DOI: https://doi.org/10.31351/vol30iss2pp261-268

Antioxidant Activity of Total phenols and Flavonoids extracted from *Echinops polyceras* roots grown in Syria

Issa Al-Assaf*,[1] and Mays Khazem*

* Department of Pharmacognosy in Faculty of Pharmacy, Damascus University, Damascus, Syria

Abstract

Free radicals are products of normal cellular metabolism, they are unstable, short-lived, and highly reactive and the excessive production of them may cause numerous degenerative diseases like cancer. Antioxidants such as polyphenols have a protective role against free radicals. *Echinops polyceras* Boiss. (Asteraceae) is one of *Echinops* genus species that spread in Syria, Lebanon, and Palestine. Phytochemicals found in this species roots have been extracted with gradient polarity solvents, and primary screening of the secondary metabolites was established. Then, the phenolic compounds content was determined with Folin-Ciocalteu reagent and flavonoids content with aluminum chloride reagent. The free radicals scavenging activity was evaluated for all extracts with DPPH• in a 96-well microplate. The selectivity study indicates that ascorbic acid and reducing sugars didn't exist in the extracts. The identification tests showed the presence of polyphenols like flavonoids and coumarins. The methanolic extract of the *E.polyceras* roots was the most effective scavengers of free radicals (84% in 30 min) with phenolic compounds content 575.5 mg GAE/g DE and flavonoids content 130 mg QE/ g DE, and the chloroform extract was the least effective as free radical scavenging (45% in 30 min) as the phenolic compounds content was 222.5 mg GAE/g DE and flavonoids content 57.5mg QE/ g DE. In conclusion, the phenolic compounds and flavonoids from *Echinops* polyceras Boiss. are effective in free radicals scavenging and protecting from diseases caused by oxidative stress.

Keywords: Antioxidants, Polyphenols, Flavonoids, Free Radicals, and *Echinops polyceras* Boiss

دراسةُ الفعاليّة المُضادة للأكسدة لعديدات الفينول والفلافونوئيدات المُستخلَصة من جذور نبات Echinops polyceras النامي في سورية

عيسى العسّاف*،[1] و ميس خازم*

* قسم العقاقير في كليّة الصيدلة، جامعة دمشق، دمشق، سورية

الخلاصة

تُعدّ الجذور الحُرّة نواتج طبيعيّة لعمليات الاستقلاب، وتتميّز أنها غير ثابتةٍ وذات عمرٍ قصير ومُتفاعلة بشدّة، ويُمكن أن يُسبب فرط إنتاجها أمراضاً تنكسيّة مثل السرطان. تتميّز مُضادات الأكسدة مثل عديدات الفينول بدورها الوقائيّ ضد الجذور الحُرّة. يُعدّ نوع Echinops polyceras Boiss. أحد أنواع جنس Echinops الذي ينتشر في سورية ولبنان وفلسطين. استُخلصت المُكوّنات الفعّالة الموجودة في جذور هذا النوع باستخدام مُحلّاتٍ مُتدرّجة القطبيّة، وقد تمّ بعد ذلك إجراء تحرٍّ أوليّ عن المُستقلبات الثانويّة. تمّ تحديد المُحتوى الفينوليّ (باستخدام كاشف فولين-سيكالتو) والفلافونوئيديّ (باستخدام كاشف كلوريد الألمنيوم)، ومن ثمّ تقييم الفعاليّة الكاسحة للجذور الحُرّة للخلاصات المدروسة باستخدام كاشف DPPH• وذلك باستخدام طبقٍ للزرع ذو ٩٦ حفرة. أظهرت نتائج دراسة الانتقائيّة خلو الخلاصات من كلٍّ من حمض الأسكوربيك والسكريات المُختزلة، كما أظهرت تفاعلات نتائج الكشوفات الأوليّة وجود عديدات الفينول مثل الفلافونوئيدات والكومارينات. وقد أبدى المُستخلص الكحوليّ أفضل نشاط في كسح الجذور الحُرّة وبتقدير (٨٤% خلال ٣٠ دقيقة) حيث بلغ المُحتوى الفينوليّ 575.5 mg GAE/g DE والفلافونوئيديّ 130 mg QE/ g DE، وكانت أدنى فعالية مُضادة للأكسدة لمُستخلص الكلوروفورم (٤٥% خلال ٣٠ دقيقة) حيث بلغ المُحتوى الفينوليّ 222.5 mg GAE/g DE والفلافونوئيديّ 57.5mg QE/ g DE. يُمكن تلخيص ما سبق بأن المُركبات الفينوليّة وخاصةً الفلافونوئيدات تمتلك فعّاليّة في كسح الجذور الحُرّة وبالتالي الوقاية من الأمراض الناتجة عن الشدة التأكسديّة.

