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Phytochemical screening and Free radicals scavenging activity of leaves of Echinops polyceras Boiss. grown in Syria

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Abstract

Free radicals are reactive compounds, their excessive production is considered to be an important cause of oxidative damage in biomolecules causing degenerative diseases. Polyphenols are one of the most important groups of secondary metabolites of plants, which have an antioxidant activity depending on their properties as hydrogen donors. Echinops polyceras Boiss. (Asteraceae) is one of Echinops genus species that spread in Syria, Lebanon, and Palestine. Phytochemicals found in this species leaves have been extracted with gradient polarity solvents, and primary screening of the secondary metabolites was established. The phenolic compounds and flavonoids contents were determined. The free radicals scavenging activity was evaluated for all extracts with DPPH• in a 96-well microplate. The specificity study indicates that ascorbic acid was absent, and reducing sugars were exist in the aqueous extract. The identification tests showed the presence of polyphenols like flavonoids and coumarins. The methanolic extract of the E. polyceras leaves was the most effective scavengers of free radicals (90.22% in 30 min) with phenolic compounds content 682.5 mg GAE/g of dried extract (DE) and flavonoids content 194.5 mg QE/ g DE. The chloroform extract was the least effective as free radical scavenging (60% in 30 min) as the phenolic compounds content was 278.5 mg GAE/g DE and flavonoids content 94 mg 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

تحرّي المُركبات الفعّالة والفعاليّة الكاسحة للجذور الحُرّة لأوراق نبات Echinops polyceras Boiss. النامي في سورية

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الخلاصة

تُعدّ الجذور الحُرّة مُركبات مُتفاعلة بشدّة، ويُمكن أن يُسبب فرط إنتاجها أذيّاتٍ تأكسديّة في الجزيئات الحيويّة مؤدّية لحدوث أمراضٍ تنكسيّة. تُعدّ عديدات الفينول إحدى أهمّ مجموعات المُستقلبات الثانويّة في النباتات، والتي تمتلك فعاليّة مُضادة للأكسدة بسبب خصائصها المانحة للهيدروجين. ينتشر نوع Echinops polyceras Boiss. (Asteraceae) الفصيلة النجميّة وهو أحد أنواع جنس Echinops في سورية ولبنان وفلسطين. استُخلصت المُكوّنات الفعّالة الموجودة في أوراق هذا النوع باستخدام مُحلّاتٍ مُتدرّجة القطبيّة، وقد تمّ بعد ذلك إجراء تحرٍّ أوليّ عن المُستقلبات الثانويّة. تمّ تحديد المُحتوى الفينوليّ والفلافونوئيديّ، ومن ثمّ تقييم الفعاليّة الكاسحة للجذور الحُرّة للخلاصات المدروسة باستخدام كاشف DPPH•وذلك باستخدام طبقٍ للزرع ذو ٩٦ حفرة. أظهرت نتائج دراسة الانتقائيّة احتواء الخلاصات المائيّة على سكاكر مُرجعة، بينما خلت هذه الخلاصة من حمض الأسكوربيك. كما أظهرت نتائج الكشوفات الأوليّة وجود عديدات الفينول مثل الفلافونوئيدات والكومارينات. وقد أبدى المُستخلص الميثانوليّ أفضل نشاط في كسح الجذور الحُرّة وبتقدير (٩٠,٢٢٪ خلال ٣٠ دقيقة) حيث بلغ المُحتوى الفينوليّ ٦٨٢,٥ mg GAE/g DE والفلافونوئيديّ ١٩٤,٥ mg QE/ g DE ، وكانت أدنى فعالية مُضادة للأكسدة لمُستخلص الكلوروفورم (٦٠٪ خلال ٣٠ دقيقة) حيث بلغ المُحتوى الفينوليّ ٢٧٨,٥ mg GAE/g DE والفلافونوئيديّ ٩٤ mg 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 reactive compounds that tend to capture electrons from stable biological molecules in order to stabilize themselves [(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)]. Polyphenols are one of the most important groups of secondary metabolites of plants, they are widely distributed in the plant kingdom and can be obtained directly from plants, foods or drug-supplements. These compounds are important agents in the prevention of several diseases. Polyphenols function as antioxidants depends on their properties as hydrogen donors. These hydrogen atoms are accepted by reactive radicals to yield much less reactive radicals and non-radical species [(4)]. The mechanism of the protective action of phenolic compounds in plants rely on the antioxidant activity that scavenges free radicals, protection of lipid peroxidation, and the chelation of toxic metals [(5)].

