IRAQI Academic Scientific Journals
Samarra Journal of Pure and Applied Science
SJPAS
مجلة سامراء للعلوم الصرفة والتطبيقية
www.sjpas.com
p ISSN: 2663-7405
e ISSN: 2789-6838

Study of preliminary qualitative screening and evaluation of alkaloids and flavonoids compounds of some Orobanche species growing in Iraq

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https://doi.org/10.54153/sjpas.2024.v6i2.698

Article Information
Received: 29/8/2023
Revised: 8/9/2023
Accepted: 30/10/2023
Published:30/6/2024

Keywords:
Orobanche, Alkaloids Phytochemicals, Phenols, Iraq.

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Abstract

Among 27 Orobanche species growing in Iraq, only *O. aegyptiaca* and *O. ramosa* (recently *Phelipanche aegyptiaca* and *P. ramosa*) have been studied phytochemically. Preliminary phytochemical screening revealed the presence of alkaloids, phenols, flavonoids, terpenes, resins, steroids, saponins (present or absent) and tannins in the areal parts of all tested species. HPLC results indicated the presence of six alkaloid compounds (oxo sparteine, isosparteine, retamine,13-hydroxylupanine, lupanine and sparteine) and three phenolic compounds (isoverbascoside,caffeic acid and verbascoside) in all *Orobanche* s.l.taxa investigated . These parasitic taxa showed high amounts of the alkaloid Lupanine and the phenylpropanoid verbascoside. In this study, *O.ramosa* is the richest broomrape species in secondary metabolites.

Introduction:

The parasitic flowering plants belong to 4500 species in 17 -30 families and represent nearly 1% of all described angiosperm species '[1-4]'. Orobanchaceae is the largest family of parasitic plants, comprising 90-115 genera and more than 2000 species '[5, 6]'. *Orobanche* (broomrape) is the largest genus in Orobanchaceae, comprising 100- 200 non – photosynthetic root holoparasitic species, mostly native to the Northern Hemisphere '[7-16]'. One of the main characteristics of *Orobanche* is the formation of the haustorium, the specialized organ that forms a structural and functional feature between the host and parasite and by which the parasite can draws water, nutrients and assimilates from the roots of the host plants and grows at the expense of its host '[17, 18]'. Some *Orobanche* species, Such as *O. aegyptiaca* (=*Phelipanche aegyptiaca*), *O. ramose* (= *P. ramose*) and *O. crenata* are considered noxious and destructive parasitic weed in cultivated crops (such as those belong to Solanaceae, Fabaceae, Cucurbitaceae, Asteraceae, Brassicaceae) '[3,15,18,19]' Most published papers on Orobanche species is focused on seed germination stimulants, host – parasite

interactions and parasitic weed control while little is known about their phytochemical components '[8,16,20,21]'. Scharenberg & Zidorn mentioned that of the 200 species of *Orobanche* s.l (*Orobanche* and *Phelipanche* species), only 27 species were studied for active compounds '[8]'. However, several recent reports exhibited the presence of different classes of secondary metabolites in *Orobanche*, including aromatic aldehyde, ketones, phenylethanoids, phenylmethanoids, phenylethanoid glycosides, phenylpropanoid glycosides, phenolic acids, lignans, flavonoids, tropone derivatives, sterols, sesquiterpenes, iridoid glycosides, mono and bicyclic monoterpenes, carotenoids, alkaloids and tannins '[8,16]'. Phenylpropanoid glycosides (like orobanchoside, verbascoside, isoverbascoside) are the prominent group of bioactive compounds in the genus *Orobanche* '[8,16]'. Orobanchaceae was represented by about 27 species in Iraq '[22-30]' However, phytochemical information for Iraqi parasitic flowering plants is very limited. In Iraq, only *O.aegyptiaca* and *O.ramosa* have been previously investigated phytochemically '[31]'.

Materials and methods

Plant collection and identification

Plant samples (*Orobanche* / host) at flowering stage were collected from different sites in Salahaddin (North central Iraq) and sulaymaniyah (North east Iraq) governorates during April-July 2019. *Orobanche* samples were identified based on morphological (by Iraqi National Herbarium /BAG) and molecular analysis (by Macrogen,Inc /south korea) while host samples were identified by BAG . All identified samples were kept in Biology Department / College Education for Pure Sciences / Tikrit University.