الكلمات المفتاحيّة: مُضادات الأكسدة، عديدات الفينول، الفلافونوئيدات، الجذور الحُرّة و*Echinops polyceras* Boiss

Introduction

Free radicals are products of normal cellular metabolism, they are atoms or molecules which contain one or more unpaired electrons in a valency shell. The odd number of electron(s) of a free radical makes it unstable, short-lived and highly reactive [(1)]. The excessive production of free radicals is considered to be an important cause of oxidative damage in biomolecules, such as proteins, lipids, and DNA, this damage leads to numerous degenerative diseases [(2)], such as cancer, atherosclerosis, gastric ulcer, and other conditions [(3)]. Antioxidants are molecules that can prevent or delay the oxidation of substrate, these compounds have a high affinity for free radicals and scavenge them to protect our health [(4)]. Polyphenols are strong antioxidants that have a protective role against oxidative stress caused by excess free radicals [(5)]. The mechanism of the protective action of phenolic compounds in plants relies on the antioxidant activity that scavenges free radicals, protection of lipid peroxidation, and the chelation of toxic metals [(6)].

[1]Corresponding author E-mail: issa.alassaf.92@gmail.com
Received: 18/ 5/2021
Accepted:11 / 7 /2021
Published Online First: 2021-12-12

DOI: https://doi.org/10.31351/vol30iss2pp261-268

Flavonoids are a large subgroup of the family of phenolic compounds, and because of the presence of multiple hydroxyl groups in their structure flavonoids have reducing properties [7].

Echinops (Asteraceae) is a genus that includes 120 species of perennials, annuals, and biennials plants. These species are found in Eastern and Southern Europe, Tropical and North Africa, and Asia [8].

Echinops polyceras Boiss. is a perennial herb, 40-60 cm, sometimes with very fine and short whitish glandular hairs in the lower part. Basal leaves congested, oblong-lanceolate, pinnatipartite into short lobes armed with short yellow spines. Heads generally abundantly, with about 4.5-5 cm in diameter (not including cornigerous bracts). Partial involucre of non-cornigerous headlets about 2 cm, pale green. Corolla is white to pale bluish, anthers are greyish-violet, and the flowering time is June – July [9]. This species spread in Syria, Lebanon, and Palestine [10]. The aim of this study was to evaluate the phytochemicals and the free radicals scavenging activity of *Echinops polyceras* Boiss root since no previous studies have distinguished the chemical constituents and the biological effects of this plant.

Materials and Methods

Chemicals

Gallic acid (was purchased from AVONCHEM), Quercetin (from Sigma-Aldrich), 2,2-diphenyl-1-picrylhydrazyl DPPH (from TCI), distilled water, absolute ethanol (from Merck), absolute methanol (from sigma-Aldrich), ethyl acetate (from SHAM LAB), chloroform (from Merck), glacial acetic acid (from BDH), hydrochloric acid (from Himedia), sulfuric acid (from Himedia), folin ciocalteu (from Sigma-Aldrich), sodium carbonate (from Scharlau), aluminum chloride (from Scharlau), potassium acetate (from Merck), ascorbic acid (from Panreac Quimica SLU), magnesium metal turnings (from Chem-Lab), ferric chloride (from Panreac), potassium iodide (from Eurolab), bismuth nitrate (from Himedia) and mercuric chloride (from Himedia), picric acid (from Panreac eu), iodine crystals (from Honeywell), 3,5-dinitrobenzoic acid (from Titan biotech), gelatin.

Apparatus

96-well microplate reader (BioTek), rotary evaporator.

Plant material

The whole plant of flowering *Echinops polyceras* Boiss. was collected from Ma'aret Sednaya (Rif-Dimashq, Syria) in July 2019, and authenticated by Dr. Imad Alkadi (Department of Plant Biology, Damascus University, Syria). The Roots were separated from the rest of the plant parts, then dried in shade and powdered.

Extraction of the plant roots:

Dried and powdered roots of *Echinops polyceras* Boiss. (20 g) were extracted with 200 ml of each of gradient polarity solvents: Distilled water, Ethanol 50%, absolute Methanol, Methanol + Ethyl Acetate (1:1), and chloroform, at room temperature with shaking for seven days.

The five extracts were evaporated separately by a rotary evaporator, and the extraction yield was calculated by the equation:

Yield% = (weight of evaporated extract/ weight of roots powder) ×100

Phytochemical identification

Echinops polyceras roots extract was assessed for the existence of flavonoids, coumarins, tannins, anthraquinones, alkaloids, saponins and cardiac glycosides.