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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 [6]. This species spread in Syria and Lebanon and Palestine [7]. The aim of this study was to evaluate the phytochemicals and the free radicals scavenging activity of *Echinops polyceras* Boiss. leaves 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), toluene (from BDH) , formic acid (from BDH), 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, and TLC silica gel 60 F_{254} (from Merck).

Apparatus

96-well microplate reader (BioTek), rotary evaporator, TLC plate heater (CAMAG), and TLC scanner (CAMAG).

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 at the Department of Plant Biology, Damascus University, Syria. The leaves were separated from the rest of the plant parts, then dried in shade and powdered.

Extraction of the plant leaves

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

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

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

Phytochemical identification

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

Test for flavonoids

Flavonoids were identified using UV (366 nm) fluorescence after aluminum chloride (5%) in ethanol was added [8].

To 5 ml of ethanolic extract, 0.5 g of magnesium metal and 1 ml of concentrated HCl were added. The pink or red color formation indicates the presence of flavones (Shinoda test).

Test for coumarins

Ethanolic extract (5 ml) was evaporated then the residue was dissolved in 2 ml of hot distilled water. Few drops of this solution were put on a clean filter paper and the fluorescence under UV light (366 nm) was examined. An intense blue fluorescence indicates the presence of coumarins.

Test for tannins

Ferric chloride test

To 1 ml of ethanolic extract, 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 [9].

Gelatin test

To 1 ml of tested extract, 2 drops of 1 % gelatin solution with 10% sodium chloride were added. The formation of white precipitate indicates the presence of tannins [10].

Test for anthraquinones

Borntrager test

E. polyceras leaves 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 [9].

Modified Borntrager test

Boil 1g of the plant material with 2ml of dilute sulphuric acid and 2ml of 5% aqueous ferric chloride solution for 5 minutes and continue the reaction as Borntrager test. The formation of red color in the aqueous layer indicates the presence of anthraquinone glycosides [10].

Test for alkaloids

Ethanolic extract (20 ml) was evaporated, and the dry residue was 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 [9, 10].

Test for saponins

To 0.25 g of the aqueous extract, 15 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 [9].

Test for cardiac glycosides

Keller killiani test

To 2 ml of the ethanolic extract, 1 ml of glacial acetic acid and one drop of 5% $FeCl_3$ and 1 ml concentrated H_2SO_4 were added. 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 [11].

Kedde's test

Evaporate the chloroform extract of the leaves, 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 [10].

Determination of total phenolic content (TPC)

Total phenolic content was determined by a micro colorimetric method described by Ainsworth & Gillespie [12]: 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 were transferred to a clear 96-well microplate, and the absorbance of each well was read at 765 nm.

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 using the regression equation between gallic acid standard concentrations and the absorbance at 765 nm (A_{765}).

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 [9]:

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.

Detection of Ascorbic acid using a spectrophotometric method

Determination of λ_{max} of ascorbic acid [13]

0.1 g of ascorbic acid 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, then 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 [13]:

1g of the aqueous extract 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 [14]: 200 mg of each extract were mixed with 1.5 mL of 95% ethanol, 100 µl of 10% AlCl3 (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 were transferred to a clear 96-well microplate and read the absorbance of each well at 420 nm.

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 using the regression equation between quercetin standard concentrations and the absorbance at 420 nm (A_{420}).

