Journal of Pharmacy & Allied Medicine

JPAM, 2(1), 40-45, 2024
https://doi.org/10.58985/jpam.2024.v02i01.20
ISSN (Print): 3005-2726

Research Article

Introduction of antioxidant potential and phytochemical compounds of *Satureja sahendica* Bornm. endemic plant from Iran

Ghader Ghasemi* and **Shirin Rahmanzadeh-Ishkeh**

Department of Horticultural Sciences, West Azerbaijan Agricultural Research and Education Centre, Urmia, Iran.

Article Information
Received: 31 October 2024
Revised: 19 November 2024
Accepted: 22 November 2024
Published: 01 December 2024

Academic Editor
Prof. Dr. Christian Celia

Corresponding Author
Prof. Dr. Ghader Ghasemi
E-mail:
gader.g1390@yahoo.com
Tell: +989149037704

Keywords
Antioxidant, endemic, flavonoid, phenol, plant pigments, *Satureja sahendica*.

Abstract

Nowadays, the search for antioxidant compounds with natural sources has been considered due to the side effects of synthetic antioxidants. *Satureja sahendica* is an Iranian endemic species from Lamiaceae family. This plant has been used in the food industry, cosmetics and medical preparations. In this study, antioxidant activity, phytochemical compounds including phenols and flavonoids and various plant pigments in *S. sahendica* extract were evaluated. The antioxidant activity of the extract was measured by two methods DPPH and FRAP and showed the values of 84.29% and 148.36±1.17 (mm Fe^{++}/100g DW), respectively. Also, the extract of this plant contained 95.85±0.50 (mg GAE/100g DW) phenol and 19.33±0.30 (mg QUE/100g DW) flavonoid. According to the results of this study, *S. sahendica* can be a good source of phytochemicals and antioxidants of natural origin.

1. Introduction

In today's crowded world, one of the smartest ways to stay healthy and fight disease is to use the potential of medicinal plants [1]. This group of low-cost plants can replace the use of a large number of drugs, fungicides and other chemical compounds [2]. One of the valuable medicinal plants is *Satureja sahendica*. This plant is from the Lamiaceae family and is the exclusive owner of Iran [3]. The antioxidant properties of foods are crucial in nutrition. Over the last decade, extractable and non-extractable compounds have become key in the evaluation/determination of the antioxidant properties of food matrices because of their relevance in human health [4]. Compounds such as phenol and flavonoids are valuable phytochemicals with antioxidant properties that can protect the human body against many diseases [5-10], for more information please see [11]. The purpose of this research is to investigate the antioxidant and phytochemical activity of *S. sahendica* extract as an endemic plant in Iran.

2. Materials and methods

2.1 Plant materials

Miandoab is one of the cities of the West Azerbaijan province in Iran, which has a classically semi-arid climate at the altitude of 1352 m above the sea level, Latitude: 38°190′ N; and Longitude: 45°180′ E. This region (Miandoab) is one of the habitats of *S. sahendica* in Iran. The aerial parts of *S. sahendica* plants were collected from Miandoab city during the flowering stage in 2019 and the plant sample was dried in the form of dry shade at room temperature (25 °C). The

voucher herbarium sample of the collected *S. sahendica* species has been deposited at herbarium of the Urmia University of Medical Sciences.

2.2. Preparation of methanol extract

The powdered aerial parts (2 g) were extracted with MeOH (80%, v/v) by ultrasound (Elmasonic, Singen, Germany) process at 120 Hz power at 25 ºC for 30 min. The recovered extract after centrifugation at 3000 *x g* for 5 min (centrifuge ROTANTA 380/380R, Hettich, Germany) was kept at 4 °C in dark glass, until analysis.

2.3. Antioxidant activity

2.3.1. Antioxidant activity by DPPH assay

The electron donation ability of the obtained methanolic extract was measured by bleaching of the purple-coloured solution of DPPH according to the literature [12]. Extracts (25, 50, and 75 µL) were added to 2 mL of 0.1 M DPPH methanolic solution. After an incubation period of 30 min at room temperature, the absorbance was determined against a blank at 517 nm. Percentage inhibition of free radical DPPH (PI%) was calculated as follows:

$$\text{DPPHsc } \% = \frac{(\text{Abs control})_{t=X\ min} - (\text{Abs sample})_{t=X\ min}}{(\text{Abs control})_{t=X\ min}} \times 100$$

Where

Abs control is the absorbance of DPPH solution mixtures without methanol extract, and Abs sample is the absorbance in the presence of the test compounds.