Test for flavonoids

lavonoids were identified by UV (366 nm) fluorescence after the addition of 1 ml of 5% aluminum chloride in ethanol [11].

- Magnesium metal (0.5 g) was added to 5 ml of ethanolic extract then 1 ml of concentrated HCl was added. A pink or red coloration that disappears on standing for 3 minutes indicates the presence of flavons (Shinoda test).

Test for coumarins

Ethanolic extract (5 ml) was evaporated, and the residue was dissolved in 2 ml of hot distilled water, then few drops of this solution were put on a filter paper and the fluorescence under UV light was examined. An intense blue fluorescence indicates the presence of coumarins.

Test for tannins

- Ferric chloride test:
  Ethanolic extract (1 ml) was added into a test tube then 2 to 3 drops of 10% of ferric chloride ($FeCl_3$) solution were added, and observed for a dark green (hydrolysable tannins) or dark blue (condensed tannins) coloration [12].
- Gelatin test:
  Tested extract (1 ml) was placed in a test tube, then 2 drops of 1 % gelatin solution with 10% sodium chloride were added. A white precipitate formation indicates the presence of tannins [13].

Test for anthraquinones:

- Borntrager test:
  E. polyceras roots powder (1 g) was extracted with 10 ml of chloroform for 10 minutes and filtered, then 2 ml of ammonia were added. The formation of red color in the aqueous layer indicates the presence of free anthraquinones [12].
- Modified Borntrager test
  Boil 1g of the plant material with 2ml of dilute sulphuric acid, 2ml of 5% aqueous ferric chloride solution for 5 minutes and continue the reaction as Borntrager test. The formation of red

DOI: https://doi.org/10.31351/vol30iss2pp261-268

color in the aqueous layer indicates the presence of anthraquinone glycosides [13].

Test for alkaloids

Ethanolic extract (20 ml) was evaporated, and the dry residue dissolved in 5 ml of HCl (2N) and filtered. Then, few drops of Mayer, Dragendroff, Wagner and Hager reagents were added. The formation of white, orange, reddish-brown and yellow precipitates respectively indicate the presence of alkaloids [12, 13].

Test for saponins

To 0.5 g of the aqueous extract, 20 ml of hot water were added into a test tube, the tube was shaken vigorously. The formation of a stable foam indicates the presence of saponins [12].

Test for cardiac glycosides

- Keller killiani test:

To 2 ml of the ethanolic extract, 1 ml of glacial acetic acid was added with one drop of 5% $FeCl_3$ and 1 ml concentrated H_2SO_4. The formation of reddish-brown color at the junction of the two liquid layers, and the bluish-green color at the upper layer indicate the presence of cardiac glycosides [14].

- Kedde's test:

Evaporate the chloroform extract of the roots, then add one drop of 90% alcohol and 2 drops of the reagent (2% 3,5-dinitro benzoic acid in 90% alcohol), an alkaline solution (20% sodium hydroxide solution) was added. Purple color is produced in the case of the presence of β-unsaturated-o lactones [13].

Determination of total phenolic content (TPC)

Total phenolic content was determined by a micro colorimetric method described by Ainsworth & Gillespie [15]: 200 mg of each extract were dissolved with 2 ml of methanol 95% (vol/vol). 100 µL of each sample were transferred to 2 ml microtubes and were mixed with 200 µL of 10% (vol/vol) Folin–Ciocalteu reagent, the mixture was vortexed thoroughly. Then 800 µL of Na_2CO_3 (700 mM) was added into each tube, and the assay tubes were incubated at room temperature for two hours. 200 µL of samples, standard (gallic acid), and blank (200 µL of 10% (vol/vol) Folin–Ciocalteu reagent with 800 µL of 700 mM Na_2CO_3) were transferred to a clear 96-well microplate, and the absorbance of each well was read at 765 nm in triplicate.

Gallic acid calibration curve

The calibration curve was established with nine dilutions of gallic acid standard at concentrations of (12, 24, 36, 48, 60, 84, 96, 108, 120) mg/L. Then the absorbance was read at 765 nm using the microplate reader.

TPC of the extracts:

Total phenolic content was calculated as gallic acid equivalents (GAE) in 1 g of dried extract (DE) using the regression equation between Gallic acid standard concentrations and absorption at 765 nm.

Specificity

The specificity of Folin–Ciocalteu method was checked by detection of the presence of some reducing compounds like reducing sugars and ascorbic acid.

- Detection of reducing sugars using Fehling's test:

1 ml of the ethanol extract was diluted with 1ml of water in a test tube, then 20 drops of boiling Fehling's solution (A and B) was added. The formation of a precipitate red-brick in the bottom of the tube indicates the presence of reducing sugars [12].