Evaluation of free radicals scavenging activity (RSA)

The DPPH• radical scavenging activity was evaluated according to Cheung *et al*. method [15] with some modification by Choi *et al*., 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 was measured at 520 nm against methanol as a blank after 10 and 30 min.

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

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

Where:

A_0: absorption of DPPH˙ solution

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

Qualitative and Quantitative determination of quercetin in the ethanolic extract

Quercetin standard (QS) and ethanolic extract of leaves (EEL) solutions were prepared at concentrations of 40 mg/L and 5 g/L respectively, by dissolving 4 mg of quercetin and 500 mg of the extract in 100 ml ethanol with ultrasound assistance for 10 min at room temperature.

15 µl of quercetin and the ethanolic extract solutions were applied to the TLC plate, toluene: ethyl acetate: formic acid (50:40:2) was used as a mobile phase in a glass chamber saturated with the mobile phase. The plate was dried using a TLC plate heater at 90 C°, and then it was visualized under UV light at 254 nm. After that, they scanned at 254 nm using a TLC scanner.

The quercetin percentage in the ethanolic extract was determined according to the equation:

$$\mathbf{Quercetin}\% = \frac{\mathbf{AUC_1 \times C_1}}{\mathbf{AUC_2 \times C_2}} \times \mathbf{100}$$

Where: AUC1: Area under the curve of the extract

AUC2: Area under the curve of quercetin

C1: The concentration of the extract

C2: The concentration of quercetin

Results

Extraction yield

The extraction yield% of the five extracts is shown in Table (1). Aqueous extract showed the highest yield (13.25 %) followed by hydroethanolic 50%, methanol, methanol: ethyl acetate (1:1), and chloroform extracts 11.25, 8.5, 6.5, and 2%, respectively.

Table 1.Extraction yield

Extracts	Yield%
DH2O	13.25
EtOH 50	11.25
MeOH	8.5
MeOH+EtOAc	6.5

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)	$\bar{A}_{765}$
0	0
12	0.174
24	0.317
36	0.428
48	0.595
60	0.721
84	1.011
96	1.13
108	1.223
120	1.378

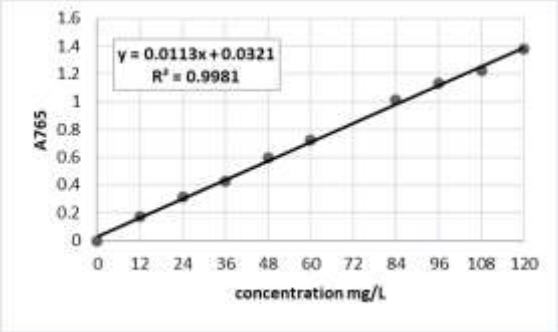

Figure 1.Gallic acid calibration curve

TPC of the extracts:

Total phenolics content in the extracts was presented in Table (3). The methanolic extract showed the highest content of phenolic compounds (682.5 mg GAE/g DE), followed by DH_2O, MeOH: EtOAc (1:1), EtOH 50%, and $CHCl_3$ extracts,

respectively. The TPC results are shown in Figure (2).

Table 3. Gallic acid concentrations and their absorbances

Concentration (mg/L)	$\bar{A}_{765}$
0	0
12	0.174
24	0.317
36	0.428
48	0.595
60	0.721
84	1.011
96	1.13
108	1.223
120	1.318

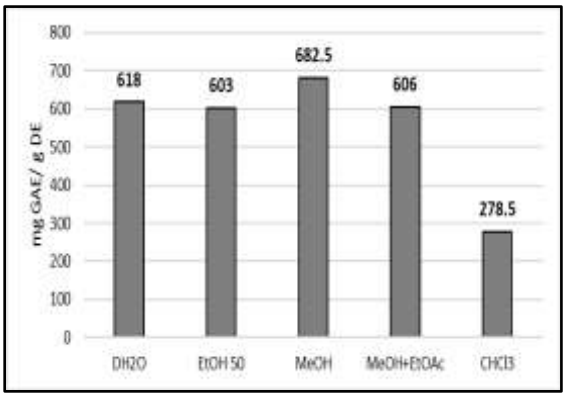

Figure 2. TPC in 1 g of the dried extracts .All results of TPC are presented as the mean of three replicates ± SD ($p < 0.05$)

Specificity

Detection of reducing sugars:

Fehling's test indicates that the extract of *E. polyceras* leaves contain reducing sugars, because of the formation of a precipitate red-brick.