2.3.2. Antioxidant activity by FRAP assay

To measure the antioxidant activity by the FRAP method, the samples along with 3 mL of FRAP fresh reagent [2.5 mL of a 10 mM TPTZ solution in 40 mM HCl, 2.5 mL of 20 mM $FeCl_3.6H_2O$ and 25 mL of 300 mM acetate buffer (pH 3.6)] were mixed together. The resulting mixture was put in a warm water bath (37 ˚C) and its absorption was read on the 593 nm wavelength by using spectrophotometer (Unico 2100UV Single Beam UV/Vis, Shanghai, China). The $FeSO_4{\cdot}7H_2O$ was used to draw the standard curve. Moreover, the obtained results were quantified based on mM Fe^{++}/100g DW plant [13].

2.4. Phytochemical compounds

2.4.1. Total phenol content (TPC)

Phenolic content was measured using Folin–Ciocalteu reagent. An aliquot of 100 µL of extract was added to 180 µL of deionised water and 1200 µL of the F–C reagent. After shaking, the mixture was incubated for 5 min at room temperature and then (7.5%) sodium carbonate was added. The samples were kept in the dark within the room temperature for 30-45 minutes. Finally, absorption was measured at 760 nm wavelength by spectrophotometer [14]. The deionized water was used as the control while gallic acid was used as standard. The standard curve was drawn according to gallic acid, and the results were reported in mg of gallic acid equivalents per 100 g of dry weight.

2.4.2. Total flavonoid content (TFC)

To assay the total flavonoid content, 150 µL of 5% sodium nitrite, 300 µL of aluminum chloride (10 %) and 1000 µL of acetate solutions (of 1 mM NaOH) were added to a specific amount of each extract. The volume obtained was adjusted to 5mL using distilled water. The absorbance of the solution was measured at 380 nm against a blank. Additionally, to draw the standard curve, quercetin was used [15]. The amount of total flavonoids in all the extracts was reported as mg of quercetin equivalents per 100 g of dry weight.

2.4.3. Total carotenoids and chlorophylls A and B

In order to measure the content of carotenoids and chlorophylls, 0.05 g of the fresh plant tissues were homogenized with 5 mL acetones in ice bath. Then, 1 g of anhydrous sodium sulfate was added to the homogenate and filtered using the filter paper. The volume of the filtered was adjusted to 10 mL with acetone and then it was centrifuged at 4000 rounds for 10 minutes. The absorption of the upper phase solution was measured at the 662, 645, 470 nm wavelengths using acetone as control. The amount of carotenoid and chlorophylls A and B for each extract was measured as reported in the literature [16].

$$C_a = 11.75\ A_{662} - 2.350\ A_{645} \quad (1)$$

$$C_b = 18.61\ A_{645} - 3.960\ A_{662} \quad (2)$$

$$C_{x+c} = 1000\ A_{470} - 2.270\ C_a - 81.4\ C_b/227 \quad (3)$$

3. Results and discussion

3.1 Antioxidant activity

Health-related uses of medicinal and aromatic plants have long been known. The compounds derived from these plants have antifungal, antimicrobial and antioxidant effects [17]. Reactive oxygen species (ROS) are highly reactive chemical molecules formed due to the electron acceptability of O_2. Examples of ROS include peroxides, superoxide, hydroxyl radical,