- Detection of Ascorbic acid using a spectrophotometric method:

- **Determination of λ_{max} of ascorbic acid** [16]

Ascorbic acid (0.1 g) was dissolved with distilled water in a volumetric flask (100 ml), then 1 ml of this solution was transferred into another 100 ml volumetric flask with the addition of 10 ml of 0.1 N of hydrochloric acid, distilled water was used to complete the rest volume to 100 ml.

The λ_{max} was determined by a spectrophotometric scan between 200-300 nm.

- **Scanning of extract solution**

Aqueous extract (1 g) was also dissolved with distilled water in a volumetric flask (100 ml), then 1 ml of the solution was transferred into another 100 ml volumetric flask with 10 ml of 0.1 N of hydrochloric acid, then distilled water was used to complete the rest volume to 100 ml.

The absorbance was detected at the λ_{max} of ascorbic acid.

Determination of total flavonoids content (TFC)

Flavonoids content was determined according to chang *et al*. protocol [17]: 200 mg of each extract were mixed with 1.5 mL of 95% ethanol, 100 µL of 10% $AlCl_3$ (w/v) solution, and 100 µL of 1 mol/L potassium acetate solution were added, and the assay tubes were incubated at room temperature for 30 min. 200 µL of samples, standard (Quercetin) and blank (100 µL of 10% $AlCl_3$ (w/v) with 100 µL of 1 mol/L potassium acetate) were transferred to a clear 96-well microplate and read the absorbance of each well at 420 nm in triplicate.

Quercetin calibration curve

The calibration curve was established with seven dilutions of quercetin standard at concentrations of (6, 12, 24, 30, 36, 48, 60) mg/L. Then the absorbance was read at 420 nm using the microplate reader.

TFC of the extracts

Total Flavonoids content was calculated as quercetin equivalents (QE) in 1 g of dried extract (DE) using the regression equation between quercetin standard concentrations and absorption at 420 nm.

Evaluation of free radicals scavenging activity (RSA)

DOI: https://doi.org/10.31351/vol30iss2pp261-268

The DPPH• radical scavenging activity was evaluated according to Cheung *et al*. method [18] with some modification by Choi *et al*. Briefly, 160 µL of 0.2 mM DPPH• in methanol were mixed with 40 µL of the extracts or standards (ascorbic acid, gallic acid, and quercetin) in a 96-well microplate. The mixtures were left to stand at room temperature, and the absorbance at 520 nm was measured against methanol as a blank after 10 and 30 min.

Free radicals scavenging activity (RSA) was determined by the equation:

$$\mathbf{RSA\% = 100 \times \frac{A_0 - A_s}{A_0}}$$

Where:

A_0: absorption of DPPH• solution

As: absorption of DPPH• solution after 10 and 30 min of the sample addition.

Results

Extraction yield

The extraction yield% of the extracts is shown in **Table (1)**. Aqueous extract showed the highest yield (10.5%) followed by hydroethanolic 50%, methanol, methanol: ethyl acetate (1:1), and chloroform extracts with yields of 7, 4, 2.75, and 1%, respectively.

Table 1. Extraction yield

Extracts	Yield%
DH2O	10.5
EtOH 50	7
MeOH	4
MeOH+EtOAc	2.75
CHCl3	1

Phytochemical identification

The results of the identification tests are shown in **Table (2)**.

Table 2. Phytochemical identification of *E. polyceras* roots

Flavonoids	Aluminum chloride	+
	Shinoda test	+
Coumarins	fluorescence	+
Tannins	Ferric chloride test	+
	Gelatin test	-
Anthraquinones	Borntrager test	-
	Modified Borntrager	-
Alkaloids	Mayer	-
	Dragendroff	-
	Wagner	-
	Hager	-
Saponins	Foam test	+
Cardiac glycosides	Keller killiani test	-
	Kedde's test	-

Gallic acid calibration curve

Gallic acid concentrations and their absorbances are shown in **Table (3)**. Also, the linearity and the regression equation are shown in **Figure (1)**.

