https://doi.org/10.48047/AFJBS.6.15.2024.10626-10634

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Chemical Profile, Radical Scavenging, Reducing Power, and Anti-Lipid Peroxidation Activities of *Lavandula stoechas* Methanol Extract and Its Fractions

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Volume 6, Issue 15, Sep 2024

Received: 15 July 2024

Accepted: 25 Aug 2024

Published: 25 Sep 2024

doi: 10.48047/AFJBS.6.15.2024.10626-10634

ABSTRACT
Lavender has long been believed to offer various therapeutic and curative benefits, such as promoting relaxation and addressing conditions like parasitic infections, burns, insect bites, and muscle spasms. In this study, the aerial parts of the plant, collected during its flowering phase, were extracted using 85% methanol over a period of three days. The resulting suspension was then filtered through a Büchner funnel, and the methanol was evaporated under reduced pressure using a rotary evaporator, yielding a crude methanol extract (ME). This extract was subsequently partitioned with hexane, chloroform, and ethyl acetate, followed by evaporation to dryness under reduced pressure, to obtain hexane (HE), chloroform (CHE), ethyl acetate (EAE), and remaining aqueous (AqE) extracts. The total phenolic and flavonoid content of each extract was then assessed. The antioxidant properties of these extracts were evaluated through three in vitro assays: ABTS radical scavenging activity, reducing power, and hydrogen peroxide scavenging. Results showed that all extracts were rich in phenolic compounds, with values ranging from 55.85 to 104 mg gallic acid equivalents per gram of dry extract. Flavonoid levels varied from 12.36 to 32.65 mg quercetin equivalents per gram of dry extract. Notably, the ethyl acetate extract demonstrated the highest radical scavenging activity in the ABTS assay (IC_{50} = 1.45 µg/mL), along with strong reducing power (EC_{50} = 23 µg/mL) and hydrogen peroxide scavenging ability (IC_{50} = 95 µg/mL). These findings suggest that lavender is a promising source of natural antioxidants.

Key words: *Lavandula stoechas* L., ABTS, reducing power, lipid peroxidation, polyphenolic compounds

1. Introduction

Plants have held a significant role in human life throughout history. For thousands of years, people have relied on various plants available in their environment to treat and manage a wide range of diseases. The World Health Organization (WHO) estimates that around 80% of the global population still uses traditional herbal preparations as a primary form of healthcare. The plant kingdom is an abundant source of diverse bioactive compounds, among which secondary metabolites are particularly notable for their therapeutic properties (Benabdallah et al., 2020).

Reactive oxygen species (ROS) is a term encompassing oxygen-derived free radicals, such as superoxide anions (O2•−), hydroxyl radicals (HO•), peroxyl (RO2•), alkoxyl (RO•), along with oxygen-derived non-radical species like hydrogen peroxide (H_2O_2) (Mohammed et al., 2020).

Antioxidants are compounds that help protect cells from the harmful effects of xenobiotics, drugs, carcinogens, and toxic radical reactions, either through direct or indirect mechanisms (Akgül et al., 2022). Numerous medicinal plants are rich in antioxidants, including phenolic compounds, nitrogen compounds, vitamins, terpenoids, and other endogenous metabolites (Kaoudoune et al., 2020; Unal et al., 2022).

The genus Lavandula includes around 20 species and over 100 varieties, with *Lavandula* stoechas, or wild lavender, known for its aromatic foliage and distinctive bracts crowning the flowers. This plant is native to the western Mediterranean region, including countries such as Algeria, Tunisia, Italy, France, Spain, Turkey, and India (Boukhatem et al., 2020). Medicinal plants like lavender offer valuable herbal resources that are often used to treat various health conditions. They are favored for their safety, affordability, effectiveness, minimal side effects, and ease of access, making them popular in traditional medicine (Bayrak et al., 2017). Lavender oil is particularly noted for its antibacterial, antifungal, carminative, antiflatulence, anticholic, sedative, and antidepressant properties (Amira et al., 2012, Ez zoubi *et al*., 2020; Benchikh et al., 2024). Additionally, lavender is believed to aid in pain management and reduce tremors (Koulivand et al., 2013). This study thus aims to investigate the polyphenolic content and evaluate the in vitro antioxidant activity of the methanolic extract and its fractions from *L. stoechas*, given its significance in traditional medicine.

