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# Effect of Drying Temperature Regime on Antioxidant Activity of *Lespedeza bicolor* Turcz. Extracts

Olena Vergun*[1], Oksana Shymanska[1], Miroslava Hrstková[2], Dzhamal Rakhmetov[1], Olga Grygorieva[1], Judita Lidiková[2]

[1]M.M. Gryshko National Botanic Garden of the National Academy of Sciences of Ukraine, Kyiv, Ukraine
[2]Slovak University of Agriculture in Nitra, Nitra, Slovakia

Olena Vergun: https://orcid.org/0000-0003-2924-1580
Oksana Shymanska: https://orcid.org/0000-0001-8482-5883
Dzhamal Rakhmetov: https://orcid.org/0000-0001-7260-3263
Miroslavá Hrstková: https://orcid.orcid/0009-0005-0906-5753
Olga Grygorieva: https://orcid.org/0000-0003-1161-0018
Judita Lidiková: https://orcid.org/0000-0001-9922-4300



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*Lespedeza bicolor* Turcz. (bush clover) is a well-known medicinal, forage, and honey plant, the plant extracts of which exhibit numerous biological activities such as antioxidant, antimicrobial, anti-inflammatory, etc. This study aimed to determine the antioxidant capacity of *L. bicolor* ethanol extracts from plant raw material dried at 45 and 65 °C. Plants were collected from the experimental sets of the M.M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine. It was determined the total polyphenol content (TPC) was determined by the Folin-Ciocalteu method, antioxidant activity by the DPPH method (with 2,2-diphenyl-1-picrylhydrazyl), FRAP method (with 2,4,6-tris(2-pyridyl)-S-triazine), and ABTS (with 2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) assay of ethanol extracts. The TPC, antioxidant activity by DPPH, FRAP, and ABTS methods of *L. bicolor* extracts obtained from plant raw dried at 45 °C were 15.16–53.18 mg GAE.g-1, 46.84–49.19 μmol TE.g$^{-1}$, 133.16–335.62 μmol TE.g$^{-1}$, and 2.11–2.39 μmol TE.g$^{-1}$, respectively, depending on plant part. The TPC, antioxidant activity by DPPH, FRAP, and ABTS methods of extracts obtained from plant raw dried at 65 °C were 22.9–106.68 mg GAE.g$^{-1}$, 48.86–51.68 μmol TE.g$^{-1}$, 130.17–345.64 μmol TE.g$^{-1}$, and 2.19–2.39 μmol TE.g$^{-1}$, respectively, depending on plant part. The Pearson's coefficients were higher between the investigated parameters of plants dried at 65 °C and found a strong correlation: $r$ = 0.858 (TPC vs. DPPH), $r$ = 0.952 (TPC vs. FRAP), and $r$ = 0.858 (TPC vs. ABTS). Thus, *L. bicolor* plant extracts are a valuable source of bioactive compounds with antioxidant activity. The study of TPC and antioxidant activity by different methods showed that the temperature regime of plant raw material drying affected the polyphenols' content 1.5–2.0 times. The drying temperature did not significantly affect the antioxidant activity results: a slight increase was observed in flower and leaf extracts by the DPPH and FRAP methods, stem and leaf extracts by the ABTS assay. The obtained data can be used in further biochemical and pharmacological investigations.

**Keywords:** bush clover, polyphenols, radical scavenging activity, reducing power of extracts, correlation

***Corresponding Author:** Olena Vergun, M.M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine, Kyiv, Ukraine
olenavergun8003@gmail.com

## Introduction

Plants of the genus *Lespedeza* Michx. are native to eastern North America, eastern and southern Asia, and Australia, consisting of around 35 plant species (Ohashi et al., 2009). The aerial parts of these plants are used for the treatment of neuralgia and rheumatism by Native Americans. They also used roots against poisoning (Cornara et al., 2016).

The study of *L. bicolor* extracts showed that this plant can be used in the treatment of skin diseases and vitiligo (Ha et al., 2020). Plants of *L. bicolor* have a wide therapeutic potential (Do et al., 2019). *L. bicolor* and *L. capitata* Michx. have been used in Ukrainian traditional medicine for the treatment of kidney diseases (Sokolova et al., 2023; Zaychenko et al., 2024; Serebrovska et al., 2025). In the methanol extracts of *L. bicolor* roots found pterocarpans, coumestans, and arylbenzofurans some of which can be used for leukaemia treatment via intracellular signalling (Thuy et al., 2019) or characterized by cytotoxic and antiproliferative activity (Kim et al., 2023). The active component of *L. bicolor* (protocatechuic acid) can be used to treat alopecia (Shin et al., 2022). The essential oil of this plant contains the terpenoids, sesquiterpenoids, and phenylpropanoids, which exhibit antioxidant, antimicrobial, and enzyme-inhibitory effects (Chitiala et al., 2025). According to Zhu et al. (2025), the main biochemical components of *L. bicolor* essential oil were β-pinene (15.41%), β-phellandrene (12.43%), and caryophyllene (7.79%).

This plant's methanol aerial part extracts exhibited antibacterial and antifungal activities against *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Candida* spp. (Samiullah et al., 2011). The methanol root extracts of this plant exhibited anticancer and antibacterial effects (Li et al., 2024).

