

Phytochemical profile and antioxidant activity potential of *Lotus sanguineus* (Vural) D.D.Sokoloff

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ABSTRACT

The *in vitro* antioxidant activity and phenol, phenolic acid, flavonoid, and proanthocyanidin content of *Lotus sanguineus* (Vural) D.D.Sokoloff extracts were investigated. The aerial parts of *Lotus sanguineus* have high antioxidant activity. While the ethyl acetate extract has the highest antioxidant activity, in contrast the hexane extract shows lowest activity. In addition, phytochemical antioxidant compounds, phenolic substance, phenolic acid, flavonoid and proanthocyanidin contents of the extracts were determined. The highest total phenolic, phenolic acid, flavonoid and proanthocyanidin contents were found in the ethyl acetate extract. The obtained data supported the result that the plant has antioxidant activity.

Keywords: antioxidant activity, *Lotus sanguineus*, Fabaceae

INTRODUCTION

The genus *Lotus* L. (Fabaceae) is represented by 125 species worldwide¹. *Lotus* species semishrubs or rarely shrubs naturally distributed in Europe, Asia, Af-

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rica, Australia, some islands of the Atlantic and Pacific Oceans and the Socotra archipelago in the Indian Ocean^{2,3}. *Lotus sanguineus* (Vural) D. D. Sokoloff (*Syn. Dorycnium sanguineum* Vural) is an endemic species and has a very limited distribution in Karaman, Turkey⁴ and is locally called kızıl gernevük⁵. According to IUCN (2019), the status of *L. sanguineus* was evaluated as CR (Critically Endangered)⁴.

In traditional medicine, aerial parts of *Dorycnium rectum* have been used to treat burns and wounds⁶ and muscular-skeletal, cardiovascular, digestive and skin disorders⁷. *D. pentaphyllum* have been used as antidiarrhoeal⁸. *D. pentaphyllum* and *D. graecum* as antihemoroidal^{9,10}.

Previous studies indicate that the extracts and compounds of *Dorycnium rectum*¹¹⁻¹³, *D. pentaphyllum*^{9,14-17}, *D. herbaceum*^{18,19} and *D. hirsutum*²⁰ have various biological activities such as antibacterial, antifungal, antiparasitic, anti-inflammatory, anthelmintic, cytotoxic and antioxidant activities.

Phytochemical components present in the genus *Dorycnium* have been reported such as sulfur-containing compounds, isoflavonoids, flavonoids, hydroquinone glucoside, phenylbutanone glucoside and polyphenolic compounds^{10,18-28}.

Oxidative stress caused by free radicals contribute to the development and progression of various chronic diseases such as diabetes, atherosclerosis, cancer, liver disease, neurodegenerative disorders and autoimmune diseases. An imbalance between antioxidant systems and the production of oxidants, including ROS, is known as oxidative stress²⁹. Exogenous antioxidants such as flavonoids and polyphenols found in fruits, vegetables, and medicinal plants act as scavenging ROS, chelating metals, and regulating enzymatic and non-enzymatic systems^{30,31}. Therefore, there has been increasing efforts to identify new antioxidants from natural resources³².

There are no reports in the literature dealing with the phytochemical profile and bioactivities of *L. sanguineus*. Aim of this study to determine the *in vitro* antioxidant activity of *L. sanguineus* extracts and their phenol, phenolic acid, flavonoid, and proanthocyanidin content.

METHODOLOGY

Plant material

The aerial parts of *Lotus sanguineus* were collected in the vicinity of Karaman-Bucaklışla in July 2019 and identified by Ömer Çeçen. A voucher specimen (KNYA 28307) was deposited in the Herbarium of Selçuk University (KONYA).

Chemicals

All chemicals, enzymes and references used in the experimental protocols were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The quality of all chemicals was of analytical grade.