2. Materials and Methods

2.1. Plant material

The fresh areal parts of *Lavandula stoechas* L. (*L. stoechas*) were collected from Setif (Northeast of Algeria) in April 2018. The taxonomic identification of the plant was carried by Professor Smain Amira, Department of Animal Biology and Physiology, University Setif 1, Algeria. A voucher number 107 LS 28/03/15 Set/SA/ was deposed at the laboratory of Phytotherapy Applied to Chronic Diseases, Faculty of Nature and Life Sciences, University Setif 1, Algeria. The collected plant was dried under shade and grounded into a fine powder using an electric mill.

2.2. Extraction and fractionation

The extraction procedure was conducted as described by Benchikh (2017). The plant dried powder was extracted with methanol (85%) at room temperature for 3 days. The resulting suspension was then filtered and concentrated by evaporation at 50°C and then was fractioned by successive washing with different solvents of increasing polarity to obtain the following fractions: methanol extract (ME), chloroform extract (CHE), ethyl acetate extract (EAE) and the remaining aqueous extract (AqE).The extracts were stored at 4°C until use.

2.3. Determination of total phenolic content

Total phenolic content was assessed by Folin Ciocalteu reagent as described by Li *et al* (2007). An aliquot of 100 µL of a suitable diluted methanolic extract ant its fractions from *L. stoechas* was mixed with 500 µL of Folin Ciocalteu reagent (diluted 10 times). After 4 min, 400 µL of 7.5% of Na_2CO_3 solution was added. The final mixture was shaken and incubated in dark at room temperature for 90 min and the absorbance of the reaction mixture was measured at 760 nm. The results were expressed as mg of gallic acid equivalent (GAE) per gram of dry plant extract.

2.4. Determination of total flavonoid content

Total flavonoid content of each extract was determined using aluminum chloride assay (Bahorun *et al*., 1996). Briefly, 1 mL of each tested extract or standard (quercetin) were mixed with 1 mL of $AlCl_3$ (2%). After 10 min of incubation, the absorbance against prepared blank was measured at 430 nm. The results were expressed as quercetin equivalent per gram of dry weight (mg QE/g DW) using a calibration curve of quercetin.

2.5. Antioxidant activities evaluation

2.5.1. ABTS radical cation decolorization assay

The radical scavenging assay against ABTS was measured using the method of Ree *et al*. (1999) with slight modification. The ABTS radical stock solution (7 mM in water) was mixed with 2.45 mM potassium persulfate and kept for 12-16 h in the dark at room temperature. The solution was then diluted with methanol to give an absorbance of ~0.7 at 734 nm. Then 50 µL of sample was mixed with 1 mL of ABTS mixture and kept for 30 min at room temperature in the dark. The absorbance of reaction mixture was measured at 734 nm. Trolox was used as positive control. All determinations were performed in replicates. Scavenging capability of test compounds was calculated from the following equation:

% inhibition = [(Absorbance of control – Absorbance of test sample)/Absorbance control] x100

To determine the IC_{50} values, a dose response curve was plotted. IC_{50} is defined as the concentration sufficient to obtain 50% of a maximum scavenging capacity.

2.5.2. Reducing power

The reducing powers of the extracts from *L. stoechas* areal parts and BHT were determined according to the method described by Chung *et al*. (2005). A volume of 0.1 mL aliquot of each extract or BHT were mixed with an equal volume of 0.2 M phosphate buffer (pH 6.6) and 1% potassium ferricyanide, and then incubated at 50°C for 20 min. 0.25 mL of 1% trichloroacetic acid was added to the mixture to stop the reaction, and then the mixture was centrifuged at 2790g for 10 min. The supernatant (250 µL) was mixed with 250 µL distilled water and 0.1% $FeCl_3$ (500 µL) and then the absorbance was measured at 700 nm. The reducing powers of the tested samples increased with the absorbance values.

2.5.3. Hydrogen peroxide-scavenging activity

The ability of *L. stoechas* extracts to scavenge H_2O_2 was determined according to the method of Ruch *et al.* (1989). A solution of H_2O_2 (40 mM) was prepared in Na_2HPO_4–NaH_2PO_4 buffer solution (pH = 7.4, 0.1 mol/L). H_2O_2 concentration was determined spectrophotometrically from absorption at 230 nm. Different concentrations of samples in distilled water were added to a H_2O_2 solution (0.6 mL). Absorbance of H_2O_2 at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without H_2O_2. The activity of all samples to scavenge H_2O_2 was calculated using the following equation: Scavenging effect (%) = (1 − Abs of sample 230 nm/Abs of control 230 nm) × 100.

