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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.Sokolof 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 worldwide[1]. *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, Turkey[4] and is locally called kızıl gernevük[5]. 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 wounds[6] and muscular-skeletal, cardiovascular, digestive and skin disorders[7]. *D. pentaphyllum* have been used as antidiarrhoeal[8]. *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 stress[29]. 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 resources[32].

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-Bucakkışla 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 Rossi[33]. 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 2016[34]. 1 mL of each of extract, Arnow 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 Salatino[35]. 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 1999[39]. 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 Strain[40]. 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 µM $FeSO_4.7H_2O$ per g material.

Cupric reducing antioxidant capacity (CUPRAC)

CUPRAC activity was determined according to the method found by Apak et al. with some modifications[41]. 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 modifications[42]. 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 ± 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 study[18]. 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 studies[19]. 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 activity[20].

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: CH_2Cl_2 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 (µg/mL)

Name of the analysis	1	2	3	4	5
DPPH radical-scavenging activity[A]	570.4 ± 3.73[a]	1846.3 ± 36.57[b]	673.6 ± 20.06[c]	550.4 ± 1.41[c]	584.8 ± 13.68[d]
DMPD radical-scavenging activity[B]	113.0 ± 1.56[a]	-	29.5 ± 3.40[c]	119.8 ± 1.63[c]	143.8 ± 2.96[c]
Ferric reducing antioxidant power[C]	1189.3 ± 27.11[a]	298.5 ± 8.33[b]	922.9 ± 61.58[c]	3880.2 ± 84.85[c]	1344.1 ± 23.35[c]
Cupric reducing antioxidant capacity[D]	289.9 ± 1.71[a]	54.3 ± 4.51[b]	235.4 ± 15.40[c]	758.3 ± 51.94[c]	222.6 ± 6.26[c]
Total antioxidant capacity[D]	122.8 ± 3.89[a]	155.6 ± 14.93[b]	117.9 ± 7.50[c]	228.6 ± 5.89[c]	92.1 ± 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 ± 50.24 µM FeSO4 eq. in 1 g dry extract. [A]Results were expressed as the mean of triplicates ± standard deviation (S.D.) and DPPH activity was expressed as EC_{50} in µg/mL equivalents. [B]Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as mg Trolox equivalents (TE) in 1 g dry extract. [C]Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as µM $FeSO_4$ equivalents in 1 g dry extract. [D]Results were expressed as the mean of triplicates ± 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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REFERENCES

1. POWO (2023). Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. Published on the Internet.
http://www.plantsoftheworldonline.org/. Accessed 16 March 2023.

2. Kramina TE, Degtjareva GV, Samigullin TH, Valiejo-Roman CM, Kirkbride Jr JH, Volis S, et al. Phylogeny of *Lotus* (Leguminosae: Loteae): Partial incongruence between nrITS, nrETS and plastid markers and biogeographic implications. Taxon, 2016;65(5):997-1018. Doi: 10.12705/655.4

3. Kramina TE, Lysova MV, Samigullin TH, Schanzer IA, Özbek MU, Sokoloff DD. Phylogenetic placement and phylogeography of large-flowered *Lotus* species (Leguminosae) formerly classified in *Dorycnium*: Evidence of pre-pleistocene differentiation of western and eastern intraspecific groups. Plants, 2021;10(2):260. Doi: 10.3390/plants10020260

4. Subaşı Ü, Şekerciler F, Vural M. Conservation assessment and action on *Lotus sanguineus* (Fabaceae), a critically endangered species endemic to Turkey. Hacquetia, 2020;19(2):307-315. Doi: 10.2478/hacq-2020-0003

5. Asal D, Yaşarkan O. New Turkish scientific names for 171 taxa where duplications have occurred in the "Checklist of the Flora of Turkey (Vascular Plants)." Bağbahçe Bilim Dergisi, 2017;4(2):1-7.

6. Pieroni A, Giusti ME, De Pasquale C, Lenzarini C, Censorii E, Gonzáles-Tejero MR. et al. Circum-Mediterranean cultural heritage and medicinal plant uses in traditional animal healthcare: a field survey in eight selected areas within the RUBIA project. J Ethnobiol Ethnomed, 2006;2(1):16. Doi: 10.1186/1746-4269-2-16

7. González-Tejero MR, Casares-Porcel M, Sánchez-Rojas CP, Ramiro-Gutiérrez JM, Molero-Mesa J, Pieroni A, et al. Medicinal plants in the Mediterranean area: synthesis of the results of the project Rubia. J Ethnopharmacol, 2008;116(2):341-357. Doi: 10.1016/j.jep.2007.11.045

8. Rigat M, Bonet MÀ, Garcia S, Garnatje T, Valles J. Studies on pharmaceutical ethnobotany in the high river Ter valley (Pyrenees, Catalonia, Iberian Peninsula). J Ethnopharmacol, 2007;113(2):267-277. Doi: 10.1016/j.jep.2007.06.004