Table 3. Gallic acid concentrations and their absorbances

Concentration (mg/L)	Ā 765
0	0
12	0.174
24	0.317
26	0.428
48	0.595
60	0.721
84	1.011
96	1.13
108	1.223
120	1.378

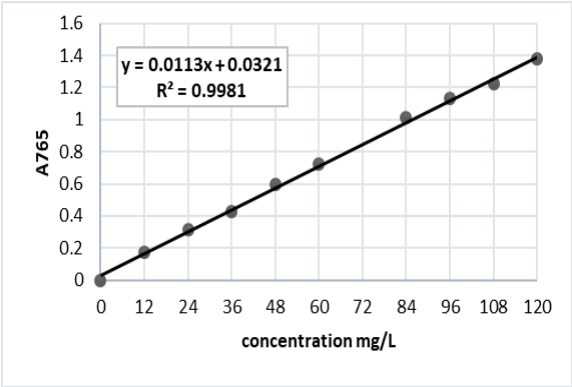

Figure 1. Gallic acid calibration curve

TPC of the extracts

Total phenolics content in the extracts was presented in **Table (4)**. The methanolic extract showed the highest content of phenolic compounds (575.5 mg GAE/g DE), followed by MeOH: EtOAc (1:1), EtOH 50%, distilled H_2O, and $CHCl_3$ extracts, respectively. The TPC results are shown in **Figure (2)**.

DOI: https://doi.org/10.31351/vol30iss2pp261-268

Table 4.TPC, TFC and RSA% of the standards and the extracts.

Samples	TPC±SD (GAE/ g DE)	TFC±SD (QE/ g DE)	RSA%		Time for RSA≥ 90%
			10 min	30 min	
Ascorbic Acid	-	-	91.21	93.85	< 10 min
Gallic Acid	-	-	83.52	87.54	> 30 min
Quercetin	-	-	85.71	89.59	> 30 min
DH_2O	403.5 ±0.335	103 ±0.541	76.61	82.65	> 30 min
EtOH 50	486 ±0.335	119 ±0.207	77.39	81.55	> 30 min
MeOH	575.5 ±0.358	130 ±0.435	78.96	84.07	> 30 min
MeOH+EtOAc	521.5 ±0.503	123 ±0.281	73.47	83.28	> 30 min
$CHCl_3$	222.5 ±0.276	57.5 ±0.405	35.16	45.27	> 30 min

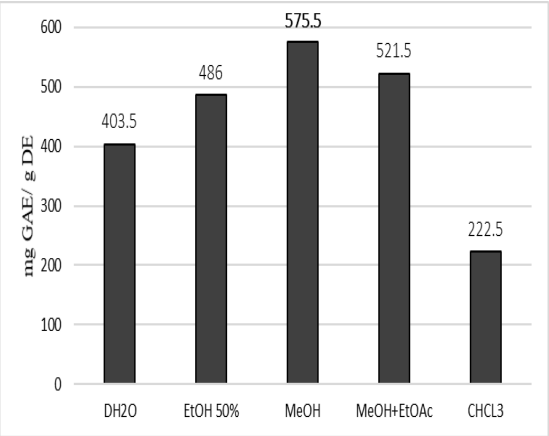

Figure 2. TPC in 1 g of the dried extracts
All results of TPC are presented as the mean of three replicates ± SD ($p < 0.05$)

Specificity

- Detection of reducing sugars:
  The result of Fehling's test indicates the absence of reducing sugars because the red-brick precipitate does not exist.

- Detection of Ascorbic acid using a spectrophotometric method:

- Determination of λ_{max} of ascorbic acid:
Ascorbic acid solution showed the maximum absorbance at 240 nm, according to **Figure (3)**.

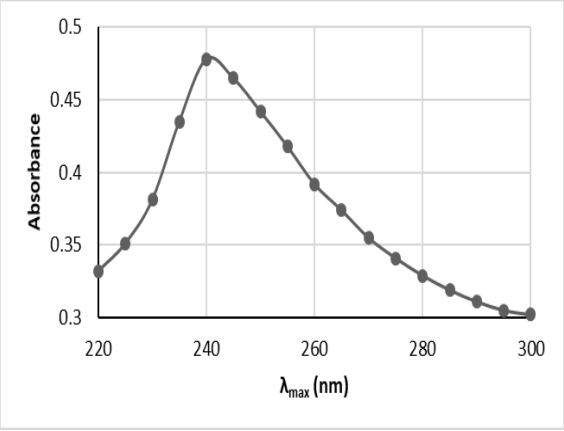

Figure 3. The scanning of ascorbic acid solution

Scanning of extract solution

The scanning of the extract solution is shown in **Figure (4)**, it indicates that the extract of *E. polyceras* roots is free of ascorbic acid.