3. Results

3.1. Polyphenol contents

The total phenolics and flavonoids contents among the different extracts of *L. stoechas* L. areal parts extracts are presented in table 1. All extracts were rich in total phenols and varied between 55.85 ± 1.77 to 104 ± 1.89 mg EGA/g of dry extract. The flavonoid content of the extracts varied between 12.36 and 32.65 mg QE/g of dry extract.

Table 1: Total phenolics and flavonoids contents of *L. stoechas* L. extracts

Extract	**Total phenolic content (µg GAE/mg DW)**	**Total flavonoids content (µg QE/mg DW)**
ME	88.33±1.85	21.19±1.26
CHE	55.85±1.77	12.36±0.48
EAE	104.07±1.89	32.65±0.27
AqE	97.59±0.73	19.47±0.63

3.2. Antioxidant activities

3.2.1. ABTS radical cation decolorization assay

The present results show also that EAE extract exhibited the highest antioxidant activity (IC_{50}=1.45 µg/mL), followed by AqE (IC_{50}=7.57 µg/mL), ME (IC_{50}=10.8 µg/mL) then CHE (IC_{50}=16.75 µg/mL). EAE showed higher activity than Trolox as standard (IC_{50}=3.17 µg/mL) as standard (Figure 1).

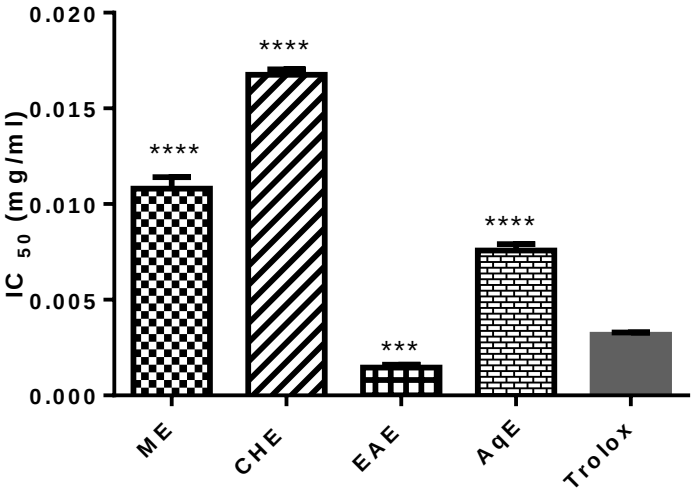

Figure 1. ABTS free radical scavenging activity of different *L. stoechas* extracts. ME: methanol extract, CHE: chloroform extract, EAE: ethyl acetate extract, AqE: aqueous extract, BHT: Butylated hydroxytoluene. Data were presented as IC_{50} means ± SD (n=3). ****($p < 0.0001$); ***($p < 0.001$) compared to Trolox as standard.

3.2.2. Reducing power

The present results show also that EAE extract exhibited the highest antioxidant activity (EC_{50}= 0.023 mg/mL), followed by AqE (EC_{50}=0.087 mg/mL), ME (EC_{50}=0.109 mg/mL) then CHE (EC_{50}=0.697 mg/mL). EAE showed higher activity than BHT as standard (EC_{50}=0.074 mg/mL) as standard (Figure 2).

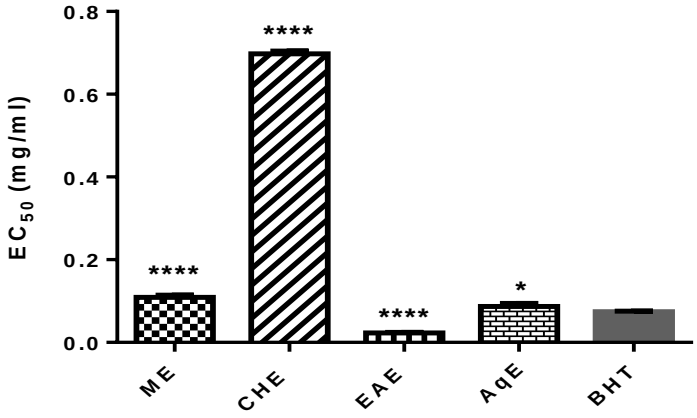

Figure 2: Reducing power of different *L. stoechas* extracts. ME: methanol extract, CHE: chloroform extract, EAE: ethyl acetate extract, AqE: aqueous extract, BHT: butylated hydroxytoluene. Data were presented as IC_{50} means ± SD (n=3). ****($p < 0.0001$); *($p < 0.05$) compared to BHT as standard.