9. Bremner P, Rivera D, Calzado MA, Obón C, Inocencio C, Beckwith C, et al. Assessing medicinal plants from South-Eastern Spain for potential anti-inflammatory effects targeting nuclear factor-Kappa B and other pro-inflammatory mediators. J Ethnopharmacol, 2009;124(2):295-305. Doi: 10.1016/j.jep.2009.04.035

10. Movsumov IS, Yusifova DY, Suleymanov TA, Garaev EA. Biologically active compounds from Dorycnium graecum growing in Azerbaijan. Chem Nat Compd, 2020;56:953-954. Doi: 10.1007/s10600-020-03198-6

11. Sivakumaran S, Molan AL, Meagher LP, Kolb B, Foo LY, Lane GA, et al. Variation in antimicrobial action of proanthocyanidins from *Dorycnium rectum* against rumen bacteria. Phytochemistry, 2004;65(17):2485-2497. Doi: 10.1016/j.phytochem.2004.08.046

12. Molan AL, Waghorn GC, Min BR, McNabb WC. The effect of condensed tannins from seven herbages on *Trichostrongylus colubriformis* larval migration *in vitro*. Folia Parasitol, 2000;47(1):39-44. Doi: 10.14411/fp.2000.007

13. Waghorn TS, Molan AL, Deighton M, Alexander RA, Leathwick DM, McNabb WC, et al. In vivo anthelmintic activity of *Dorycnium rectum* and grape seed extract against Ostertagia (Teladorsagia) circumcincta and Trichostrongylus colubriformis in sheep. N Z Vet J, 2006;54(1):21-27. Doi: 10.1080/00480169.2006.36599

14. Stefanović O, Radojević I, Vasić S, Čomić L. *Antibacterial activity of naturally occurring compounds from selected plants*. Antimicrobial Agents, Chapter 1, Books on Demand, Hırvatistan, 2012; pp. 1-25.

15. Usta C, Yıldırım AB, Türker AU. Antibacterial and antitumour activities of some plants grown in Turkey. Biotechnol Biotechnol Equip, 2014; 28(2): 306-315. Doi: 10.1080/13102818.2014.909708

16. Türker H, Yıldırım AB. Screening for antibacterial activity of some Turkish plants against fish pathogens: a possible alternative in the treatment of bacterial infections. Biotechnol Biotechnol Equip, 2015;29(2):281-288. Doi: 10.1080/13102818.2015.1006445

17. Koygun G, Arslan E, Zengin G, Orlando G, Ferrante C. Comparison of anticancer activity of *Dorycnium pentaphyllum* extract on MCF-7 and MCF-12A cell line: Correlation with invasion and adhesion. Biomolecules, 2021;11(5):671. Doi: 10.3390/biom11050671

18. Stefanović OD, Tešić JD, Čomić LR. *Melilotus albus* and *Dorycnium herbaceum* extracts as source of phenolic compounds and their antimicrobial, antibiofilm, and antioxidant potentials. J Food Drug Anal, 2015;23(3):417-424. Doi: 10.1016/j.jfda.2015.01.003

19. Nicolaescu O, Varut RM, Patruna S. Determination of the polyphenolic content of *Dorycnium herbaceum* plant extract and testing *in vivo* antioxidant effect, comparative with Bilberry. Rev Chim, 2019;70(2):667-670.

20. Pistelli L, Noccioli C, Martera M, Giamperi L, Bucchini A, Fraternale D, et al. Antioxidant flavonol glycosides from *Dorycnium hirsutum*. Chem Nat Compd, 2006;42(3):281-284. Doi: 10.1007/s10600-006-0099-1

21. Zurabishvili TS, Kemertelidze EP. Flavonoids of *Dorycnium intermedium*. Chem Nat Compd, 1974;10:265. Doi: 10.1007/BF00563640

22. Kazantzoglou G, Magiatis P, Panoutsopoulos G, Skaltsounis AL. Dorycnioside, a new phenylbutanone glucoside from *Dorycnium pentaphyllum* subsp. *herbaceum*. Z Naturforsch C, 2004;59(1-2):23-26. Doi: 10.1515/znc-2004-1-205

23. Hanganu D, Vlase L, Olah N. Phytochemical analysis of isoflavons from some Fabaceae species extracts. Not Bot Horti Agrobot Cluj-Napoca, 2010;38(1):57-60.

24. Varut RM, Rotaru LT. GC-MS, HPLC, TLC of *Dorycnium herbaceum* tincture species and synergistic/antagonist effect testing in combination with antibiotics. Rev Chim, 2017;68(2):228-231.