Preparation of specimens

After *Orobanche* collection, aerial part samples were air dried and powdered by electrical mill and kept until use.

Preliminary phytochemical screening

Phytochemical test was employed on the preliminary phytochemical screening for different secondary metabolites such as alkaloids (Wagner test), flavonoids (H_2SO_4 test), phenols (terpenes, resins, tannins, steroids (salvoski test) and saponins (Foam test) '[32]'.

Apparatus and Chromatographic conditions

The separation of alkaloids carried out on liquid chromatography Shimadzu 10AV-LC equipped with binary delivery pump and the eluted peaks monitored by spectrophotometer. Column,3µm particle size, Phenomenex C-18 (50 × 4.6 mm I.D),

A mobile phase;

solvent A was 0.01M phosphate buffer, pH 6.2, solvent B acetonitrile using linear gradient from 0% B-100 % B for 10-minute, detection UV set at 297 nm, flow rate 1.4 ml / min, temperature 30^{o} C, each standard at 25 µg/ml, and the dilution factor 25. For phenolics, the separation done in the same apparatus used in separation of alkaloid. Column,3µm particle size, Phenomenex C-18 (50 × 4.6 mm I.D), mobile phase, solvent A 0.1M phosphoric acid, pH

6.2, solvent B (6:3:1, v/v) of acetonitrile : methanol :0.1% phosphoric acid gradient program from 0% B-100 % B for 15 minute, detection UV set at 280 nm, flow rate 1.2 ml / min, temperature 30° C, each standard was 25 µg/ml, dilution factor 25. Samples were measured at the Ministry of Science and Technology by HPLC / Shimadzu company 10AV-LC.

Extraction of alkaloids and sample preparation

1g of *Orobanche* powder macerated several times with 25 ml methanol for 1 hour. The extract then filtered and evaporated using rotary evaporator at a temperature of 45° C. The residue then treated with 25 ml HCl solution (2% v/v), filtered and extracted several times with 10 ml petroleum ether to remove pigments and fat, the aqueous acid layer then basified to pH 10 by ammonia and extracted three times with 25ml chloroform. The organic solvent then evaporated to yield a total alkaloid extract of 0.17g, then the obtained residue dissolved in 10 methanol and filtered through 0.45 µm polypropylene filter. A 20 µL aliquot injected into HPLC column under optimum separation condition compared with standard '[33]'. 'Table 1'. The concentration was determined by comparing between area and retention time of standard with the sample using the same separation conditions, and calculated by the equation:

Con.of sample µg/ml = Area of sample / Area of standard × conc. Of standard ×dilution factor. '[33]'

Table 1: alkaloids standards with retention time, area and concentration.

No	Alkaloids	Retention time (min)	Area UV	Concentration 25 µg /ml
1	Sparteine	1.83	102802	25
2	Lupanine	2.97	113041	25
3	13-hydroxy lupanine	3.88	122389	25
4	Retamine	5.08	116484	25
5	Isosparteine	5.97	134318	25
6	Oxo sparteine	6.81	131350	25

Extraction of Phenolics and sample preparation

1g of *Orobanche* powder was dissolved in 20 ml hexane to exclude fat layer, then the organic layer dissolved in 100 ml of 80 % methanol and the extract was exposed to ultra-sonication (Branson sonifer, USA) at 60% duty cycle for 25 min at 25° C then centrifuged at 7500 rpm for 15 min, the clear supernatant of each sample was subjected to charcoal to remove pigments before evaporation under vacuum (Buchi Rotavapor Re Type). Dried specimens were re-suspended in 1.0 ml HPLC grade methanol by vortex. The mixture passed through 2.5 µm filter and kept at 4° C until use then 20 µl of sample injected into HPLC system according the optimum condition. The concentration for each constituent was calculated by comparing the peak area and retention time of standard with sample 'Table 2'under the same condition '[34]'.

Table 2: Phenolics standards with retention time, area and concentration.