Also, this species is known as the honey plant, and honey has demonstrated antioxidant and anti-inflammatory activities (Ren et al., 2024). The major polyphenol compounds are rutin, hyperoside, and kaempferol-3-O-rutinoside (Ren et al., 2023). Kaempferol-3-O-galactoside is recommended as a chemical marker of *L. bicolor* honey authenticity (Ren et al., 2022).

According to Sun et al. (2023), *L. bicolor* plants exhibited aluminum tolerance in acid soils. *L. bicolor* is a forage plant characterized by the highest nutritional value in the vegetative stage (Zhang et al., 2025). This study aimed to determine the antioxidant potential of *L. bicolor* extracts using dried plant raw material at different temperatures.

## Material and Methodology

### Plant Material

Plants of *Lespedeza bicolor* Turcz. were collected from experimental sets of the Department of Cultural Flora of the M.M. Gryshko National Botanical Garden of the National Academy of Sciences of Ukraine (Kyiv) in the flowering stage. The investigated plants' leaves, flowers, and stems were separated and dried at 45 and 65 °C.

### Extract Preparation

One gram of dried plant raw material was mixed with 50 ml of 80% ethanol. The mixtures were shaken for 12 hours on the Unimax 2010 horizontal shaker (Heidolph Instrument GmbH, Germany). Obtained samples were filtered via Munktell No. 390 filtrating paper (Munktell & Filtrac, Germany).

### The Total Polyphenol Content

The polyphenol content of plant extracts was determined by the Folin-Ciocalteu assay (Lachman et al., 2006). The volumetric flask (50 ml) was filled with extract (0.1 ml), with the following addition of Folin-Ciocalteu reagent (0.85 ml). After 3 min, add 20% $NA_2CO_3$ (5 ml). After stirring the mixture, water was added till the mark (50 ml). Experimental flasks were placed in the dark for 2 hours, and after they were analyzed on a Shimadzu UV-1800 UV/Visible Scanning Spectrophotometer (Shimadzu, Kyoto, Japan) at 765 nm. The gallic acid was used as a standard, and results were expressed as mg GAE.$g^{-1}$. Four replicates were used in the experiment.

### DPPH Radical Scavenging Activity

A working DPPH solution was prepared using methanol and 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma Aldrich, USA). DPPH solution (3.9 mL) and plant extract (0.1 mL) were mixed, stirred, and left in the dark for 10 min. The solution was measured at 515.6 nm against a blank solution, using a Shimadzu UV-1800 UV/Visible Scanning Spectrophotometer (Shimadzu, Kyoto, Japan). Based on the calibration curve, antioxidant activity was expressed as the mmol of Trolox equivalent per gram of fresh matter (mmol TE.$g^{-1}$ DW) (Brand-Williams et al., 1995). Analyses were performed in 4 replicates.

### ABTS Radical Scavenging Assay

According to Re et al. (1999), the ABTS radical scavenging assay was conducted. Working ABTS solution was created using the ABTS·+ radical cation

(2,2'-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) (Sigma Aldrich, USA), potassium persulfate ($K_2S_2O_8$) (Sigma Aldrich, USA), and acetate buffer (pH = 4.3). 3 mL of ABTS solution and 0.05 mL of extract were mixed, stirred, and left in the dark for 20 min. The solution was measured at 734 nm against a blank solution, using a Shimadzu UV-1800 UV/Visible Scanning Spectrophotometer (Shimadzu, Kyoto, Japan). Based on the calibration curve, antioxidant activity was expressed in mmol of Trolox equivalent per gram of dry matter (mmol TE.g$^{-1}$ DW).

### FRAP Assay

The FRAP assay determined antioxidant activity (Pedersen et al., 2000). FRAP solution was created using 2,4,6-tris(2-pyridyl)-S-triazine (Sigma Aldrich, USA), ferric chloride ($FeCl_3$) (Sigma Aldrich, USA), and acetate buffer (pH = 3.5). 3 mL of working FRAP solution and 0.5 mL of extract were mixed, stirred, and left in the dark for 20 min. The solution was measured at 593 nm against a blank solution, using a Shimadzu UV-1800 UV/Visible Scanning Spectrophotometer (Shimadzu, Kyoto, Japan). Based on the calibration curve, antioxidant activity was expressed as the mmol of Trolox equivalent per g of dried matter (mmol TE.g$^{-1}$). Analyses were performed in 4 replicates.

### Statistical Analysis

The results are expressed as mean values of three replications ±standard deviation(SD). Data were analysed with the ANOVA test, and differences between means were compared through the Tukey-Kramer test ($p < 0.05$).

## Results and Discussion

Polyphenol content has become one of the most popular and interesting topics among plant biochemists, botanists, ecologists, etc. Polyphenol compounds are well-known secondary metabolites widely distributed in the plant world (Rasouli et al., 2017). They have been found in fruit plants (Swallah et al., 2020), medicinal herbs (Kulig et al., 2019), wines (Gutiérrez-Escobar et al., 2021), honey (Jibril et al., 2019), etc. According to Ren et al. (2023), the *L. bicolor* honey is rich in polyphenol compounds. Extracts of these plants are rich in polyphenol compounds, in particular water extracts, such as rutin, quercetin, chlorogenic acid, etc. (Yaromiy et al., 2025).