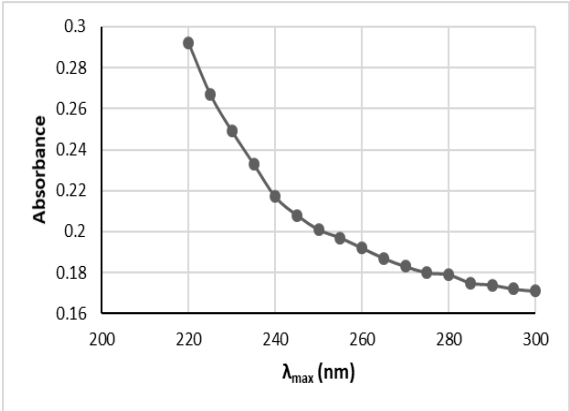

Figure 4. The scanning of roots extract solution.

Quercetin calibration curve

The concentrations of quercetin and their absorbances are shown in **Table (5)**. Also, the linearity and the regression equation are shown in **Figure (5)**.

DOI: https://doi.org/10.31351/vol30iss2pp261-268

Table 5. Quercetin concentrations and their absorbances

Concentration (mg/L)	Ā 420
0	0
6	0.058
12	0.109
24	0.196
30	0.235
36	0.285
48	0.363
60	0.456

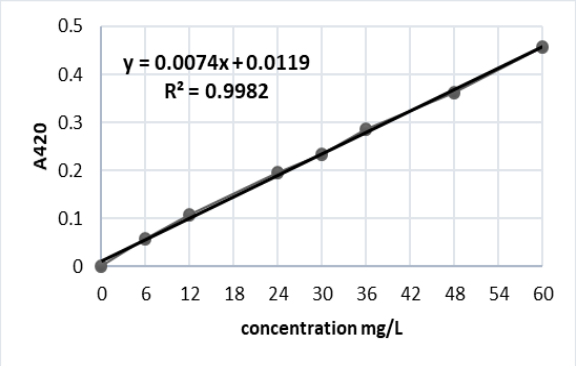

Figure 5. Quercetin calibration curve

TFC of the extracts

Flavonoids content in the extracts is also presented in **Table (4)**. As the methanolic extract showed the highest phenolic amount, it contained the highest flavonoid content (38.9 mg QE/ g DE), followed by MeOH: EtOAc (1:1), EtOH 50%, distilled H_2O and $CHCl_3$ extracts, respectively. The TFC results are shown in **Figure (6)**.

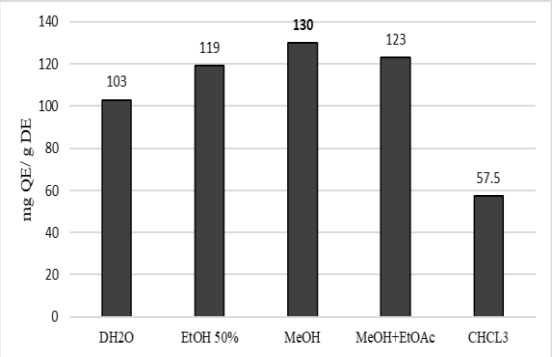

Figure 6. TFC in 1 g of the dried extracts

All results of TFC are presented as the mean of three replicates ± SD ($p < 0.05$)

RSA of the extracts

DPPH• assay revealed that the methanolic extract was the most effective in free radicals scavenging after 30 min (≈ 84.1 %), compared with the other extracts: MeOH: EtOAc (1:1), DH_2O, EtOH 50%, and $CHCl_3$, they scavenge 83.28, 82.65, 81.55, and 45.27 % of DPPH• radical, respectively. The scavenging activities after 10 and 30 min for the standards: [ascorbic acid (AA), gallic acid (GA) and quercetin (Q)] and the studied extracts are shown in **Figure (7)**.

The DPPH• scavenging activities after 10 and 30 min, total phenolic, and flavonoid contents of the extracts is shown in **Table (4)**.

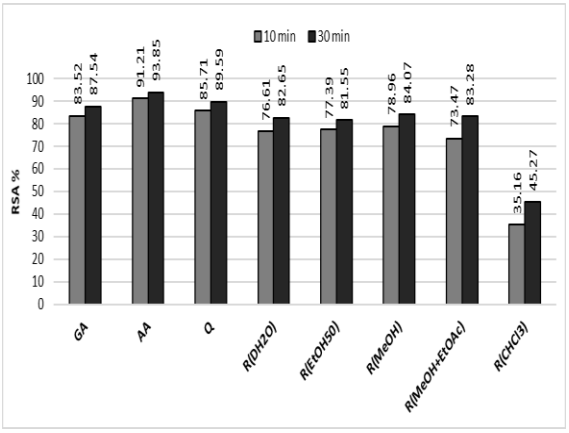

Figure 7.RSA% of standards and studied extracts after 10 and 30 min

Statistical analysis

All experiments were accomplished in triplicate. The results were expressed as the mean± standard deviation (SD). One-way analysis of variance (ANOVA) was carried out to identify significant differences between experimental groups, using Microsoft excel 2019. Differences were considered significant ($p < 0.05$).