No	Alkaloids	Retention time (min)	Area UV	Concentration 25 µg /ml
1	Verbascoside	1.97	157645	25
2	Caffeic acid	3.46	152496	25
3	Isoverbascoside	4.21	175414	25

- The equation of flavonoids calculation is the same of alkaloids'[34]'

Result and discussion

Preliminary phytochemical tests on the seven *Orobanche* species indicated the presence of alkaloids, flavonoids, tannins, phenolics, steroids, terpenes, resins and saponins 'Table 3'. This confirms the previous reports that mentioned the presence of these secondary metabolites in *Orobanche* species '[8,16,35,36]'. Of the seven species studied, only *O.alba* ,*O.anatolica and O.ramosa* gave a negative test for saponins 'Table 3' In this regard , several studies indicated the absence of saponins in some *Orobanche* members '[37,38]'. The HPLC results revealed the presence of six alkaloid compounds (oxosparteine, isosparteine, retamine,13-hydroxylupanine, lupanine and sparteine) belong to quinolizidine group 'Table 4', Fig. 1 and three phenolic compounds (isoverbascoside,caffeic acid and verbascoside) 'Table 5', Fig.2 in all parasitic taxa investigated These taxa showed varied amounts of alkaloid and phenolic compounds . In all studied parasitic plants, lupanine showed the highest concentrations compared to other identified compounds where the highest content of this alkaloid was found in *O.ramosa* (1671.2 µg/ml) and the lowest was in *O.anatolica* (950.1 µg/ml) . Compared to other compounds, 13-hydroxy lupanine (342.3 µg/ml and retamin (380 µg/ml) showed the lowest concentrations in all *Orobanche* species studied. Results indicated that the alkaloid content of the studied *Orobanche* species was influenced by host species (as in case of *O.crenata* growing on different hosts), parasite species (as in case of *O.cernue* and *O.crenata* parasitizing the same host) and by geographical distribution and climate (as in case of *O.crenata* growing in different districts and climate). In general *Phelipanche* species (formerly *O.aegyptiaca* and *O.ramosa*) showed more total alkaloid contents (5188 – 5532.6 µg/ml)than those of *Orobanche* species (3571.5 – 5113.2 µg/ml) . However, *O.crenata* ,*O.aegyptiaca* and*O.ramosa* , the most serious parasitic weeds , showed the highest alkaloid contents compared to rest of the parasitic species studied . In regard to phenolic compounds 'Table 3' Verbascoside in general showed the highest concentrations in all studied plant species while caffeic acid showed the lowest. Compared with rest of the species, *O.ramosa* (1218.5µg/ml) and *O.reticulata* (1247.9µg/ml). exhibited the highest amount of verbascoside . Table 3 showed that *O.cernua* (2558.9 µg/ml) and *O.ramosa* (2482.1 µm/ml) contained the highest phenolic contents compared to other species investigated. However, all studied *Orobanche* taxa especially *O.ramosa* represent a rich source of active compounds. According to literatures, alkaloids from quinolizidin group and phenylpropanoids are the most promenant secondary metabolites in the genus *Orobanche* s.l.

'[8,16,20,31,35,36,39,40,41]'. These metabolites were reported to include several biological activities such as antioxidant, antimicrobial, anti-inflammatory and antitumor '[8,16]'.

Table 3: phytochemical screening of *Orobanche* species.

No	Species	Flavonoids	Alkaloids	Phenols	Terpenoid	Resins	Saponin	Tannin	Steroid
1	*O. alba*	+	++	+	++	++	-	++	+
2	*O.aegyptica*	++	+	++	+	+	+	+	+
3	*O.anatolica*	+	+	++	++	+	-	++	+
4	*O.cernua*	++	+	++	+	+	+	++	+
5	*O.crenata*	+	+	++	+	+	+	++	+
6	*O.ramosa*	+	+	+	++	+	-	+	+
7	*O.reticulata*	+	+	++	++	+	+	++	++