According to Belwal et al. (2022), numerous drying techniques exist, each of which can have positive or negative aspects affected on plant raw materials depending on plant species. The study of the drying process of plant raw material with ascorbic acid, polyphenols, glycosides, and volatile compounds showed optimal temperatures for retaining these bioactive compounds in the range from 40 to 70 °C. In this case, the plant is raw dried at 55–60 °C, optimal for polyphenol content determination, and 60–70 °C for flavonoids (ElGamal et al., 2023). The optimal temperature for polyphenol retention during water extraction is 60–80 °C (Antony and Farid, 2022).

The total polyphenol content of different *L. bicolor* extracts are represented in Figure 1. All investigated extracts demonstrated an increase in total polyphenol content in plant extracts with raw dried at a higher temperature (65 °C). So, in stem, flower, and leaf extracts of *L. bicolor* dried at 65 °C, the polyphenol

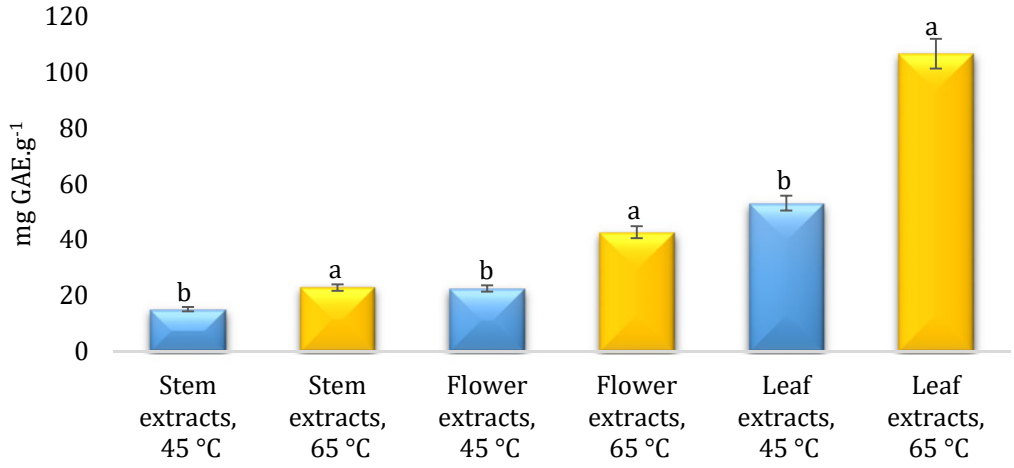


**Figure 1** The total polyphenol content of *Lespedeza bicolor* Turcz. ethanol extracts depending on the temperature regime
GAE – gallic acid equivalent. Means in each column followed by different letters are significantly different ($p < 0.05$)

content increased 1.46, 1.89, and 2 times, respectively. The TPC of stem, flower, and leaf extracts of the plant dried at 45 °C was 15.5, 22.59, and 53.18 mg GAE.g$^{-1}$, respectively.

Kim and Kim (2010) found a significant difference between total polyphenol content in different extracts of *L. cuneata*, most of them determined in the ethyl-acetate extracts (359.54 mg GAE.g$^{-1}$) and the least in the water extract (21.24 mg GAE.g$^{-1}$). According to Samiullah et al. (2012), the total polyphenol compounds of *L. bicolor* methanol extracts were 1.66 and 1.23 mg GAE.g$^{-1}$ in the aerial and root parts, respectively. Also, the methanol extract of this plant was effective against pathogenic strains. The study of *L. bicolor* methanolic extracts showed that the aerial part had 3.32 mg GAE.g$^{-1}$ and root extracts 2.46 mg GAE.g$^{-1}$ of total polyphenol content (Ullah, 2017). The comparative analysis of three *Lespedeza* species showed that *L. bicolor* ethanol extracts had the most content of total polyphenol content, while *L. cuneata* exhibited the highest antioxidant potential (Chitiala et al., 2025).

The numerous methods of antioxidant activity evaluation exist, among which DPPH, FRAP, ABTS, etc. (Alam et al., 2013). One of the most widely used methods of antioxidant activity evaluation is DPPH (α,α-diphenyl-β-picrylhidrazyl), that based on measuring of DPPH concentration in the investigated solution (Kedare and Singh, 2011). The unreacted DPPH is a measure of the antioxidant activity of the investigated extract and depends on the quantity and nature of the used solvent, which can affect reaction kinetics (Dawidowicz et al., 2012). The antioxidant activity determined by the DPPH method in the investigated *L. bicolor* stem extracts was approximately the same (Figure 2). The flower and leaf extracts of plants dried at 65 °C had antioxidant activity 1.08 and 1.05 times higher than those of plants dried at 45 °C. In this case, the antioxidant activity by the DPPH method didn't depend on the temperature regime of raw plant drying. In total, the results of this parameter for plants dried at 45 °C and 65 °C were as follows: stem extracts 48.92 and 48.86 μmol TE.g$^{-1}$, flower extracts 46.84 and 51 μmol TE.g$^{-1}$, and leaf extracts 49.16 and 51.86 μmol TE.g$^{-1}$.