Detection of Ascorbic acid using a spectrophotometric method

Determination of λ_{max} of ascorbic acid

Ascorbic acid solution showed a maximum absorbance at 240 nm, according to Figure (3).

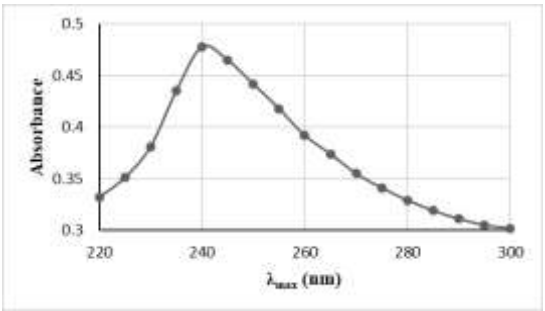

Figure 3. The scanning of ascorbic acid solution

Scanning of extract solution

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

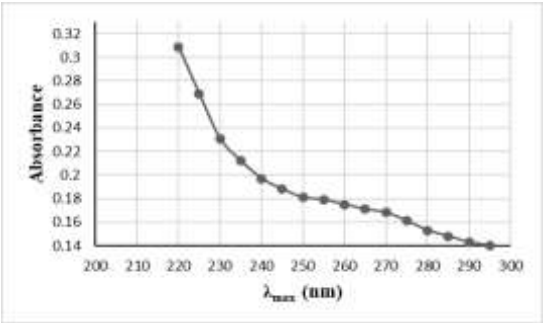

Figure 4.The scanning of leaves extract solution

Quercetin calibration curve

The concentrations of Quercetin and there absorbances are shown in Table (4). Also, the linearity and the regression equation are shown in Figure (5).

Table 4. Quercetin concentrations and their absorbances

Concentration (mg/L)	$\bar{A}_{420}$
0	0
6	0.058
12	0.109
24	0.196
30	0.235
36	0.285
48	0.363
60	0.456

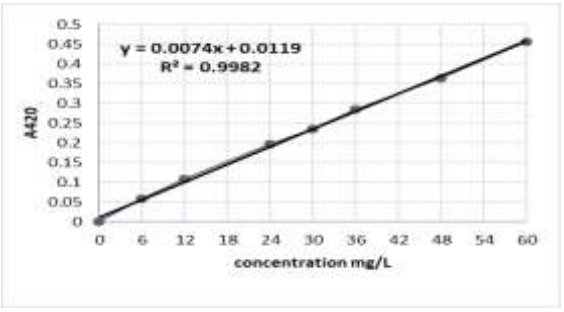

Figure 5. Quercetin calibration curve

TFC of the extracts

Flavonoids content in the extracts is also presented in Table (5). 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%, DH_2O and $CHCl_3$ extracts, respectively. The TFC results are shown in Figure (6).

Table 5.TPC, TFC and RSA% of the standards and the extracts

Samples	Yield%	TPC	Samples		Yield%
			10 min	30 min	
Ascorbic Acid	-	-	-	91.21	93.85
Gallic Acid	-	-	-	83.52	87.54
Quercetin	-	-	-	85.71	89.59
DH2O	13.25	618 ±0.177	155.5 ±0.282	72.84	77.6
EtOH 50	11.25	603 ±0.223	167.5 ±0.475	75.98	80.76
MeOH	8.5%	682.5 ±0.135	194.5 ±0.475	85.4	90.22
MeOH+EtOAc	6.5%	606 ±0.541	176.5 ±0.206	78.02	82.65
CHCl3	2%	278.5 ±0.28	94 ±0.207	52.28	60.09