Correlation ratio was calculated between:

- Total phenolic content and flavonoids content of the roots extracts, it was ≈ 98%.
- Free radical scavenging activity and total phenolic content, it was ≈ 82%
- Free radical scavenging activity and flavonoids content, it was ≈ 89%

Discussion

Neither the chemical composition nor the biological effects of *Echinops polyceras* Boiss. have been studied previously. In this study, a primary chemical screening has been established, the polyphenols and flavonoids contents have been determined and the free radicals scavenging activity has been evaluated for five different extracts of this plant.

The results of extraction yield of *E. polyceras* Boiss. roots (H_2O> EtOH 50%> MeOH 100% > MeOH+ EtOAc (1:1) > $CHCl_3$) -which descended according to the polarity of the solvent- may refer to the polarity of the extracted compunds, or the presence of the secondary metabolites as glycosides more than free aglycons because the solvents have a crucial role in the type of the secondary metabolites found in the extracts [19].

The phytochemical screening revealed the existence of polyphenols as an important component in the

DOI: https://doi.org/10.31351/vol30iss2pp261-268

extract, especially flavonoids and coumarins, because of that the content of total phenols and flavonoids were determined.

Ascorbic acid and reducing sugars are reducing compounds that may interfere with the antioxidant activity of the phenolic compounds in the extracts [20], so, they were identified in the specificity tests, which indicates the absence of ascorbic acid and the reducing sugars.

The methanolic extract of *E. polyceras* roots showed the highest content of total phenols (575.5 GAE/ g DE) and flavonoids (194.5 QE/ g DE), while the chloroform extract showed the lowest contents 222.5 GAE/ g DE and 94 QE/ g DE respectively. Based on these results, the examined extracts have significant antioxidant and free radicals scavenging effects, the most effective extract among them was the methanolic extract (84% in 30 minutes), while the chloroform extract was the less effective (45% in 30 minutes), this may be explained by the content of phenols and flavonoids in the extracts.

According to the correlation ratio, the free radicals scavenging activity is correlated to the phenolic content and flavonoids content with correlation ratio 82% and 89%, respectively, these results indicate that the phenolic compounds especially flavonoids are effectively contributed to the scavenging activity.

Also, we noticed that there is a high correlation between phenolic compounds and flavonoids (98%), this may indicate that the majority of phenols in the studied extracts are flavonoids and these compounds were responsible for most of the activity. The phenolic content may contribute directly to the antioxidant activity [21].

Studied extracts scavenging activities of free radicals were close to the scavenging activities of gallic acid and quercetin standards, this may be explained by the obvious content of phenolic compounds especially flavonoids.

Depending on the positive result of Shinoda test, the extract of roots contains flavone type of flavonoids, the structure-activity relationship study of these compounds indicates that the hydroxyl groups, the 3,4-catechol structure in the B-ring, the 2-3 double bond, and 4-oxo function are key factors for the antioxidant activity of the flavonoids [22].

The aqueous extract which can use as an infusion in traditional medicine [23] showed a good effect in scavenging free radicals (≈83 in 30 minutes).

All the results of this study showed that *E. polyceras* roots can be a valuable source of polyphenols such as flavonoids, which have a crucial role as antioxidants and free radicals scavengers, this can predict the ability to use this species in the treatment of oxidative stress illnesses.

This plant needs more studies about the safety, the toxicological effects and the determination of the therapeutic dose.

Conclusion

The study was concluded that our study is the first report about *Echinops polyceras Bois.* roots, where primary identification tests of secondary metabolites were established, and the phenolic compounds and flavonoids contents were determined in different extracts, using micro methods in a 96-well microplate. Also, the scavenging activity of free radicals was evaluated using the DPPH• radical. Our *in vitro* results were good enough to make this species a good source of effective antioxidants which can be used to prevent oxidative stress illnesses. The *in vivo* and the safety studies should be fulfilled.

Acknowledgments

The authors would like to thank the staff of the department of pharmacognosy in the Faculty of pharmacy Damascus University and the staff of the Leishmania Center of Epidemiological and Biological Studies, Damascus University, especially, prof. Chadi Soukkarieh and Dr. Hassan Alkhouri for providing research facilities.