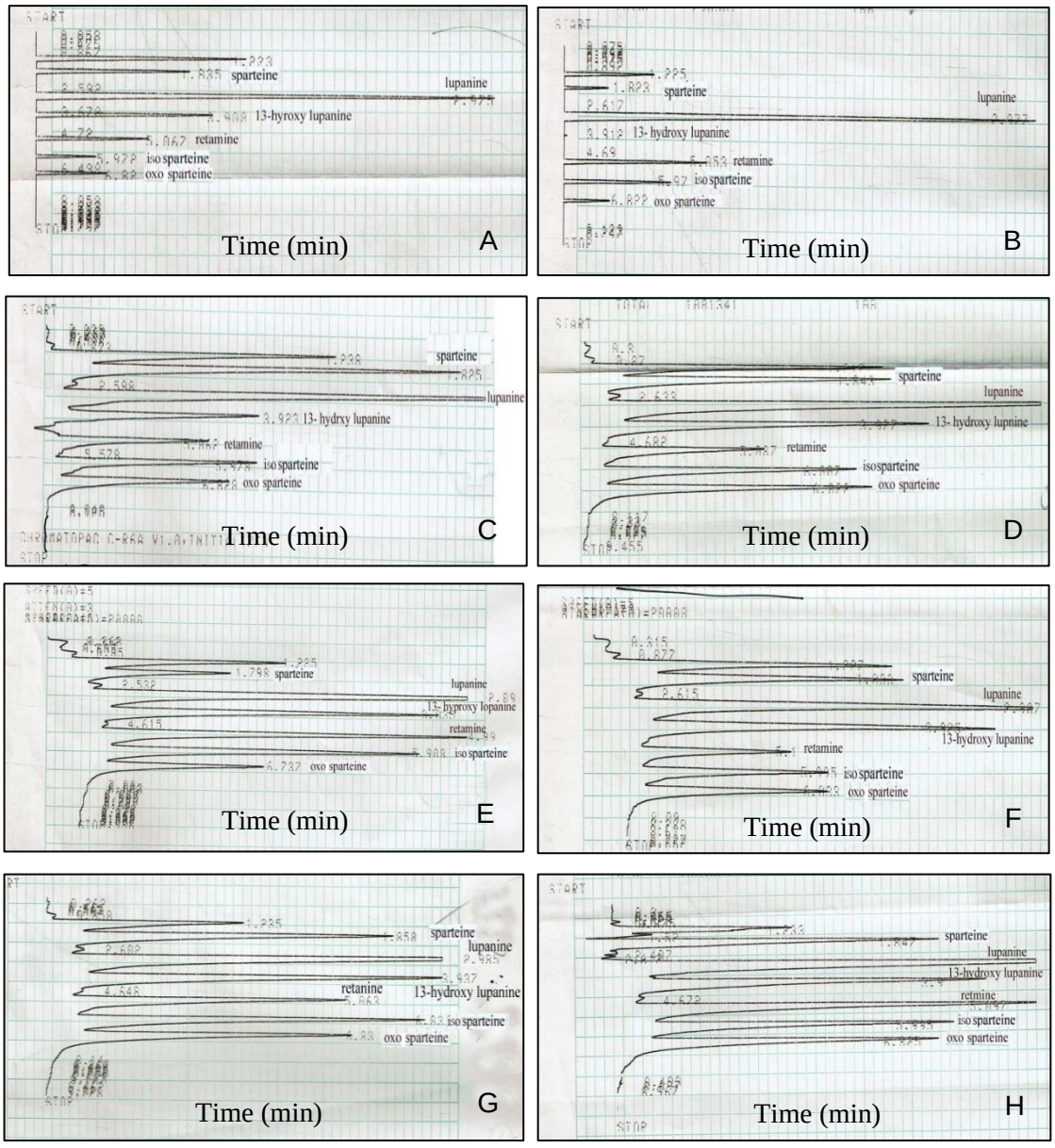

Fig. 1 chromatograms of Quinolizidine alkaloid (QA) in *Orobanche* species. E,F,G and H : are *O.anatolica*, *O.reticulata*, *O.crenata, O.aegyptica and O.ramosa* respectively.

Table 4: Quinolizidine alkaloid (QA) compounds in *Orobanche* species.