https://doi.org/10.58985/jpam.2024.v02i01.20

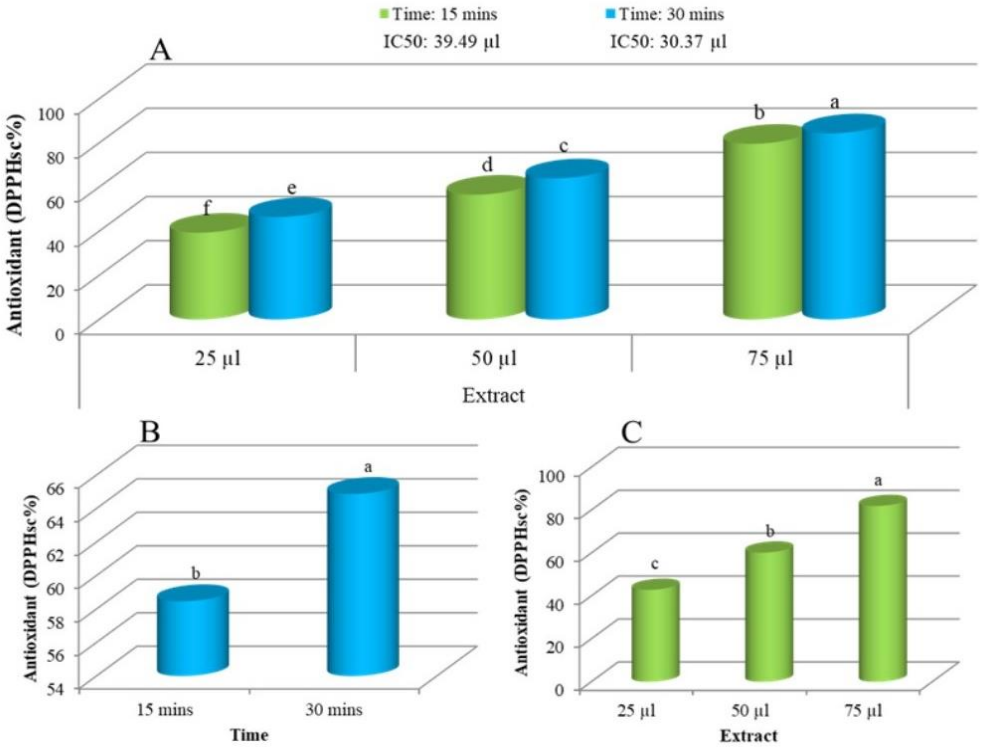

Figure 1. Antioxidant activity of different concentration of methanol extraction during studied time. (A) Interaction, (B) Main effect (Time) and (C) Main effect (concentration).

singlet oxygen and alpha-oxygen [18]. Antioxidants are compounds that inhibit oxidation. Oxidation is a chemical reaction that can produce ROS, thereby leading to chain reactions that may damage the cells of organisms [19]. Antioxidants have two sources: synthetic and natural. Medicinal plants, including *S. sahendica*, are valuable sources of natural antioxidants [20]. The results of the antioxidant activity of *S. sahendica* extract by DPPH method showed that it was raising the concentration of the extract and time increases the amount of antioxidant activity. So that, the highest antioxidant activity was observed in concentration of 75 µL at 30 minutes (84.29 %) (Fig. 1A). The study of the simple effect of time on the amount of antioxidant activity showed that the increase in the amount of antioxidant activity between different treatments (time 15 and 30 mines) was significant. The highest antioxidant activity was observed at 30 minutes (64.87 %) (Fig. 1B). Furthermore, the study of the simple effect of concentration on the level of antioxidant activity showed that there was a statistically significant difference between different treatments (concentrations 25, 50 and 75 µL). The highest antioxidant activity was observed in concentration of 75 µL (81.93 %) (Fig. 1C). For DPPH test, the lowest IC_{50} value was observed for time 15 min (30.37 µL) and the highest IC_{50} value (39.49 µL) was recorded for the time 30 min (Fig. 1A). In addition, the results of measuring the antioxidant activity by FRAP method showed a value of 148.36±1.17 (mm Fe^{++}/100g DW) in *S. sahendica* extract (Table 1).

The Ferric Reducing Antioxidant Power, well-known as FRAP assay and (DPPH) are valuable method for evaluation of total antioxidant power of the herbal extracts [21]. The antioxidant properties of medicinal plants may be due to the presence of phenolic compounds or other polar compounds such as terpenoid-glycosides in their aqueous extract, which are traditionally used as a decoction or infusion [22, 23].

3.2. Phytochemical content

3.2.1. Phenol and flavonoid content

A review of articles shows that medicinal plants, especially the large family Lamiaceae, have been used to treat patients since ancient times because they contain active medicinal and biologically active compounds [24]. Polyphenols and flavonoids are the

https://doi.org/10.58985/jpam.2024.v02i01.20

Table 1. Biochemical composition and antioxidant activities of *S. sahendica* extract

Characteristics	Content
Antioxidant activity (FRAP)	148.36±1.17 (mm Fe^{++}/100g DW)
Total Phenolic Content	95.85±0.50 (mg GAE/100g DW)
Total Flavonoid Content	19.33±0.30 (mg QUE/100g DW)
Total Carotenoid Content	1161.90±13.60 (mg/100g DW)
Chlorophyll A	7.80±0.02 (mg/100g DW)
Chlorophyll B	2.20±0.06 (mg/100g DW)

common antioxidant natural products found in medicinal plants [21]. Previous research shows that both internal and external factors of genotype and environment along with plant growth stage and extraction method play an important role in the amount of phenolic compounds in plants [25]. Based on the measurements, the amount of phenol and flavonoids in the whole samples was equal to 95.85±0.50 (mg GAE/100g DW) and 19.33±0.30 (mg QUE/100g DW) respectively (Table 1). Extracts from some species of the Lamiaceae family may be recommended for the treatment of complications of diabetes due to their polyphenolic compounds [26]. It has been reported that phenolic compounds have antimicrobial and antioxidant properties, helping plants prevent infections from pathogens and pathogenic microorganisms [27]. Additionally, their presence in plant tissues protects them against the toxic effects of reactive oxygen species [28].

