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CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF THE ESSENTIAL OIL AND FLAVONOIDS OF *PHLOMIS FLOCCOSA* D. DON.

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The present study aimed to investigate the antioxidant activities of essential oils and different extracts from *Phlomis floccosa* parts. Where the essential oil was detected in the leaves of *Phlomis floccosa* only, while it was absent in stem and flower parts. The hydro-distillation of the leaves of *Phlomis floccosa* yielded 0.18% of clear yellowish liquid oil and it didn´t has antioxidants activity. On the other hand, the methanol extracts of different parts of the plant (stems, leaves and flowers) showed high free radical scavenging activity. The DPPH radical scavenging activity of methanol extracts probably related to their flavonoids contents. Hence, methanol extract was analyzed by using HPLC, which revealed that, the plant contained 21 flavonoid compounds in different plant parts (stems, leaves and flowers). The major detected compound of flavonoids was luteolin-6- glucose -8- arabinose with percentages of 1234.3 mg/100 g, 1098.2 mg/100 g and 440.17 mg/100 g for stems, leaves and flowers of *Phlomis floccosa*, respectively. The second major compound detected in stems and flowers was luteolin-6- arabinose -8- glucose (144.47 mg/100 g and 375.3 mg/100 g, respectively). On the other hand, the second major compound detected in leaves was naringenin (278.13 mg/100 g). According to the obtained results, the methanolic extract of *Phlomis floccosa* can be considered as an interesting source of natural antioxidant.

Keywords: *Phlomis floccosa*, antioxidant, essential oil, flavonoids, DPPH scavenging activity

Phlomis floccosa belonging to family Lamiaceae, which includes about 200 genera and 3000 species. Several studies have reported on the antioxidant activity of the Lamiaceae growing in various regions of the world (Barros et al., 2010 and Kamatou et al., 2010). Antioxidants are

important in reducing heart disease and could prevent damage of DNA in human. Many synthetic antioxidant components have toxic and/or mutagenic effects, which have attracted most of the attention on the natural source of antioxidants. Plants are sources of natural antioxidant, because they contain phenolic compounds; such as phenolic acid, flavonoids, tannins and anthocyanins (Azad et al., 2015). Yumrutas and Saygideger (2012) showed that, the methanol extract of *Phlomis armeniaca* has high activities in antioxidant assays due to the phenolic content. Delnavazi et al. (2014) demonstrated that, the plants with terpene rich in their oils were suggested as potential sources of free radical-scavenging compounds. Phytochemical investigations of the *Phlomis* species has been the subject of several studies, which resulted in the isolation of essential oils, flavonoids, iridoids, phenylethylalcohol glycosides and other components and with flavonoids being the major phyto-constituents, isolated from the *Phlomis* genus (Amor et al., 2009 and Li et al., 2010). The leaves of *Phlomis aurea* proved to contain the 7-glucosides, 7-rutinosides and 7-*p*-coumaroylglucosides of naringenin, apigenin, luteolin and chrysoeriol, hispidulin 7-glucoside, luteolin 7-diglucoside, vicenin-2 and lucenin-2. The microscopic hairs on the leaves only contained the 7-monoglucosides and their acylated derivatives. *Phlomis floccosa* showed a similar flavonoid pattern, but with no flavanones (El-Negoumy et al., 1986). This research aimed to determine the antioxidant activity of essential oil and compare antioxidant activity of the different extracts of *Phlomis floccosa* to determine the highest antioxidant activity extract of different plant parts, which it was analyzed by HPLC.

MATERIALS AND METHODS

1. Plant Material

Phlomis floccosa was collected at full flowering stage from Mersa-Mattruh during April 2011, separated to its different parts (stems, leaves and flowers). An amount of 200 g of each fresh plant part was used to obtain the essential oils. Meanwhile, all collected parts of the plant were air dried, ground to fine powder and kept for further investigations.