3.2.3. Hydrogen peroxide scavenging activity

The present results show also that EAE extract exhibited the highest antioxidant activity (IC_{50}=50.95 µg/mL)), followed by AqE (IC_{50}=70.21 µg/mL), CHE (IC_{50}=81.98 µg/mL) then ME (IC_{50}=88.09 mg/mL). All the results were significantly different from BHT (IC_{50}=3 µg/ml) as standard (Figure 3).

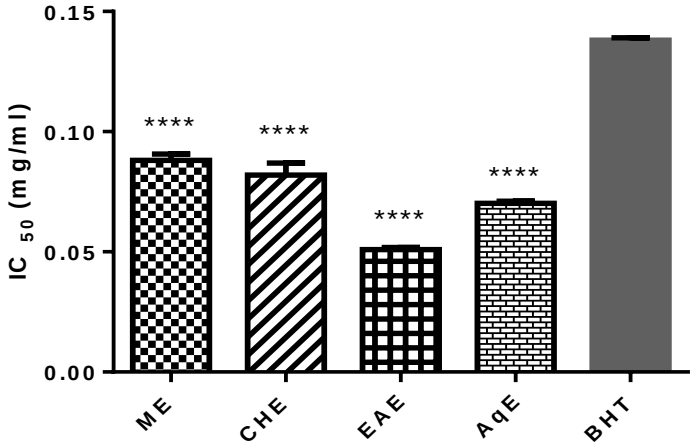

Figure 3: Hydrogen peroxide scavenging activity of different *L. stoechas* extracts. ME: methanol extract, CHE: chloroform extract, EAE: ethyl acetate extract, AqE: aqueous extract, BHT: Butylated hydroxytoluene. Data were presented as IC_{50} means± SD (n=3). (****$P \leq 0.0001$) *vs* BHT as standard.

Discussion

The extracts of *L. stoechas* were analysed for their total polyphenol and flavonoid contents, revealing significant polyphenol concentrations, with the highest levels found in the ethyl acetate extract (EAE). These findings surpass those from previous studies by Algieri et al. (2016) and Guesmi et al. (2017), which focused on the methanolic extract. The current results align with Ceylana et al. (2015) for methanolic extract analysis. According to Amira et al. (2023), polar solvents are particularly effective for extracting polyphenols and flavonoids, likely due to increased polarity in flavonoids as they form glycoside conjugates with hydroxyl groups, enhancing solubility in such solvents. The choice of extraction solvent plays a crucial role in accurately isolating botanical compounds from plant material (Ounis et al., 2023). Polyphenolic compounds are abundant in the plant and primarily contribute to its potent antioxidant activity. Consequently, the antioxidant properties of the plant extracts were assessed through three complementary methods: ABTS radical cation decolorization, reducing power, and H_2O_2 and β-carotene bleaching assays, providing a comprehensive evaluation of the extracts' antioxidant potential.

The ABTS assay evaluates the reduction in absorbance of the ABTS radical cation (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonate), or ABTS+) when it encounters an antioxidant. The ability to scavenge the ABTS radical reflects the extract's capacity to act as an electron or hydrogen donor in free radical reactions (Benchikh et al., 2022b). In this study, the *L. stoechas* extracts demonstrated a notably strong ABTS+ radical scavenging activity, surpassing the performance observed in the reducing power and hydrogen peroxide assays. This suggests that *L. stoechas* extracts exhibit significant potential for scavenging ABTS radicals effectively.

The reducing power assay is commonly used to assess the capability of natural antioxidants to donate electrons or hydrogen atoms. In this assay, antioxidant reductants in the extracts reduce the Fe^{3+}/ferricyanide complex to its ferrous form, monitored by measuring the formation of Perl's Prussian blue at a wavelength of 700 nm (Ounis et al., 2023). The composition of phenolic compounds, particularly those with multiple hydroxyl groups, contributes to variations in antioxidant activity. These hydroxyl groups are largely responsible for the potent antioxidant effects. Additionally, factors such as extraction method, temperature, solvent polarity, concentration, and time can influence antioxidant activity (Amira et al., 2020). This study's findings revealed a dose-dependent increase in reducing capacity across different extracts, with the EAE fraction showing the highest scavenging activity. This enhanced activity may be attributed to the EAE fraction's high phenolic acid (104.07±1.89 mg GAE/kg DW) and flavonoid content (32.65±0.27 mg QE/kg DW) compared to other fractions.