The same results were obtained in the extracts to determine the antioxidant activity by the FRAP method (Figure 3). A slight increase was found in the obtained values in the flower and leaf extracts of plants dried at 65 °C, while in the stem extracts, the opposite was observed – the stem extracts had a slight decrease in plants dried at 65 °C. The antioxidant activity by the FRAP method of stem extracts of *L. bicolor* dried at 45 °C and 65 °C was 133.16 and 130.17 μmol TE.g$^{-1}$, the flower extracts were 230.6 and 234.61 μmol TE.g$^{-1}$, and the leaf extracts were 335.62 and 345.64 μmol TE.g$^{-1}$, respectively.

According to Zhu et al. (2025), the *L. bicolor* essential oil demonstrated 81.96 μmol.g$^{-1}$ of antioxidant activity by the FRAP method.

The ABTS method of antioxidant capacity determination is based on radical cation absorbance and scavenging in the aqueous phase (Khade et al., 2023). The antioxidant activity determination by the ABTS method of stem, flower, and leaf extracts of *L. bicolor*, as represented in Figure 4, showed no significant difference between extracts of plants dried at 45 and 65 °C. Stem and leaf extracts showed a slight increase in antioxidant activity in extracts dried at 65 °C compared to those dried at 45 °C. In comparison, the extracts of flowers demonstrated a slight decrease

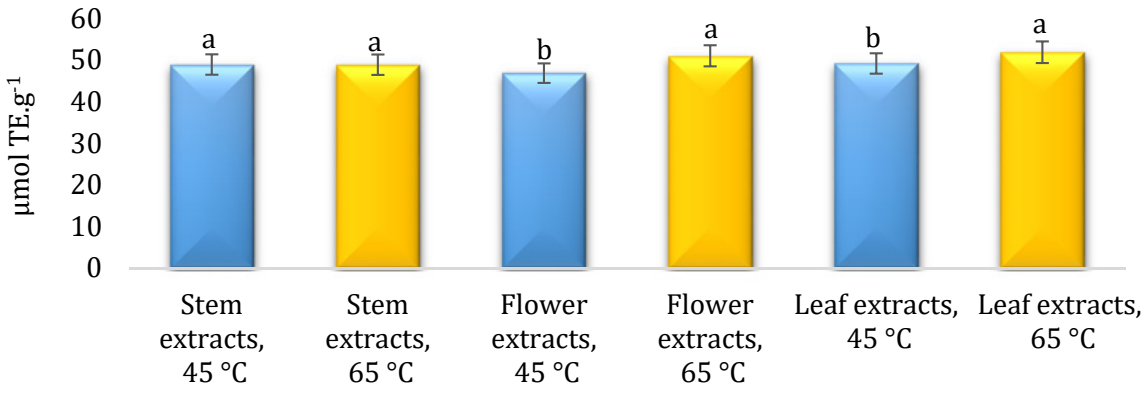


**Figure 2** The antioxidant activity by the DPPH method of *Lespedeza bicolor* Turcz. ethanol extracts depending on the temperature regime
TE – Trolox equivalent. Means in each column followed by different letters are significantly different ($p < 0.05$)

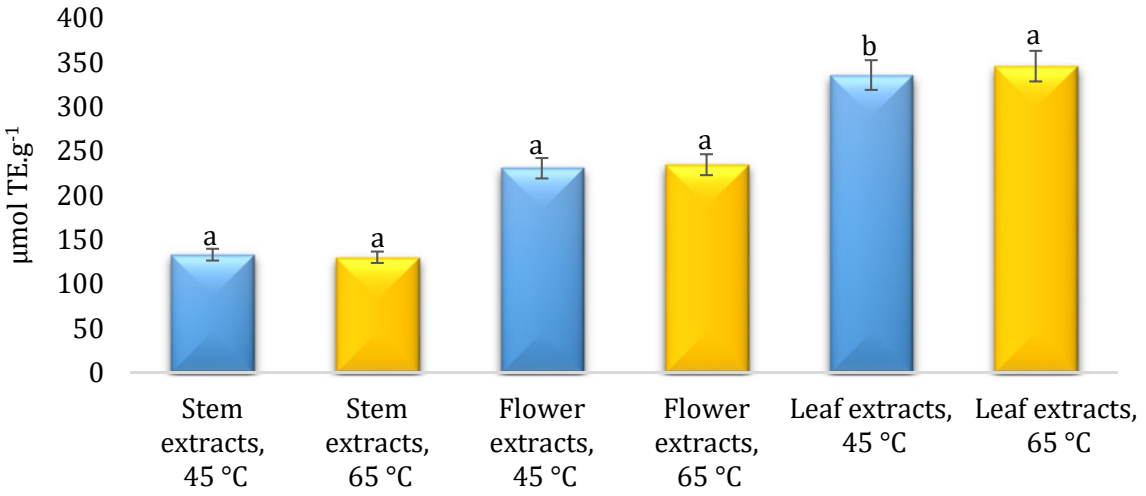


**Figure 3** The antioxidant activity by the FRAP method of *Lespedeza bicolor* Turcz. ethanol extracts depending on the temperature regime
TE – Trolox equivalent; 45 °C – plant raw dried at 45 °C; 65 °C – plant raw dried at 65 °C. Means in each column followed by different letters are significantly different ($p < 0.05$)

in antioxidant activity by the ABTS method in plants dried at 65 °C. So, stem extracts of plants dried at 45 and 65 °C had 2.11 and 2.19 µmol TE.g$^{-1}$, flower extracts 2.39 and 2.33 µmol TE.g$^{-1}$, and 2.38 and 2.39 µmol TE.g$^{-1}$ of antioxidant activity by the ABTS method.