Extraction

Coarsely powdered aerial parts (50 g) of the plant were sequentially extracted at room temperature with hexane and 80% methanol. The extracts were individually concentrated in a rotary evaporator under reduced pressure to dryness. Hexane and methanol extracts of the aerial parts were 0.1975 g, 0.40% and 6.4958 g, 12.99%, respectively. The methanol extract was redissolved in a mixture of methanol/water (10:90) and then sequentially partitioned with dichloromethane and ethyl acetate; the resulting extracts were separately concentrated in vacuo to dryness and aqueous-methanol extract was lyophilized. Dichloromethane, ethyl acetate and aqueous-methanol extracts of the aerial parts were 0.63 g (1.26%, w/w), 0.1860 g (0.37%, w/w) and 6.4958 g (12.99%, w/w) respectively.

Quantitative assessment of phytochemical profile

Total phenolic content

The total phenolic content of the extract was determined according to the method of Singleton and Rossi³³. 75 μL of Na_2CO_3 and 100 μL of Folin-Ciocalteu Reagent were added to 20 μL of freshly diluted extract. Then the composite was left to incubate in the dark at room temperature for 30 minutes. The absorbance was measured spectrophotometrically at 690 nm using a 96-well microplate reader. The total phenolic content of the extracts was expressed as mg gallic acid equivalents (GAE) in 1 g dry extract.

Total phenolic acid content

The total phenolic acid content of the extracts was detected spectrophotometrically with respect to the method declared by Mihailović et al. in 2016³⁴. 1 mL of each of extract, Arnou reagent, 0.1 M HCl, and 1 M NaOH solutions were mixed. Later, the eventual volume was adjusted to 10 mL with distilled water. The absorbance of samples was measured immediately at 490 nm. The total phenolic acid content of the extracts was stated as caffeic acid equivalents (CAE) in 1 g dry extract.

Total flavonoid content

The total flavonoid content of the extracts was calculated according to the aluminum chloride colorimetric method developed by Woisky and Salatino³⁵. 50 μL extracts were mixed with 150 μL of 75% ethanol, 10 μL of 10% aluminum chloride solution, 10 μL of 1M potassium acetate. The mixture was incubated in the dark at room temperature for 30 minutes. The absorbance was read spectrophotometrically at 405 nm. The results were expressed as quercetin equivalents (QE) in 1 g dry extract.

Total proanthocyanidin content

The total proanthocyanidin content of the extracts was explored with regard to the vanillin-HCl method emphasized by Ariffin et al.³⁶ 2.5 mL of 1% vanillin and 2.5 mL of 9 M HCl were added to 1 mL of the sample in a capped glass tube. The mixture was allowed to incubate in the dark for 20 minutes at 30 °C. The absorbance was read spectrophotometrically at 492 nm. The total proanthocyanidin content of the extracts was expressed as (-)-epicatechin equivalents (ECE) in 1 g dry extract.

Estimation of antioxidant activity based on free radical-scavenging activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging activity

The scavenging activity of the extracts against DPPH radical was assessed using the method described by Akter et al.^{37,38} 25 μL of extracts were separately added to 200 μL 0.1 mM DPPH solution prepared in methanol before use. The mixture was incubated at room temperature in the dark for 50 minutes. The absorbance was read at 540 nm. MeOH was used in the control group and butylated hydroxy toluene (BHT) was used as the reference material.

DMPD (N, N'-dimethyl-p-phenylenediamine) radical-scavenging activity

The scavenging activity of the extracts against DMPD radical was expressed by using the method described by Fogliano et al. in 1999³⁹. 15 μL of extracts were separately added to 210 μL of the DMPD solution prepared before use. The mixture was incubated at room temperature in the dark for 50 minutes. The absorbance was measured at 492 nm. The results were given as mg Trolox equivalent (TE) per g material.

Estimation of antioxidant activity based on metal-related activity

Ferric reducing antioxidant power (FRAP)

FRAP assay was performed according to a method described by Benzie and Strain⁴⁰. 10 μL of extracts and 30 μL of distilled water were mixed with a working FRAP reagent in a microplate. The mixture was incubated at 37°C for 30 minutes. Later, the absorbance was recorded at 593 nm using a 96-well microplate reader. BHT was used as the reference substance. The results were expressed as $\mu\text{M FeSO}_4 \cdot 7\text{H}_2\text{O}$ per g material.