2. Isolation of the Essential Oils

Fresh stems, leaves and flowers of *Phlomis floccosa* (200 g) were hydro-distilled in a cleveger-type apparatus (Denny, 1989). After 3 hours of distillation, the essential oils were separated from water, stored in sealed dark glass vials and kept in refrigerator for further analysis.

3. Extraction of Total Extract

About 80 g from each of the stems, leaves and flowers were extracted with 70% methanol. The obtained residue from each part was dried and weighed.

4. Extraction Using Different Organic Solvents

Successive extraction technique

About 700 g of the stems, leaves and 250 g of the flowers were subjected to extraction with successive selective organic solvents using soxhlet apparatus, in order of increasing polarity including petroleum ether (b.p. 40-60 ºC), chloroform, methanol and 50% methanol. The obtained residue from each solvent was dried and weighed.

5. Determination of Free Radical Scavenging Activity

Free radical scavenging assay using 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) was carried out according to Yildirim et al. (2001). This method is based on the reduction of alcoholic DPPH solution. The odd electron of DPPH shows an absorption maximum at 517 nm (purple color). As the odd electron of the radical becomes paired off in the presence of a hydrogen donor (the antioxidant), the absorption strength vanishes, and the resulting de-colorization is stoichiometric with respect to the number of electrons captured.

Reagents

1 mM 1, 1 Diphenyl-2-picrylhydrazyl (DPPH) (Sigma). A weight of 0.394 g DPPH was dissolved in 1L of 95% ethyl alcohol.

Ascorbic acid (vitamin C).

Procedure

Different concentrations (125, 250, 500 and 1000 µg/ml) of each tested extract, volatile oil and ascorbic acid as a reference antioxidant (reference control) were prepared in 80% (v/v) ethanol. A volume of 3 ml from each extract and ascorbic acid concentrations were mixed with 1 ml of 1 mM of DPPH radical. A control tube was prepared by mixing 3 ml of 80% ethyl alcohol with 1 ml of alcoholic solution of DPPH radical. The tubes were kept at room temperature in the dark for 30 min. The degree of disappearance of purple color was measured against blank (80% ethyl alcohol) at 517 nm.

Calculation

$$\% \text{ Scavenged DPPH} = \frac{\text{Absorbance of control} - \text{Absorbance of extract}}{\text{Absorbance of control}} \times 100$$

IC_{50} was defined as the concentration that reduced 50% DPPH free radicals compared with the reference antioxidant compound, and calculated by means of Graph Pad Prism software (Ver. 4).

6. Qualitative and Quantitative Analysis of the Highest Antioxidant Activity Containing Extracts by HPLC

The highest antioxidant activity containing extract of different parts of *Phlomis floccosa* were analyzed by HPLC. The analytical HPLC system

employed consisted of HP 1090M Series II high performance liquid chromatography equipped with an HP 1090M Series II diode array and an eight-channel electrochemical colorimetric array detector (EC; Esa Inc., USA). The EC was operated using 100-800 mV potentials (100 mV intervals). The detector array was housed in a temperature-regulated compartment at 35ºC. Flavonoid separation was done by ODS-3 (4.0 × 150 nm, 3 µm) column with a C-18 guard column, with temperature set at 35ºC. The flow rate of the mobile phase was 0.7 ml/min, and the injection volumes were 10 µl of the standards and sample extracts. All flavonoids were quantified using the external standard method. Quantification was based on peak area (DAD) or beak height (EC) (Mattila et al., 2000).

RESULTS AND DISCUSSION

1. Essential Oils

The essential oil was detected in the leaves of *Phlomis floccosa* only, while it was absent in stem and flower parts. The hydro distillation of the leaves of *Phlomis floccosa* yielded 0.18% of clear yellowish liquid oil.

2. Extract Residues of Different Parts of *Phlomis floccosa*

The obtained data show that, total extract residues (70% methanol) of stems, leaves and flowers of *Phlomis floccosa* were 8.36, 15.0 and 14.62%, respectively. The successive extraction using different organic solvents revealed that, the methanol extract residues had the highest values of 4.75, 5.36 and 7.51%, obtained from stems, leaves and flowers, respectively (Table 1).