The radical scavenging activity of *L. cuneata* different extracts was from 92.63 to 833.24 µg.mL$^{-1}$ and from 26.82 to 161.36 µg.mL$^{-1}$ by DPPH and ABTS methods, respectively (scavenging radical by 50%) (Shin and Song, 2017). According to Mariadoss et al. (2023), the antioxidant capacity by the ABTS method of hexane, ethyl acetate, and methanol leaf extracts of *L. cuneata* exhibited 149.86, 58.32, and 237.23 µg.mL$^{-1}$, respectively. The study of *L. cuneata* extract showed a strong anti-oxidative effect against free radicals by the DPPH and ABTS methods (Wahab et al., 2023). The total antioxidant capacity by the ABTS assay in the leaf extracts of *Paulownia elongata* was higher at drying regime 40 °C during 6 hours (6,065.8 µmol TE.g$^{-1}$) than 80 °C during 2 hours (3,637.1 µmol TE.g$^{-1}$) (Shahin et al., 2025).

Numerous studies showed a high positive correlation between total polyphenol content and different radical scavenging activities of extracts depending on plant species, extraction solution, and plant growth conditions (Akkari et al., 2016). In our experiment correlation between total polyphenol compounds and antioxidant capacity of extracts by DPPH and ABTS

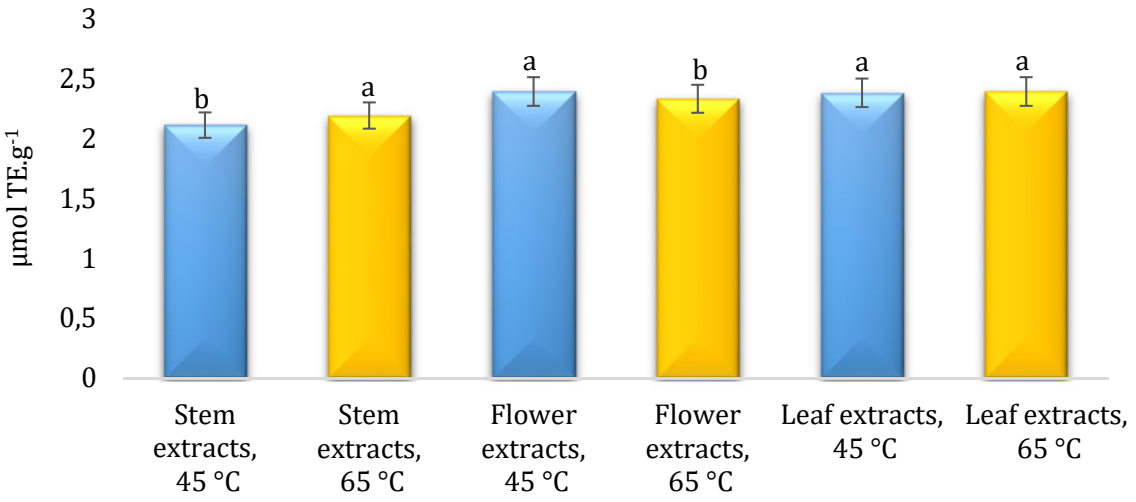


**Figure 4** The antioxidant activity by the ABTS method of *Lespedeza bicolor* Turcz. ethanol extracts depending on the temperature regime
TE – Trolox equivalent. Means in each column followed by different letters are significantly different ($p < 0.05$)

**Table 1** The Pearson's criterion between investigated parameters

| Parameter | TPC | DPPH | FRAP | ABTS |
|---|---|---|---|---|
| | | **Drying at 45 °C** | | |
| **TPC** | 1.000 | | | |
| **DPPH** | 0.419 | 1.000 | | |
| **FRAP** | 0.950 | 0.116 | 1.000 | |
| **ABTS** | 0.651 | -0.416 | 0.855 | 1.000 |
| | | **Drying at 65 °C** | | |
| **TPC** | 1.000 | | | |
| **DPPH** | 0.858 | 1.000 | | |
| **FRAP** | 0.952 | 0.966 | 1.000 | |
| **ABTS** | 0.858 | 0.999 | 0.966 | 1.000 |

Notes: TPC – total content of polyphenol compounds; DPPH – antioxidant activity by the DPPH method; FRAP – antioxidant activity by the FRAP method; ABTS – antioxidant activity by the ABTS method

assays was stronger in extracts prepared from plant raw dried at 65 °C (Table 1). The relation between total polyphenol content and antioxidant capacity by the FRAP method was very strong, but didn't depend on the drying regime of plant raw preparation. The correlation between DPPH and FRAP methods increased in extracts of plant raw dried at 65 °C (from $r$ = 0.116 to $r$ = 0.966). The correlation between FRAP and ABTS slightly increased with a higher temperature regime of plant raw drying.

Kim et al. (2010) found a high correlation between total polyphenol content and radical scavenging activity (by DPPH and ABTS) in *Lespedeza* spp. extracts.