Cupric reducing antioxidant capacity (CUPRAC)

CUPRAC activity was determined according to the method found by Apak et al. with some modifications⁴¹. 85 μL of each of 10 mM CuSO_4 , 7.5 mM neocuproine, and 1 M ammonium acetate buffer (pH 7.0) solutions were mixed in a microplate. Later, 51 μL of distilled water and 43 μL of extracts were added respectively. The mixture was incubated at room temperature for 1 hour. After the incubation period, the absorbance was read at 450 nm. The results were given as mg ascorbic acid equivalent (AAE) per g material.

Determination of total antioxidant capacity by phosphomolybdenum method (TOAC)

The total antioxidant capacities of the samples were measured with regard to the phosphomolybdenum method found by Prieto et al. with small modifications⁴². 300 μL of the reagent solution were mixed with 30 μL of warrantably diluted extracts. The microplate containing the mixture was incubated at 95°C for 90 minutes in a water bath. After the incubation period, the samples were cooled to room temperature, and the absorbance was read at 690 nm using a 96-well microplate reader. The results were given as mg ascorbic acid equivalent (AAE) per g material.

Statistics

The experiments were performed in triplicate. The results were assessed as mean \pm standard deviation. Statistical comparisons were made using one-way analysis of variance (ANOVA) followed by Students–Newman–Keuls post hoc test for multiple comparisons. In addition, Pearson correlation coefficients were calculated. The statistically significant difference was detected as $p < 0.05$.

RESULTS and DISCUSSION

This work is the first report concerning the *in vitro* antioxidant activity and phenol, phenolic acid, flavonoid, and proanthocyanidin contents of the aerial

parts of *Lotus sanguineus* (i.e., *Dorycnium sanguineum*). *D. herbaceum* acetone, ethyl acetate, and ethanol extracts were investigated for their *in vitro* antioxidant activity with quantification of phenolic compound contents and the ethanol extract showed highest antioxidant activity and total phenolic content in a previous study¹⁸. The total polyphenolic and flavonoid content and the antioxidant effect of the hydroalcoholic extract of *D. herbaceum* were also investigated and the total polyphenolic content was found higher than the flavonoid content and the extract showed significant antioxidant activity in earlier studies¹⁹. The total flavonoid content and the antioxidant effects of the methanol, ethyl acetate and n-butanol extracts of *D. hirsutum* were determined in previous studies. The amounts of flavonoids in the ethyl acetate extract was found considerably higher than in the other extracts and, showed highest antioxidant activity²⁰.

The phytochemical profiles observed with the extracts of *L. sanguineus* are shown in Table 1. The highest total phenolic, phenolic acid, flavonoid and proanthocyanidin contents were found in the ethyl acetate extract.

Table 1. Total phytochemical profile of the extracts

Extracts	1	2	3	4	5
Total Phenolic Content^A	320.5 ± 15.08 ^a	339.4 ± 4.29 ^b	340.5 ± 9.45 ^c	422.5 ± 3.79 ^c	381.8 ± 8.33 ^c
Total Phenolic Acid Content^B	117.4 ± 2.80 ^a	254.4 ± 9.43 ^b	238.3 ± 0.79 ^c	283.3 ± 9.49 ^c	60.6 ± 0.79 ^c
Total Flavonoid Content^C	28.1 ± 5.35 ^a	34.5 ± 5.43 ^a	58.5 ± 2.71 ^b	90.1 ± 5.60 ^b	3.2 ± 1.86 ^b
Total Proanthocyanidin Content^D	117.03 ± 2.29 ^a	160.1 ± 6.05 ^b	131.3 ± 3.15 ^c	324.6 ± 2.10 ^c	153.7 ± 0.52 ^d

1: MeOH extract; 2: Hexane extract; 3: CH₂Cl₂ extract; 4: EtOAc extract; 5: aqueous-methanol extract

^A Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as mg gallic acid equivalents (GAE) in 1 g dry extract. ^B Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as mg caffeic acid equivalents (CAE) in 1 g dry extract. ^C Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as mg quercetin equivalents (QE) in 1 g dry extract. ^D Results were expressed as the mean of triplicates ± stand-

ard deviation (S.D.) and as mg (-)-Epicatechin equivalents (ECE) in 1 g dry extract. ^{a-d} Values with different letters within a row were significantly different ($p < 0.05$)

In vitro antioxidant activity potential observed with the extracts of *L. sanguineus* are shown in Table 2.