Table (1). Successive extract residues (%) of stems, leaves and flowers of *Phlomis floccosa*.

Solvent used	Residue percentage (%)		
	Stems	Leaves	Flowers
Petroleum ether	1.17	4.77	2.40
Chloroform	1.18	1.03	1.50
Methanol	4.75	5.36	7.51
Methanol (50%)	2.80	3.04	4.95

3. Free Radical Scavenging Activity of Different Parts of *Phlomis floccosa*

Antioxidant activity of volatile oil and for each of the stems, leaves and flowers different extracts of *Phlomis floccosa* were illustrated in tables (2, 3 and 4) and figs. (1, 2 and 3).

Table (2). The percentages of scavenging activities for total extract and successive extraction residues of *Phlomis floccosa* stems.

Concentration (µg/ml)	% Sacvenging of total extract	% Scavenging of petroleum ether fraction	% Scavenging of chloroform fraction	% Scavenging of methanol fraction	% Scavenging of 50% methanol fraction	% Scavenging of ascorbic acid
1000	94.411± 0.320	78.368± 0.579*	86.215± 0.167	91.907± 0.229	86.372± 0.898	97.167± 0.177
500	93.033± 0.100	59.765± 0.883*	83.307± 0.132	89.367± 0.308	83.517± 0.547	96.230± 0.165
250	87.834± 0.184	39.535± 0.423*	59.713± 0.160	81.205± 0.577	74.114± 0.264	94.100± 0.211
125	81.000± 0.058	18.620± 0.369*	36.539± 0.219	69.413± 1.130	62.512± 0.421	90.500± 0.188

Values are given as mean ± S. D. (n=3). *Significant at $p < 0.05$, p-value was calculated by comparing with standard (ascorbic acid) by ANOVA followed by Dunnett's test.

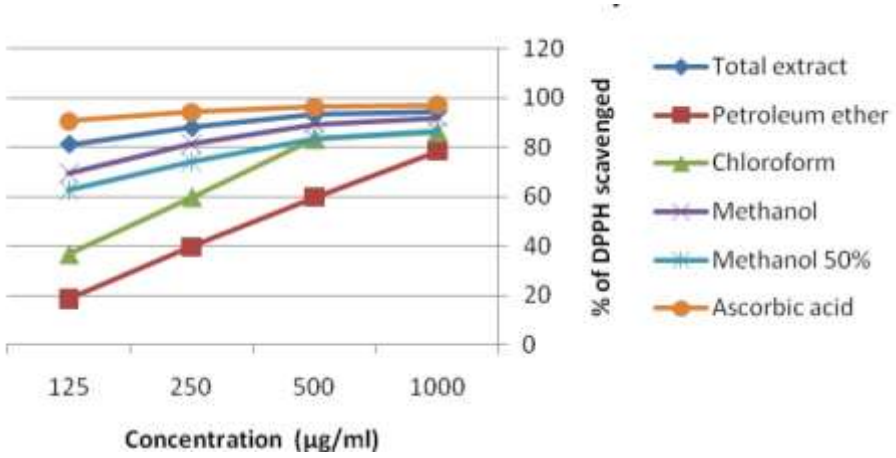

Fig. (1). The percentages of DPPH scavenging activities for total extract and successive extraction residues of *Phlomis floccosa* stems.

Table (3). The percentages of scavenging activities for essential oil, total extract and successive extraction residues of *Phlomis floccosa* leaves.