## Conclusions

Thus, *L. bicolor* plant extracts are a valuable source of bioactive compounds with antioxidant activity. The study of TPC and antioxidant activity by different methods showed that the temperature regime of plant raw material drying affected the polyphenols' content. In this case, the TPC of extracts significantly increased in plant raw material dried at 65 °C compared to those dried at 45 °C. Antioxidant capacity of extracts by the different methods wasn't affected by the temperature regime of the plant raw material investigated. The obtained data can be used in further biochemical and pharmacological investigations.

### Conflict of Interest

The authors declare no conflict of interest.

### Ethical Statement

This article does not contain any studies that would require an ethical statement.

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## References

Akkari, H., Hajaji, S., B'chin, F., Rekik, M., & Gharbi, M. (2016). Correlation of polyphenolic content with radical-scavenging capacity and anthelmintic effects of Rubus ulmifolius (*Rosa*ceae) against *Haemonchus contortus*. *Veterinary Parasitilogy*, 221, 46–53. http://dx.doi.org/10.1016/j.vetpar.2016.03.007

Alam, M. N., Bristi, N. J., & Rafiguzzaman, M. (2013). Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. *Saudi Pharmaceutical Journal*, 21, 143–152. http://dx.doi.org/10.1016/j.jsps.2012.05.002

Antony, A., & Farid, M. (2022). Effect of temperatures of polyphenols during extraction. *Applied Sciences*, 12, 2107. https://doi.org/10.3390/app12042107

Belwal, T., Cravotto, C., Prieto, M. A., Venskutonis, P., Daglia, M., Devkota Prasad, H., Baldi, A., Ezzat, S.M., Gómez-Gómez, L., Salama, M. M., Campone, L., Rastrelli, L., Echave, J., Jafari, S. M., & Cravotto, G. (2022). Effects of different drying techniques on the quality and bioactive compounds of plant-based products: a critical review on current trends. *Drying Technology*, 1, 1–24. https://doi.org/10.1080/07373937.2022.2068028

Brand-Williams, W., Cuvekier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT – Food Science and Technology*, 28(1), 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

Chitiala, R. D., Lungu, I. I., Marin, G.-A., Mitran, A. M., Caba I.-C., Lungu, A., Robu, S., Mircea, C., Stefanache, A., Hancianu, M., & Cioanca, O. (2025). Comparative analysis of *Lespedeza* species: traditional uses and biological activity of the Fabaceae family. *Molecules*, 30, 2013. https://doi.org/10.3390/molecules30092013

Cornara, L., Xiao, J., & Burlando, B. (2016). Therapeutic potential of temperate forage legumes: a review. *Critical Reviews in Food Science and Nutrition*, 56, 149–161. https://doi.org/10.1080/10408398.2015.1038378

Dawodowicz, A., Wianowska, D., & Plszowy, M. (2012). On practical problems in estimation of antioxidant activity of compounds by DPPH method (Problems in estimation of antioxidant activity). *Food Chemistry*, 131, 1037–1043. https://doi.org/10.1016/j.foodchem.2011.09.067

Do, H. M., Lee, H. J., Cho, K., Kang, M. C., Subedi, L., Parveen, A., & Kim, S. Y. (2019). Therapeutic potential of *Lespedeza bicolor* to prevent methylglyoxal-induced glucotoxicity in familiar diabetic nephropathy. *Journal of Clinical Medicine*, 8(8), 1138. https://doi.org/10.3390/jcm8081138

ElGamal, R., Song, C., Rayan, A.M., Liu, C., Al-Rijaie, S., & ElMasry, G. (2023). Thermal degradation of bioactive compounds during drying process of horticultural and agronomic products: a comprehensive overview. *Agronomy*, 13, 1580. https://doi.org/10.3390/agronomy13061580

Gutiérrez-Escobar, R., Aliaño-Gonzales, M. J., & Cantos-Villar, E. (2021). Wine polyphenol content and its influence on wine quality and properties: a review. *Molecules*, 26, 718. https://doi.org/10.3390/molecules26030718

Ha, S. Y., Jung, J. Y., Kang, H. Y., Kim, T.-H., & Yang, J.-K. (2020). Tyrosinase activity and melanogenic effects of *Lespedeza bicolor* extract *in vitro* and *in vivo*. *BioResources*, 15(3), 6244–6261.

Jibril, F. I., Hilmi, A. B., & Manivannan, L. (2019). Isolation and characterization of polyphenols in natural honey for the treatment of human diseases. *Bulletin of the National Research Centre*, 43(4), 1–9. https://doi.org/10.1186/s42269-019-0044-7

Kedare, S. B., & Singh, R. P. (2011). Genesis and development of DPPH method of antioxidant assays. *Journal of Food Science and Technology*, 48(4), 412–422. https://doi.org/10.1007/s13197-011-0251-1

Khade, O. S., Sruti, K., Sonkar, R. M., Gade, P. S., & Bhatt, P. (2023). Plant secondary metabolites: extraction, screening, analysis and their bioactivity. International *Journal of Herbal Medicine*, 11(2), 1–17. https://doi.org/10.22271/flora.2023.v11.i2a.855