References

1. Phaniendra A, Jestadi DB, Periyasamy L. Free radicals: properties, sources, targets, and their implication in various diseases. Indian J Clin Biochem. 2015 Jan;30 (1):11-26.
2. Supasuteekul C, Nonthitipong W, Tadtong S, Likhitwitayawuid K, Tengamnuay P, Sritularak B. Antioxidant, DNA damage protective, neuroprotective, and α-glucosidase inhibitory activities of a flavonoid glycoside from leaves of Garcinia gracilis. Rev. bras. farmacogn. 2016 Jun;26(3):312-320.
3. Shon MY, Kim TH, Sung NJ. Antioxidants and free radical scavenging activity of Phellinus baumii (Phellinus of Hymenochaetaceae) extracts. Food Chem. 2003 Sep;82(4):593-7.
4. Ramos-Tovar E, Muriel P. Free radicals, antioxidants, nuclear factor-E2-related factor-2 and liver damage. J Appl Toxicol. 2020 Jan;40(1):151-68.
5. Tsao R. Chemistry and biochemistry of dietary polyphenols. Nutrients. 2010 Dec;2(12):1231-46.
6. Kalinowska M, Gryko K, Wróblewska AM, Jabłońska-Trypuć A, Karpowicz D. Phenolic content, chemical composition and anti-/pro-oxidant activity of Gold Milenium and Papierowka apple peel extracts. Sci Rep. 2020 Sep;10(1):1-5.
7. Vicente O, Boscaiu M. Flavonoids: Antioxidant compounds for plant defence... and for a healthy human diet. Not Bot Horti Agrobo. 2018 Jan;46(1):14-21.
8. Khadim EJ, Abdulrasool AA, Awad ZJ. Phytochemical investigation of alkaloids in the Iraqi *Echinops* heterophyllus (Compositae). Iraqi J Pharm Sci. 2014;23:26-34.

DOI: https://doi.org/10.31351/vol30iss2pp261-268

9. Feinbrun-Dothan N. Flora Palaestina/3. Ericaceae to compositae/by Naomi Feinbrun-Dothan Text Text. Israel Academy of Sciences and Human.; 1978.

10. Mouterde P. Nouvelle flore du Liban et de la Syrie.

11. Sabatier S, Amiot MJ, Tacchini M, Aubert S. Identification of flavonoids in sunflower honey. J. Food Sci. 1992 May;57(3):773-4.

12. Zohra SF, Meriem B, Samira S, Muneer MA. Phytochemical screening and identification of some compounds from mallow. J Nat Prod Plant Resour. 2012;2(4):512-6.

13. De S, Dey YN, Ghosh AK. Phytochemical investigation and chromatographic evaluation of the different extracts of tuber of *Amorphaphallus paeoniifolius* (Araceae). Int J Pharm Biol Res. 2010;1(5):150-7.

14. Bhatt S, Dhyani S. Preliminary phytochemical screening of *Ailanthus excelsa* Roxb. Int J Curr Pharm Res. 2012;4(1):87-9.

15. Ainsworth EA, Gillespie KM. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. Nat Protoc. 2007 Apr;2(4):875-7.

16. Davey MW, Montagu MV, Inze D, Sanmartin M, Kanellis A, Smirnoff N, Benzie IJ, Strain JJ, Favell D, Fletcher J. Plant L-ascorbic acid: chemistry, function, metabolism, bioavailability and effects of processing. J Sci Food Agric. 2000 May;80(7):825-60.

17. Mammen D, Daniel M. A critical evaluation on the reliability of two aluminum chloride chelation methods for quantification of flavonoids. Food Chem. 2012 Dec;135(3):1365-8.

18. Choi Y, Jeong HS, Lee J. Antioxidant activity of methanolic extracts from some grains consumed in Korea. Food Chem. 2007 Jan;103(1):130-8.

19. Azwanida NN. A Review on the Extraction Methods Use in Medicinal Plants, Principle, Strength and Limitation. Med Aromat Plants. 2015; 4: 196.

20. Sánchez-Rangel JC, Benavides J, Heredia JB, Cisneros-Zevallos L, Jacobo-Velázquez DA. The Folin–Ciocalteu assay revisited: improvement of its specificity for total phenolic content determination. Anal. Methods. 2013;5(21):5990-9.

21. Rammohan A, Bhaskar BV, Camilo Jr A, Gunasekar D, Gu W, Zyryanov GV. In silico, in vitro antioxidant and density functional theory based structure activity relationship studies of plant polyphenolics as prominent natural antioxidants. Arab J Chem. 2020 Feb;13(2):3690-701.

22. Heim KE, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. J Nutr Biochem. 2002 Oct;13(10):572-84.

23. Alachkar A, Jaddouh A, Elsheikh MS, Bilia AR, Vincieri FF. Traditional medicine in Syria: folk medicine in Aleppo governorate. Nat. Prod. Commun. 2011 Jan;6(1).