Concentration (µg/ml)	% Sacvenging of volatile oil	% Sacvenging of total extract	% Scavenging of petroleum ether fraction	% Scavenging of chloroform fraction	% Scavenging of methanol fraction	% Scavenging of 50% methanol fraction	% Scavenging of ascorbic acid
1000	0	94.822 ± 0.204	60.267 ± 0.145***	54.389 ± 0.317***	97.633 ± 0.034	81.689 ± 0.201*	97.167 ± 0.177
500	0	92.600 ± 0.241	48.967 ± 0.134***	47.967 ± 0.219***	96.000 ± 0.167	69.333 ± 0.088*	96.230 ± 0.165
250	0	88.689 ± 0.279	26.889 ± 0.386***	20.901 ± 0.147***	92.567 ± 0.120	55.111 ± 0.102*	94.100 ± 0.211
125	0	83.055 ± 0.267	16.967 ± 0.121***	8.011 ± 3.197***	87.333 ± 0.318	35.689 ± 0.234*	90.500 ± 0.188

Values are given as mean ± S. D. (n=3). *Significant at $p < 0.05$, p-value was calculated by comparing with standard (ascorbic acid) by ANOVA followed by Dunnett's test.

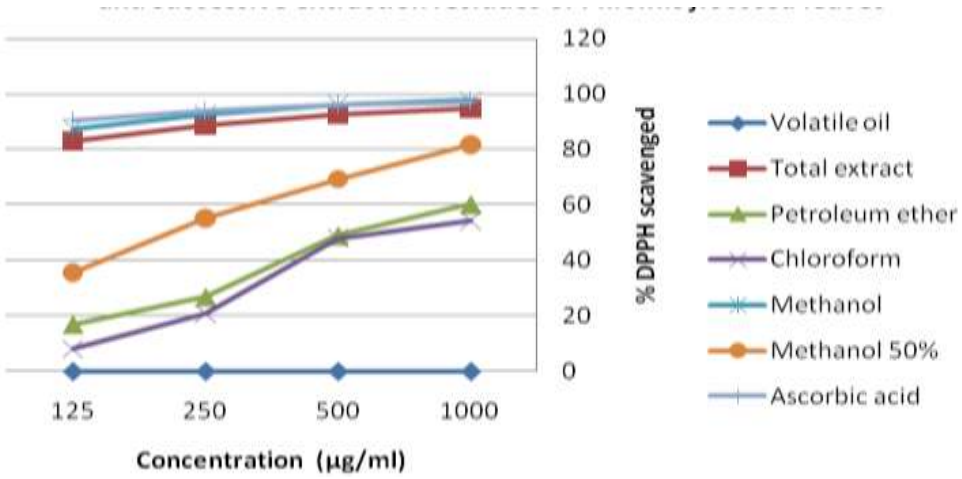

Fig. (2). The percentages of scavenging activities for essential oil, total extract and successive extraction residues of *Phlomis floccosa* leaves.

Table (4). The percentages of scavenging activities for total extract and successive extraction residues of *Phlomis floccosa* flowers.

Concentration (µg/ml)	% Scavenging of total extract	% Scavenging of petroleum ether fraction	% Scavenging of chloroform fraction	% Scavenging of methanol fraction	% Scavenging of 50% methanol fraction	% Scavenging of ascorbic acid
1000	93.667± 0.134	51.478± 0.246***	44.611± 0.350***	95.167± 0.318	88.355± 0.267	97.167± 0.177
500	92.800± 0.119	31.589± 0.342***	38.567± 0.153***	94.700± 0.186	85.744 0.084	96.230± 0.165
250	90.544± 0.269	12.389± 0.336***	34.834± 0.153***	93.444± 0.164	81.278± 0.234	94.100± 0.211
125	88.145± 0.204	2.255± 0.227***	32.656± 0.269***	90.167± 0.233	75.522± 0.395	90.500± 0.188

Values are given as mean ± S. D. (n=3). *Significant at $p < 0.05$, p-value was calculated by comparing with standard (ascorbic acid) by ANOVA followed by Dunnett's test.

