	101.0416667	50520.8333	50.52083333	505.2083
O. coelestis	0.67	500	4	0.565	39.23611111	78472.2222	78.47222222	784.7222
O. mutilii	0.55	500	4	0.445	30.90277778	61805.5556	61.80555556	618.0555

4. Conclusion

This study concluded that the differences in soluble carbohydrate content of all tested plant samples extends between 19.618-87.84mg/gm in *Geranium* species and 33.506-174.479mg/gm in *Orobanche* species, the lowest carbohydrate content was recorded in *G. purpureum* (19.61 mg/gm) and the highest in *G. rotundifolium* (87.84 mg/gm) of *Geranium* species as well in *Orobanche* the lowest amount was determined *O. aegyptiaca* (33.50mg/gm) and the highest in *O. kurdica* (174.47 mg/gm), based on carbohydrate content, the studied species can divided into three groups in both tested species (high content, medium content and low content), that be useful in chemical classification process of the species and may succeed in solving some of the taxonomic problems among convergent morphological species .

5. Recommendations

This work recommends additional researches include other investigations in fields of chemotaxonomy to solving some problems in classification process among plant species.

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