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

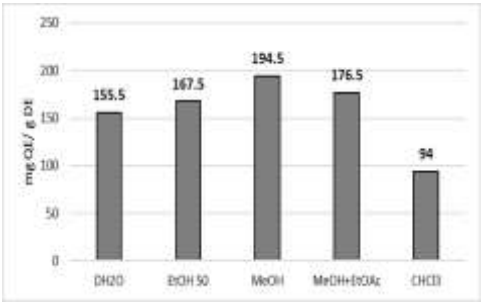

Figure 6. TFC in 1 g of the dried extracts

RSA of the extracts

DPPH• assay revealed that the methanolic extract was the most effective in free radicals scavenging after 30 min (90.22 %), compared with the other extracts: MeOH: EtOAc (1:1), EtOH 50%, DH_2O and $CHCl_3$, they scavenge 82.65, 80.76, 77.6 and 60.09 % of DPPH• radical, and the scavenging activity after 10 and 30 min for the standards and studied extracts is shown in Figure (7).

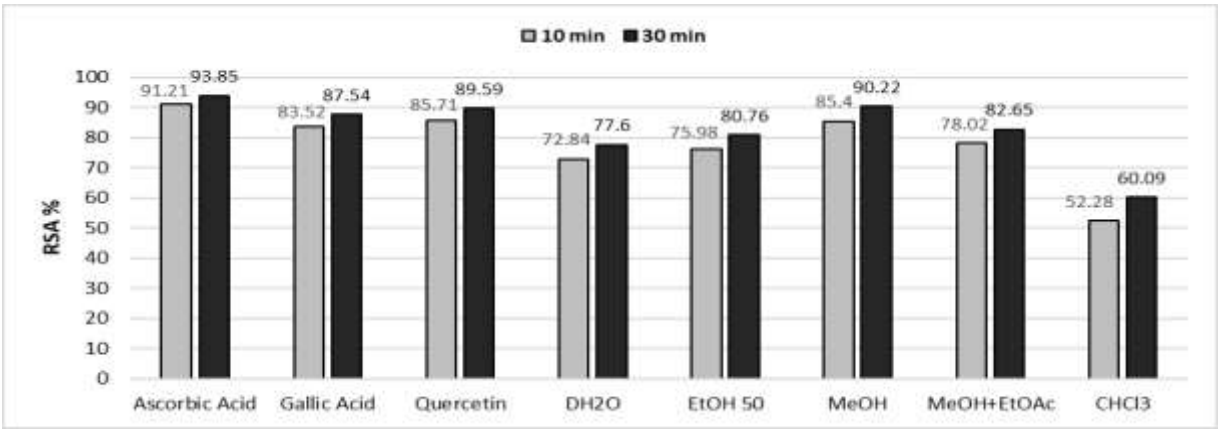

Figure 7.RSA% of standards and studied extracts after 10 and 30 min

The DPPH• scavenging activity after 10 and 30 min, total phenolic and flavonoid contents of the extracts is shown in Table (5).

Qualitative detection and Quantitative determination of quercetin in the ethanolic extract **(16)**

TLC plate used for detection of quercetin in the ethanolic extract under UV light at 254 nm is shown in Figure (8), and the Rf value of Quercetin was 6.2

The quantitative determination of quercetin depends on the AUC of the chromatogram of quercetin in the standard solution and the sample solution Figure (9).