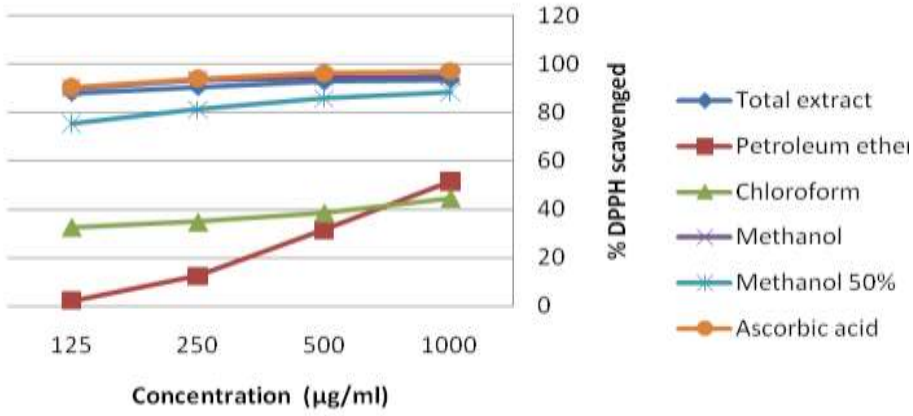

Fig. (3). The percentages of scavenging activities for total extract and successive extraction residues of *Phlomis floccosa* flowers**.**

4. IC_{50} of Different Parts of *Phlomis floccosa*

The obtained results showed that, the essential oils doesn´t have antioxidant activity. The highest percentage of DPPH scavenging activity for total and successive extracts residues of different parts (stems, leaves and flowers) of *Phlomis floccosa* was methanol extracts (Table 5). The DPPH radical scavenging activity of methanol extract may be mostly related to the presence of flavonoids. Hence methanol extracts was analyzed by HPLC.

Table (5). IC_{50} (µg/ml) of different parts of *Phlomis floccosa*.

Solvent used	IC_{50} (µg/ml)		
	Stems	**Leaves**	**Flowers**
Total extract	42.36	67.41	92.90
Petroleum ether	291.4	399.0	906.9
Chloroform	49.44	217.6	2713
Methanol	36.36	40.07	27.49
Methanol 50%	47.68	153.4	96.52
Ascorbic acid	17.63	17.63	17.63

5. Qualitative and Quantitative Analysis of the Methanol Extracts of Different Parts of *Phlomis floccosa*

Investigation of methanol extracts by HPLC revealed the presence of 21 flavonoid compounds at different parts (stems, leaves and flowers) of *Phlomis floccosa*, where the major compounds were luteolin-6-glucose-8-arabinose (1234.3 mg/100 g, 1098.2 mg/100 g and 440.17 mg/100 g) in stems, leaves and flowers of *Phlomis floccosa*, respectively. While luteolin-6-arabinose-8-glucose was the second major compound in stems and flowers (144.47 mg/100 g and 375.3 mg/100 g), respectively, but the second major compound in leaves was naringenin (278.13 mg/100 g) (Table 6).

Flavonoids have great importance as antioxidants *in vitro*, due to the high number of hydroxyl groups in their molecules. The glycosylation of flavonoids reduces their *in vitro* antioxidant activity, when compared with the corresponding aglycons. The main structural features of flavonoids required for efficient radical scavenging could be summarized as follows: an *ortho*-dihydroxy (catechol) structure in the B ring, for electron delocalization, 2, 3-double bond in conjugation with a 4-oxo function in the C ring provides electron delocalization from the B ring, hydroxyl groups at positions 3 and 5 provide hydrogen bonding to the oxo group (Prochazkova et al., 2011).

HPLC analysis of the methanol fractions showed that, the highest content of the different parts of the plant was luteolin-6-glucose-8-arabinose. Concerning its structure, the previously mentioned features required for free radical scavenging activity are fulfilled.

Table (6). HPLC analysis of the methanol extracts of different parts (stems, leaves and flowers) of *Phlomis floccosa*.