Kim, J.-S., & Kim, M.-J. (2010). In vitro anti-oxidant activity of *Lespedeza cuneata* methanolic extracts. *Journal of Medicinal Plant Research*, 4(8), 674–679. https://doi.org/10.5897/JMPR09.464

Kim, S. M., Yung, Y.-J., Pan, C.-H., & Um, B.-H. (2010). Antioxidant activity of methanol extracts from the genus *Lespedeza*. *Journal of Korean Society of Food Science and Nutrition*, 39(5), 769–775. https://doi.org/10.3746/jkfn.2010.39.5.769

Kim, S., Choi, Y. J., Goh, M. S., Kim, Y., Li, Z., Park, J. U., Ahn, Y., Seon, J. H., Yoo, H. M., Ko, K. Ch., & Cho, N. (2023). Seven new secondary metabolites isolated from roots of *Lespedeza bicolor*. *Fitoterapia*, 170, 105671. https://doi.org/10.1016/j.fitote.2023.105671

Kulig, D., Matysiak, M., Baldovská, S., Stefaniková, J., Maruniabova, N., Mnahončaková, E., Árvay, Yu., Gabavý, D., & Kolesárová, A. (2019). Screening of polyphenolic compounds from traditional medicinal herbs. *Journal of Microbiology, Biotechnology and Food Sciences*, 9, 487–491. https://doi.org/10.15414/jmbfs.2019.9.special.487-491

Lachman, J., Hamouz, K., Čepl, J., Pivec, V., Šulc, M., & Dvořák, P. (2006). The effect of selected factors on polyphenol content and antioxidant activity in potato tubers. *Chemicke Listy*, 100, 522–527.

Li, Z., Yao, L., Saravanakumar, K., Thuy, N. T. T., Kim, Y., Xue, Ch., Zheng, X., & Cho, N. (2024). *Lespedeza bicolor* root extract exerts anti-TNBC potential by regulating the FAK-related signalling pathway. *American Journal of Cancer Research,* 14(9), 4265–4285. https://doi.org/10.62347/MYPG4066

Mariadoss, A. V. A., Park, S. J., Saravanakumar, K., Sathiyaseelan, A., & Wang, M.-H. (2023). Phytochemical profiling, *in vitro* antioxidant, and antidiabetic efficacy of ethyl acetate fraction of *Lespedeza cuneata* on streptozotocin-induced diabetic rats. *Environmental Science and Pollution Research*, 30, 60976–60993. https://doi.org/10.1007/s11356-023-26412-8

Ohashi, H., Nemoto, T., & Ohashi, K. (2009). A revision of *Lespedeza* subgenus *Lespedeza* (Leguminosae) of China. *Journal of Japanese Botany*, 84, 143–166.

Pederson, C. B., Kyle, J., Jenkinson, A., Gardner, P. T., McPhail, D. B., & Duthie, G. G. (2000). Effects of blueberry and cranberry juice consumption on the plasma antioxidant capacity of healthy female volunteers. *European Journal of Clinical Nutrition*, 54(5), 405–408. https://doi.org/10.1038/sj.ejcn.1600972

Rasouli, H., Farzaei, M. H., & Khodarahmi, R. (2017). Polyphenols and their benefits: a review. International *Journal of Food Properties*, 20(2), 1700–1741. https://doi.org/10.1080/10942912.2017.1354017

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, 26(9–10), 1231–1237. https://doi.org/10.1016/S0891-5849(98)00315-3

Ren, C., Wang, K., Luo, T., Xue, X., Wang, M., Wu, L., & Zhao, L. (2022). Kaempferol-3-O-galactoside as a marker for authenticating *Lespedeza bicolor* Turcz. monofloral honey. *Food Research International,* 160, 111667. https://doi.org/10.1016/j.foodres.2022.111667

Ren, C., Li, Q., Luo, T., Betti, M., Wang, M., Qi, S., Wu, L., & Zhao, L. (2023). Antioxidant polyphenols from *Lespedeza bicolor* Turcz. honey: anti-inflammatory effects on lipopolysaccharide-treated RAW 264.7 macrophages. *Antioxidants*, 12, 1809. https://doi.org/10.3390/antiox12101809

Ren, C., Zhu, Y., Li, Q., Wang, M., Qi, S., Sun, D., Wu, L., & Zhao, L. (2024). *Lespedeza bicolor* Turcz. honey prevents inflammation response and inhibits ferroptosis by Nrf2/HO-1 pathway in DSS-induced human Caco-2 Cells. *Antioxidants*, 13(8), 900. https://doi.org/10.3390/antiox13080900

Samiullah, Bano, A., Naz, R., & Yasmin, H. (2011). In vitro inhibition potential of *Lespedeza bicolor* Turcz. against selected bacterial and fungal strains. *Journal of Medicinal Plant Research*, 5(16), 3708–3714.