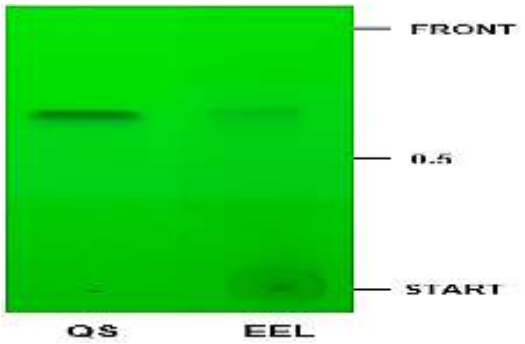

Figure 8. TLC plate of quercetin and ethanolic extract

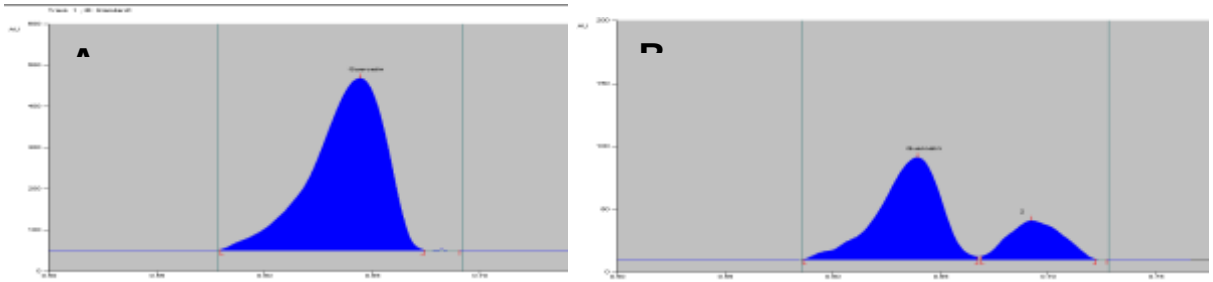

Figure 9. Chromatogram of quercetin in the standard solution (A) and the sample solution (B)

According to the table obtained from the TLC scanner: AUC_1 = 4428.39, AUC_2 = 16554.55 and C_1 = 40 mg/L, C_2 = 5000 mg/L. Then, quercetin percentage was: 0.214 % of dried ethanolic extract of leaves and 0.024% of dried leaves.

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 Total flavonoids content of the leaves extracts, it was ≈ 97%.

Free radicals scavenging activity and total phenolic content, it was ≈ 96%.

Free radicals scavenging activity and flavonoid content, it was ≈ 99%

Discussion

Neither the chemical composition nor the biological effects of *Echinops polyceras* Boiss. have been studied previously. In this study, the chemical constituents have been identified, the polyphenols and flavonoids content has been determined and the free radicals scavenging activity has been evaluated. The results of extraction yield of *E. polyceras* Boiss. leaves (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 materials, or to the presence of the secondary metabolites as glycosides more than aglycons because the solvents have a crucial role in the type of the secondary metabolites found in the extracts [17, 18].

The phytochemical screening revealed the existence of polyphenols as a major component in the extract, especially flavonoids and coumarins, because of that the content of total phenols and flavonoids were determined.

Phenolic compounds especially flavonoids have a notable antioxidant and free radicals scavenging activities. A structure activity relationship study of flavonoids such as quercetin indicates that the hydroxyl groups, the 3,4-catechol structure in the B-ring, the 2-3 double bond and 4-oxo function [19] which found in the quercetin structure are key factors for the antioxidant activity. Quercetin was identified qualitatively and determined quantitively in the dried ethanolic extract (0.214 %), so this extract showed the highest activity.

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

The methanolic extract showed the highest content of total phenols (683 GAE/ g DE) and flavonoids (195 QE/ g DE), while the chloroform extract showed the lowest contents 279 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 (90.2% in 30 minutes), while the chloroform extract was the less effective (60% in 30 minutes), this may be explained by the content of phenols and flavonoids in the extracts.

According to the correlation ratio, the most of the phenolic compounds in the extracts were flavonoids, and these compounds were responsible of the most of the activity. The phenolic content may contribute directly to the antioxidant activity [21].

The aqueous extract which can use in traditional medicine as an infusion [22], showed a good effect in scavenging of free radicals (≈78 in 30 minutes).

All the results of this study showed that *E. polyceras* leaves 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

This study is the first report about *Echinops polyceras Bois.* leaves, 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 $DPPH^{\bullet}$ 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.

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