Hydrogen peroxide is a mild oxidizing agent known for its ability to inactivate enzymes by oxidizing essential thiol (SH-) groups. It can easily cross cell membranes, where it reacts with Fe^{2+} and Cu^{2+} ions to produce hydroxyl radicals, which can be detrimental to cellular structures. Therefore, cells benefit from controlling the accumulation of hydrogen peroxide to avoid potential damage (Benchikh et al., 2018). In this study, the ability of *L.* stoechas

extracts to inhibit H_2O_2 increased proportionally with their concentration. This scavenging activity is likely due to the presence of phenolic compounds in the extracts, which readily donate electrons to neutralize hydroxyl radicals. Comparisons with existing literature on these specific antioxidant assays are challenging, as studies investigating the antioxidant potential of *L. stoechas* fractions are limited and difficult to locate.

Conclusion

Lavandula stoechas L. methanol extract and its fractions are rich in polyphenols and flavonoids. They have significant antioxidant and free radical scavenging activities in different in vitro assay systems. However, whether these compounds work singularly or in synergy with other constituents deserve further investigations. These findings give strong support for expanding the investigations of *L. stoechas* for utilization in food and cosmetic industries as well as in traditional folk medicine.

Acknowledgements

This work was supported by the Algerian Ministry of Higher Education and Scientific Research (MESRS). We express our gratitude to these organizations**.**

Competing Interests

Authors have declared that no competing interests exist

References

1. Akgül, H., Mohammed, F.S., Kına E, Uysal, İ., Sevindik, M. & Doğan, M. (2022). Total Antioxidant and Oxidant Status and DPPH Free radical activity of Euphorbia eriophora. Turkish Journal of Agriculture-Food Science and Technology, 10(2): 272-275.
2. Amira, H., Amira, I., Benchikh, F., Benabdallah, H., Mamache, W. & Amira, S. (2020). Journal of Drug Delivery & Therapeutics. 2020; 10(5):45-48. Evaluation of Antioxidant activities and total phenolic content of hydro-ethanol extract from *Phlomis bovei* De Noé areal parts. Journal of Drug Delivery & Therapeutics; 10(5):45-48.
3. Amira, H., Benabdallah, H., Mamache, W., Benchikh, F., Ounis, R., Chawki, B., Amira, S. (2023). Evaluation of the phytochemical content and antioxidant properties of different extracts of *Achillea odorata* L. Revista Bionatura; 8 (3): 31. http://dx.doi.org/10.21931/RB/2023.08.03.31
4. Amira, S., Dade, M., Schinella, G. & Rios, J.L. (2012). Anti-inflammatory, antioxidant and apoptotic activities of four plant species used in folk medicine in the Mediterranean basin. Pakistan Journal of Pharmaceutical Sciences; 25: 65-72.
5. Bahorun, T., Gressier, B., Trotin, F., Brunete, C., Dine, T., Vasseur, J., Luyckx, M., Cazin, M., Pinkas, M. (1996)**.** Oxygen species scavenging activity of phenolic extract from Hawthorn fresh plant organs and pharmaceutical preparations. *Arzneim Forsch / Drug Res*; 46: 1086-1089.
6. Bayrak, D., Okmen, G. & Arslan, A. (2017). The Biological Activities of *Lavandula stoechas* L. against Food Pathogens. International Journal of Secondary Metabolite, Vol. 4: 3 (2017) pp. 270-279.
7. Benchikh, F. (2017). Pharmacological effects of *Myrtus communis* L. on the gastrointestinal tract of rats and mice. Thesis presented for the Fulfilment of the requirements for the degree of Doctor of Sciences. University Ferhat Abbas Sétif-1-, 53p.
8. Benchikh, F., Amira, S. & Benabdallah, H. (2018). The evaluation of antioxidant capacity of different fractions of Myrtus communis L. leaves. Annual Research & Review in Biology, 22(5): 1-14. DOI:10.9734/ARRB/2018/39217