Samiullah, Bano, A., Girmay, S., & Tan, G. (2012). Total phenolic content, antioxidant, antimicrobial and anticancer activities of *Lespedeza bicolor* Turcz. (Papilionaceae). *International Conference on Applied Life Sciences*, 259–264. https://doi.org/10.5772/intechopen.84088

Serebrovska, Z. O., Tolstun, D. O., Sykalo, N. V., Farkhidinov, I. O., Kropyva, V., Myhovan, S. A., Chyzhova, V. P., Kovtonyuk, T. I., Samots, I. A., & Mankovsky, B. N. (2025). Synergichnyi vplyv ekstraktiv lespedezy ta artishoku v terapii chronichnogo zahvoryuvannya nyrok: mehanizmy ta perspektyvy [Synergistic effects of *Lespedeza* and artichoke extracts in the therapy of chronic kidney disease: mechanisms and perspectives]. *Kidneys*, 14(1), 63–70. https://doi.org/10.2214/2307-1257.14.1.2025.499 [In Ukrainian]

Shahin, L., Maharatra, A. K., & Joshee, N. (2025). Effect of drying methods on the leaf and flower tissues of *Paulownia elongata* and *P. fortune* and resultant antioxidant capacity. *Antioxidants*, 14, 280. https://doi.org/10.3390/antiox14030280

Shin, S. Y., Kwon, J.-E., Kim, S., Lee, Y.-G., Park, K.S., & Kang, S.-Ch. (2022). Hair regeneration effects of *Lespedeza bicolor* extracts *in vivo* and *in vitro*. *Applied Sciences*, 12, 2863. https://doi.org/10.3390/app12062863

Shin, Y. H., & Song, Ch.-K. (2017). Antioxidant and metalloproteinase inhibitory activities of ethanol extracts from *Lespedeza cuneata* G. Don. *Korean Journal of Environmental Agriculture*, 36(4), 263–268. https://doi.org/10.5338/KJEA.2017.36.4.40

Sokolova, L. K., Pushkarev, V. M., & Tronko, M. D. (2023). Perspektyvy vykorystannya preparative lespedey (*Lespedeza*) dla likuvannya patologii nyrok ta inshyh diabetychnyh uskladnen [Prospects of the use of lespedeza (*Lespedeza* sp.) preparation for the treatment of kidney pathology and other diabetic complications]. *International Journal of Endocrinology*, 19(5), 6–15. [In Ukrainian]

Sun, Q. B., Yin, C.-Q., Zheng, H., Dong, X.-Y., Shen, R.-F., & Zhao, X.-Q. (2023). Higher aluminum tolerance of *Lespedeza bicolor* relative to *Lespedeza cuneata* is associated with saccharide components of root tips. *Agronomy*, 13(3), 629. https://doi.org/10.3390/agronomy13030629

Swallah, M. Sh., Sun, H., Affoh, R., Fu, H., & Yu, H. (2020). Antioxidant potential overviews of secondary metabolites (polyphenols) in fruits. *International Journal of Food Science*, 9081686. https://doi.org/10.1155/2020/9081686

Thuy, N. T. T., Lee, J.-E., Yoo, H. M., & Cho, N. (2019). Antiproliferative pterocarpans and coumestans from *Lespedeza bicolor*. *Journal of Natural Products*, 82(11), 3025–3032. https://doi.org/10.1021/acs.jnatprod.9b00567

Ullah, S. (2017). Methanolic extract from *Lespedeza bicolor*: potential candidates for natural antioxidant and anticancer agent. *Journal of Traditional Chinese Medicine*, 37(4), 444–451.

Wahab, A., Sim, H., Choi, K., Kim, Y., Lee, Y., Kang, B., No, Y. S., Lee, D., Lee, I., Lee, J., Cha, H., Kim, S. D., Saba, E., & Rhee, M. H. (2023). Antioxidant and anti-inflammatory activities of *Lespedeza cuneata* in Coal fly ash-induced murine alveolar macrophage cells. *Korean Journal of Veterinary Research*, 63(3), e27. https://doi.org/10.14405/kjvr.20230022

Yaromiy, M., Bevz, N., Mykhailenko, O., Kovalov, V., Polovko, N., & Marchenko, Y. (2025). Development of the composition of aqueous extract of *Lespedeza bicolor* and gel with extract and mangiferin. *ScienceRise: Pharmaceutical Science*, 53(1), 4–13. https://doi.org/10.15587/2519-4852.2025.322720

Zaychenko, G. V., Kyslychenko, V. S., Protska, V. V., Fedosov, A. I., & Simonov, P. V. (2024). Perspektyvy zastosuvannya nefroprotektoriv roslynnoho pohodzhennya na osnovi lespedezy dvokolirnoji [Prospects for the application of nephroprotectors of plant origin based on *Lespedeza bicolor*]. Family Medicine. *Family Practices*, 108(2), 55–61. https://doi.org/10.30841/2786-720X.2.2024.307535 [In Ukrainian]

Zhang, J., He, Y., Zhang, H., Xing, Y., Li, A., Guo, D., & Wang, J. (2025). Effects of condensed tannins in *Lespedeza bicolor* leaves on *in vitro* rumen fermentation and methane emission. *New Zealand Journal of Agricultural Research*, 1, 1–19. https://doi.org/10.1018/00288233.2025.2486021

Zhu, J., Xu, Z., & Liu, X. (2025). Chemical composition, antioxidant activities, and enzyme inhibitory effects of *Lespedeza bicolor* Tircz. essential oil. *Journal of Enzyme Inhibition and Medicinal Chemistry*, 40(1), 2460053. https://doi.org/10.1080/14756366.2025.2460053