No	Species	Host	Oxo sparteine	Isosparteine *	Retamine	13-hydroxy lupanine	Lupanine	Sparteine	Total concentration
1	*O.alba*	*Vicia cracca*	527.5	452.7	**380.9**	668.2	1373.9	661.9	4064.5
2	*O.aegyptica*	*Solanum melongena*	677	711.4	697.2	908.2	1354	839.9	5188
3	*O.anatolica*	*Artemisia sp*	507.4	386.2	483.8	602.1	950.1	669.8	3599.4
4	*O.cernua [1]*	*Lactuca serriola*	427.4	469.4	610.6	342.3	1236.7	485	**3571.5**
5	*O.crenata [2]*	*Lactuca serriola*	428.7	410	414.2	854.3	1139.1	735.4	3982
6	*O.crenata [3]*	*Vicia cracca*	449.4	989.4	953.9	806.7	1447	466.8	5113.2
7	*O.ramosa*	*Solanum lycopersicon*	703.7	649.1	971.9	798.3	**1671.2**	738	**5532.6**
8	*O.reticulata*	*Vicia cracca*	436	472.5	459.3	708.6	1642.7	745.4	4464.8

*: conc µg /ml.;1. Collected from Salahaddin Province.; 2. Collected from Penjwin / Sulymaniyah provence. ; 3. Collected from Piramagrun mountain / Sulymaniyah provence.

Table 5: phenolic compounds in *Orobanche* species.

No	Species	Host	Isoverbascoside *	Caffeic acid	Verbascoside	Total concentration
1	*O.alba*	*Vicia cracca*	492.4	251.7	476.8	**1221**
2	*O.aegyptica*	*Solanum melongena*	403.6	326.8	883.3	1613.9
3	*O.anatolica*	*Artemisia sp*	**245.5**	484	744	1470.6
4	*O.cernua* [1]	*Lactuca serriola*	634.2	486.1	1138.4	**2558.9**
5	*O.crenata* [2]	*Lactuca serriola*	424.5	289.9	990.6	1705
6	*O.crenata* [3]	*Vicia cracca*	309.9	418	986.4	1714.3
7	*O.ramosa*	*Solanum lycopersicon*	754.1	509.4	1218.5	2482.1
8	*O.reticulata*	*Vicia cracca*	584.5	474.1	**1247.9**	2306.6

* : conc µg /ml.; 1. Collected from Salahaddin Province.; 2. Collected from Penjwin / Sulymaniyah provence. ; 3. Collected from Piramagrun mountain / Sulymaniyah provence.