Table 2. *In vitro* antioxidant activity potential of the extracts expressed as EC50 values ($\mu\text{g/mL}$)

Name of the analysis	1	2	3	4	5
DPPH radical-scavenging activity^A	570.4 \pm 3.73 ^a	1846.3 \pm 36.57 ^b	673.6 \pm 20.06 ^c	550.4 \pm 1.41 ^c	584.8 \pm 13.68 ^d
DMPD radical-scavenging activity^B	113.0 \pm 1.56 ^a	.	29.5 \pm 3.40 ^c	119.8 \pm 1.63 ^c	143.8 \pm 2.96 ^c
Ferric reducing antioxidant power^C	1189.3 \pm 27.11 ^a	298.5 \pm 8.33 ^b	922.9 \pm 61.58 ^c	3880.2 \pm 84.85 ^c	1344.1 \pm 23.35 ^c
Cupric reducing antioxidant capacity^D	289.9 \pm 1.71 ^a	54.3 \pm 4.51 ^b	235.4 \pm 15.40 ^c	758.3 \pm 51.94 ^c	222.6 \pm 6.26 ^c
Total antioxidant capacity^D	122.8 \pm 3.89 ^a	155.6 \pm 14.93 ^b	117.9 \pm 7.50 ^c	228.6 \pm 5.89 ^c	92.1 \pm 5.56 ^c

1: MeOH extract; 2: Hexane extract; 3: CH_2Cl_2 extract; 4: EtOAc extract; 5: Aqueous-methanol extract

P.S. 1) EC_{50} value of the reference compound “butylated hydroxytoluene (BHT)” in DPPH scavenging activity is found to be 827.41 ± 1.66 . 2) FRAP activity of the reference compound “butylated hydroxytoluene (BHT)” is found to be $2556.85 \pm 50.24 \mu\text{M FeSO}_4$ eq. in 1 g dry extract. ^AResults were expressed as the mean of triplicates \pm standard deviation (S.D.) and DPPH activity was expressed as EC_{50} in $\mu\text{g/mL}$ equivalents. ^BResults were expressed as the mean of triplicates \pm standard deviation (S.D.) and as mg Trolox equivalents (TE) in 1 g dry extract. ^CResults were expressed as the mean of triplicates \pm standard deviation (S.D.) and as $\mu\text{M FeSO}_4$ equivalents in 1 g dry extract. ^DResults were expressed as the mean of triplicates \pm standard deviation (S.D.) and as mg ascorbic acid equivalents (AAE) in 1 g dry extract. ^{a-d} Values with different letters within a row were significantly different ($p < 0.05$).

The results obtained from this study showed that the aerial parts of *L. sanguineus* have high antioxidant activity. Extracts have been shown to have free radical scavenging activity and the capacity to reduce metal ions involved in

free radical production by *in vitro* antioxidant tests. While the ethyl acetate extract has the highest antioxidant activity, in contrast the hexane extract shows lowest activity. The data obtained for the phenol, phenolic acid, flavonoid, and proanthocyanidin contents of the extracts supported the result that the plant has antioxidant activity.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization, Fatma Tosun, Esra Acar Şah, Sümeyye Albayrak and Ömer Çeçen; methodology, Esra Acar Şah and Sümeyye Albayrak; formal analysis and investigation, Esra Acar Şah and Sümeyye Albayrak; resources, Fatma Tosun, Sümeyye Albayrak, Esra Acar Şah and Ömer Çeçen; writing—original draft preparation, Fatma Tosun, Esra Acar Şah and Sümeyye Albayrak; writing—review and editing, Fatma Tosun, Sümeyye Albayrak, Esra Acar Şah and Ömer Çeçen. All authors have read and agreed to the published version of the manuscript.

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