No.	Flavonoids	Concentration (mg/100 g)		
		Stem	Leaves	Flowers
1	Luteolin-6- arabinose-8- glucose	144.47	119.35	375.30
2	Luteolin-6- glucose -8- arabinose	1234.30	1098.2	440.17
3	Apigenin-6- arabinose -8-glactose	65.70	27.91	48.19
4	Apignin-6- rhamnose -8- glucose	7.50	15.29	31.32
5	Apignin-6- glucose -8- rhamnose	76.79	43.82	187.08
6	Luteolin-7- glucose	6.58	20.40	12.76
7	Narengin	13.04	24.32	73.14
8	Rutin	4.39	1.61	13.24
9	Hespiridin	31.41	11.15	15.59
10	Rosmarinic	1.24	1.69	8.75
11	Apigenin-7-O- neohespiroside	1.67	3.53	2.58
12	Kampferol-3,7-dirhamoside	5.64	4.05	2.48
13	Apigenin-7- glucose	5.64	11.98	5.43
14	Quercetrin	4.30	1.74	2.47
15	Quercetin	3. 31	5.56	0.66
16	Naringenin	78.96	278.13	254.46
17	Hespirtin	6.62	12.98	26.58
18	Kampferol	0.84	1.30	0.77
19	Rhamnetin	1.73	3.27	1.77
20	Apignin	1.05	0.93	4.79
21	Acacetin	15.05	9.48	9.06

CONCLUSION

This study on the antioxidant activity of *Phlomis floccosa* showed that, the hydro-distillation of the leaves of *Phlomis floccosa* yielded 0.18% of clear yellowish liquid oil and it didn´t has antioxidant activity. On the other hand it could be concluded that the methanol extract exhibited promising free radical scavenging activity compared with ascorbic acid. This antioxidant activity is due to the high content of the detected flavonoids (21 compounds).

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التركيب الكيميائي والنشاط المضاد للأكسدة للزيت العطري وفلافونيدات نبات ضرس الشايب

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يهدف هذا البحث إلى دراسة الفاعليات المضادة للأكسدة من زيوت أساسية ومستخلصات مختلفة لأجزاء نبات ضرس الشايب. حيث أن الزيت الأساسى تم تعينه فى أوراق نبات ضرس الشايب فقط، بينما لوحظ غيابه فى السيقان والأزهار. وقد اتضح من خلال التقطير المائى لأوراق نبات ضرس الشايب بان نسبة الزيت الأساسي هى ٠.١٨٪ ويتميز بأنه زيت سائل ذو لون اصفر شفاف كما أوضحت النتائج التي تم التوصل إليها أنه ليس له نشاط مضاد للأكسدة. ويمثل مستخلص الميثانول أفضل المستخلصات النباتية المضادة للأكسدة لأجزاء النبات المختلفة (سيقان ، أوراق وأزهار). ويعزى ذلك لإحتواء مستخلص الميثانول على الفلافونيدات. وبتحليل مستخلص الميثانول بإستخدام HPLC، أوضحت النتائج تواجد ٢١ مركب فلافونيدي في مختلف أجزاء نبات ضرس الشايب (سيقان، أوراق وأزهار). وكان المكون الأساسي للفلافونيدات، ليتيولين-٦-جلوكوز-٨-أرابينوز بتركيزات ١٢٣٤,٣ ملليجرام/ ١٠٠جرام، ١٠٩٨,٢ ملليجرام/١٠٠ جرام، ٤٤٠,١٧ ملليجرام/١٠٠ جرام لكل من السيقان، والأوراق، والأزهار، على التوالي. بينما كان المكون الأساسي الثاني للسيقان والأزهار مركب ليتيولين-٦-أرابينوز-٨-جلوكوز (١٤٤,٤٧ ملليجرام /١٠٠ جرام، ٣٧٥,٣ ملليجرام / ١٠٠جرام، على التوالي). بينما كان المكون الأساسي الثاني للأوراق هو نارينجينين (٣٧٨,١٣ ملليجرام/ ١٠٠ جرام). وقد إتضح طبقًا للنتائج التي تم الحصول عليها أن مستخلص الميثانول لنبات ضرس الشايب يمكن أن يكون مصدرا طبيعيا لمضادات الأكسدة.