25. Uysal Ş, Aktümsek A, Picot CM, Sahan A, Mollica A, Zengin G, et al. A comparative in vitro and in silico study of the biological potential and chemical fingerprints of *Dorcycinum pentapyllum* subsp. *haussknechtii* using three extraction procedures. New J Chem, 2017;41(22):13952-13960. Doi: 10.1039/C7NJ03497K

26. Aliyazicioğlu Y, Demir S, Yaman SÖ, Şener SÖ, Demir EA, Aliyazicioğlu R, et al. Phytochemical analysis of *Dorycnium pentaphyllum* and its antiproliferative effect on cervix cancer cells. J Agric Nat, 2019;22(2):365-373. Doi: 10.18016/ksutarimdoga.vi.579938

27. Demir S, Turan İ, Mısır S, Aliyazicioğlu Y. Selective cytotoxic effect of *Dorycnium pentaphyllum* extract on human breast, liver, and lung cancer cells. KSU J Agric Nat, 2019;22(3):473-479. Doi: 10.18016/ksutarimdoga.vi.497868

28. Demir S, Özer Yaman S, Şener SÖ, Ayazoğlu Demir E, Aliyazıcıoğlu R, Özgen U, at al. *Dorycnium pentaphyllum* extract has antiproliferative effect on human cervix and colon cancer cells. Nutr Cancer, 2020;72(3):504-512. Doi: 10.1080/01635581.2019.1636100

29. Davalli P, Mitic T, Caporali A, Lauriola A, D'Arca D. ROS, cell senescence, and novel molecular mechanisms in aging and age-related diseases. Oxid Med Cell Longev, 2016;2016. Doi: 10.1155/2016/3565127

30. Nazir N, Nisar M, Zahoor M, Uddin F, Ullah S, Ullah R, et al. Phytochemical analysis, in vitro anticholinesterase, antioxidant activity and in vivo nootropic effect of *Ferula ammoniacum* (*Dorema ammoniacum*) D. Don. in scopolamine-induced memory impairment in mice. Brain Sci, 2021;11:259. Doi: 10.3390/brainsci11020259

31. Balasundram N, Sundram K, Samman S. Phenolic compounds in plants and agri-industrial by-products: Antioxidant activity, occurrence, and potential uses. Food Chem, 2006;99(1):191-203. Doi: 10.1016/j.foodchem.2005.07.042

32. Nediani C, Giovannelli L. Oxidative stress and inflammation as targets for novel preventive and therapeutic approches in non communicable diseases. Antioxidants, 2020;9(4):290. Doi: 10.3390/antiox9040290

33. Singleton VL, Rossi JAJ. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acids reagents. Amer J Enol Viticult, 1965;16:144-158. Doi: 10.5344/ajev.1965.16.3.144

34. Mihailović V, Kreft S, Benković ET, Ivanović N, Stanković MS. Chemical profile, antioxidant activity and stability in stimulated gastrointestinal tract model system of three *Verbascum* species. Ind Crops Prod, 2016;89:141-151. Doi: 10.1016/j.indcrop.2016.04.075

35. Woisky RG, Salatino A. Analysis of propolis: some parameters and procedures for chemical quality control. J Apic Res, 1998;37(2):99-105. Doi: 10.1080/00218839.1998.11100961

36. Ariffin F, Heong Chew S, Bhupinder K, Karim AA, Huda N. Antioxidant capacity and phenolic composition of fermented *Centella asiatica* herbal teas. J Sci Food Agric, 2011;91(15):2731-2739. Doi: 10.1002/jsfa.4454

37. Akter MS, Ahmed M, Eun JB. Solvent effects on antioxidant properties of persimmon (*Diospyros kaki* L. cv. Daebong) seeds. Int J Food Sci Techno, 2010;45(11):2258-2264. Doi: 10.1111/j.1365-2621.2010.02400.x

38. Celep E, Aydin A, Yesilada E. A comparative study on the *in vitro* antioxidant potentials of three edible fruits: Cornelian cherry, Japanese persimmon and cherry laurel. Food Chem Toxicol, 2012;50(9):3329-3335. Doi: 10.1016/j.fct.2012.06.010

39. Fogliano V, Verde V, Randazzo G, Ritieni A. Method for measuring antioxidant activity and its application to monitoring the antioxidant capacity of wines. J Agric Food Chem, 1999;47(3):1035-1040. Doi: 10.1021/jf980496s

40. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "Antioxidant Power": the FRAP assay. Anal Biochem, 1996;239(1):70-76. Doi: 10.1006/abio.1996.0292

41. Apak R, Güçlü K, Özyürek M, Karademir SE. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. J Agric Food Chem, 2004;52(26):7970-7981. Doi: 10.1021/jf048741x

42. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdinum complex: specific application to the determination of vitamin E. Anal Biochem, 1999;269(2):337-341. Doi: 10.1006/abio.1999.4019