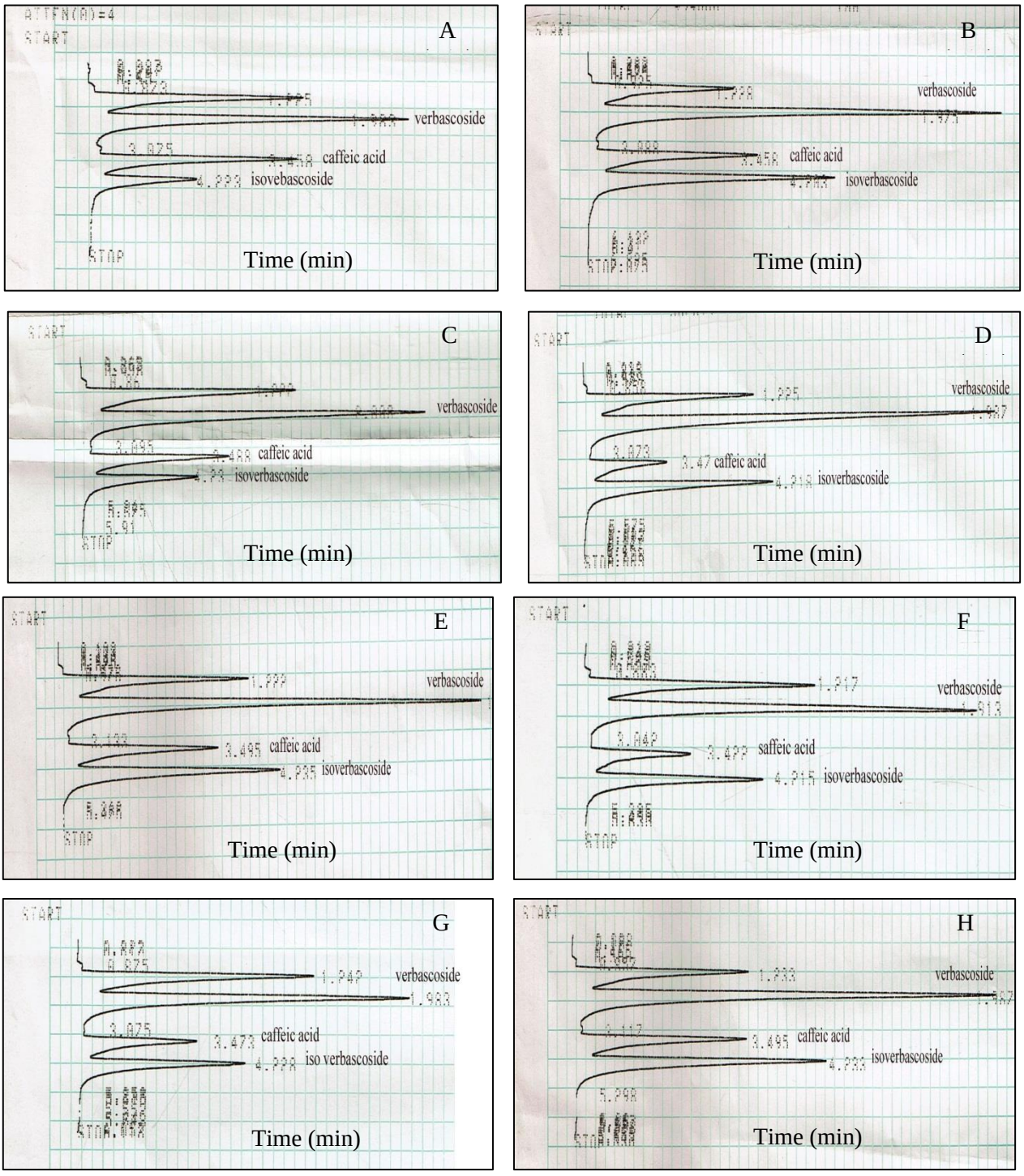

Fig. 2 chromatograms of phenolic compounds in *Orobanche* species.,E,F,G and H : are *O.reticulata*, *O.crenata*, *O.aegyptica* and *O.ramosa* respectively.

Conclusion

This study reports for the first time detailed information on the phytochemicals of seven Orobanche s.l. taxa (*O.aegyptiaca ,O.alba ,O.anatolica ,O.cernua ,O.crenata,O.ramosa* and *O.reticulata*) growing in Iraq. These taxa showed variety of phytochemicals including alkaloids (such as sparteine, retamine and lupanine) phenolics (such as isoverbascoside and verbascoside), sterols, tannins, saponins (present or absent) and flavonoids. All studied species, especially *O.ramosa* are rich in active metabolites which could be responsible for the diverse biological activities of these plants .

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مجلة سامراء للعلوم البحتة والتطبيقية
www.sjpas.com
p ISSN: 2663-7405
e ISSN: 2789-6838

دراسة الكشوفات الأولية الاستدلالية وتقدير مركبات القلويدات والفلافونويدات في بعض أنواع الهالوك النامية في العراق

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معلومات البحث:
تأريخ الاستلام:
تاريخ التعديل :
تأريخ القبــول:
تاريخ الـنشر:
الكلمات المفتاحية:
الهالوك، القلويدات، المحتوى الكيمونباتي، الفينولات، العراق

معلومات المؤلف
الايميل:
الموبايل:

الخلاصة:
من بين 27 نوع من الهالوك النامي في العراق فقط الهالوك المصري *O.aegyptica* والمتفرع *O.ramosa* ومؤخرا الفلبانكي المصري *Phelipanche aegyptica* والمتفرع *P.ramosa* قد درس محتواهم الكيميائي. الكشوفات الاستدلالية قد كشفت احتواء الأجزاء الهوائية لانواع الدراسة على القلويدات والفينولات والفلافونويدات والتربينات والراتنجات والستيرويدات والصابونيات والتانينات. وبأستخدام تقنية الكروماتوغرافيا السائلة عالية الأداء HPLC تم الكشف عن ستة مركبات قلويدية (oxo sparteine وisosparteine و13,retamine-و hydroxylupanine وlupanine وsparteine) وثلاثة مركبات فينولية (isoverbascoside وcaffeic acid وverbascoside) في كل عينات الدراسة وبتراكيز مختلفة. هذه الطفيليات الزهرية أظهرت احتوائها على تراكيز عالية من المركب القلويدي lupanine والمركب الفينولي phenylpropanoid: verbascoside. في هذه الدراسة تبين ان النوع O.ramosa هو الهالوك الاغنى بالأيضات الثانوية.