9. Benchikh, F., Benabdallah, H., Amira, H., Walid Mamache, W., & Smain, Amira. (2022). Free Radical Scavenging, Reducing Power and Lipid Peroxidation Inhibition Activities of M. communis Berries Methanol Extract and its Fractions. Turkish Journal of Agriculture - Food Science and Technology; 10(4): 760-765. DOI: https://doi.org/10.24925/turjaf.v10i4.760-765.5060
10. Benchikh, F., Mamache, M., Benabdallah, H., Amira, H., I Amira, I., & Amira, S. (2024). Acute toxicity, antidiarrheal and antisecretory properties of methanol extract of *Lavandula stoechas* L aerial part in mice. Tropical Journal of Pharmaceutical Research; 23 (3): 501-507.
11. Boukhatem, M.N., Sudha, T., Darwish, N.H., Chader, H., Belkadi, A., Rajabi, M., Houche, A., Benkebailli, F., Oudjida, F. & Mousa, S.A. (2020). A new eucalyptol-rich lavender (*Lavandula stoechas* L.) essential oil: Emerging potential for therapy against inflammation and cancer. Molecules; 25(16): 3671. https://doi:10.3390/molecules25163671.
12. Chung, Y.C., Chen, S.J., Hsu, C.K., Chang, C.T., Chou, S.T. (2005). Studies on the oxidative activity of *Graptopetalum paraguayense.* Food Chemistry; 91:419-424.
13. Ez Zoubi, Y., Bousta, D. & Farah, A. (2020). A Phytopharmacological review of a Mediterranean plant: *Lavandula stoechas* L. Clinical Phytoscience; 6: 1-9. https://doi.org/10.1186/s40816-019-0142-
14. Francesca, A., Rodriguez-Nogales, A., Vezza, T. et al. (2016). Anti-inflammatory activity of hydroalcoholic extracts of *Lavandula dentata* L. and *Lavandula stoechas* L, Journal of Ethnopharmacology, http://dx.doi.org/10.1016/j.jep.2016.05.063.
15. Guesmi, F., Ben Hadj, A. S. & Landoulsi, A. (2017). Investigation of extracts from tunisian ethnomedicinal. Biomedical and Environmental Sciences; 30(11): 811-824.
16. Kaoudoune, C., Benchikh, F., Benabdallah, H., Loucif, K., Mehlous, S. & Amira, S. (2020). Gastroprotective effect and in vitro Antioxidant Activities of the Aqueous Extract from Artemisia absinthium L Aerial Parts. Journal of Drug Delivery & Therapeutics. 2020; 10(4):153-156
17. Li, H.B., Cheng, K.W., Wong, C.C., Fan, K.W., Chen, F., Jiang, Y. (2007). Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. Food Chemistry; 102: 771-776.
18. Mohammed, F.S., Şabik, A.E., Sevindik, E., Pehlivan, M. & Sevindik, M. (2020). Determination of Antioxidant and Oxidant Potentials of Thymbra spicata Collected from Duhok-Iraq. Turkish Journal of Agriculture-Food Science and Technology; 8(5): 1171-1173. https://doi.org/10.24925/turjaf.v8i5.1171-1173.3341
19. Ounis, R., Benchikh, F., Amira, S., Benabdallah, H., Amira, H., Mamache, W., Bensouissi C. & Hellal, K. (2023). Impact of Extraction Solvent Polarity: Antioxydant Activity of Methanolic, Hydromethanolic and Aqueous Decocted Extracts of Algerian *Thymelaea hirsuta* (L.) Endl. Areal Parts. Turkish Journal of Agriculture - Food Science and Technology, 11(6): 1161-1167, 2023. DOI: https://doi.org/10.24925/turjaf.v11i6.1161-1167.5931
20. Re, R. (1999). Antioxidant activity applying an improved ABTS radical cation decolourization assay. Free Radical Biology and Medicine; 26:1231-1237.
21. Ruch, R.J., Cheng, S.J., Klaunig, J.E. (1989). Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis. 1989; 10:1003-1008.
22. Unal, O., Eraslan, E.C., Uysal, I., Mohammed, F.S., Sevindik, M. & Akgul, H. (2022). Biological activities and phenolic contents of Rumex scutatus collected from Turkey. Fresenius Environmental Bulletin, 31(7): 7341-7346.