3.2.2. Plant pigments

Plant pigments protect plants from damage caused by UV and visible light [29]. Photosynthetic pigments (chlorophyll and carotenoids) play an important role in photosynthesis, chlorophyll is the most important pigment needed for photosynthesis and resistance to environmental stresses, chlorophylls are complexed with proteins that serve to absorb light and transfer energy to the photochemical center [30]. Carotenoids can absorb sunlight and are also essential as a pigment for photosynthetic function [31, 32]. Carotenoids have good effects on human health. They are precursors of vitamin A in the body and their antioxidant, anti-obesity and anti-cancer properties have been proven, they also have anabolic effects on bone components. Nowadays, carotenoids are used as food additives, animal feed supplements, and natural food coloring and dietary supplements. More recently, they have been used commercially as nutrients for cosmetic and medicinal purposes [33]. *S. sahendica* is a valuable source of plant pigments which *S. sahendica* plant extract contained 1161.90 ± 13.60 (mg/100 g DW) carotenoid pigment, 7.80±0.02 (mg/100g DW) chlorophyll A and 2.20 ± 0.06 (mg/100g DW) chlorophyll B pigment (Table 1).

4. Conclusions

The present study showed that the *S. sahendica* plant has a high TPC (95.85±0.50 mg GAE/100g DW) and TFC (19.33±0.30 mg QUE/100g DW). This has caused a very strong antioxidant activity (DPPH: 84.29% and FRAP: 148.36±1.17 mm Fe^{++}/100g DW). Overall, the results of the present study showed that *S. sahendica* due to its phytochemical compounds can be a good candidate for natural antioxidants and a good alternative to synthetic antioxidants. Also, the various pigments in this plant are a good source for use in the food and pharmaceutical industries. However, additional studies are needed to find out the other potentials of this plant.

Authors' contributions

Data curation, formal analysis, validation, writing – original draft preparation and funding acquisition, G.G.; Methodology, project administration, software, writing – review and editing, S.R.

Acknowledgements

This research was supported by the Urmia University, Urmia, Iran.

Funding

Funding has been provided by Urmia University.

Availability of data and materials

All relevant data are within the paper and its supporting information files. Additional data will be made

https://doi.org/10.58985/jpam.2024.v02i01.20

available on request according to the journal policy.

Conflicts of interest

Authors declare that there is no conflict of interest.

References

1. Shukla, S.; Mehta, A. Anticancer potential of medicinal plants and their phytochemicals: A review. Rev. Bras. Bot. 2015, 38, 199-210. https://doi.org/10.1007/s40415-015-0135-0
2. Frankel, E.N. Recent advances in lipid oxidation. J. Sci. Food Agric. 1991, 54, 495-511. https://doi.org/10.1002/jsfa.2740540402
3. Sefidkon, F.; Akbarinia, A. essential oil content and composition of *Satureja sahendica* Bornm. at different stages of plant growth. J. Essent. Oil Res. 2009, 21, 112–114. https://doi.org/10.1080/10412905.2009.9700126
4. Plumb, J.; Durazzo, A.; Lucarini, M.; Camilli, E.; Turrini, A.; Marletta, L.; Finglas, P. Extractable and non-extractable antioxidants composition in the eBASIS Database: A key tool for dietary assessment in human health and disease research. Nutrients. 2020, 12, 3405. https://doi.org/10.3390/nu12113405
5. Ciani, M.; Menghini, L.; Mariani, F.; Pagiotti, R.; Menghini, A.; Fatichenti, F. Antimicrobial properties of essential oil of *Satureja montana* L. on pathogenic and spoilage yeasts. Biotechnol. Lett. 2000, 22, 1007–1010. https://doi.org/10.1023/a:1005649506369
6. Abdollahi, M.; Salehnia, A.; Mortazavi, S. H.; Ebrahimi, M.; Shafiee, A.; Fouladian, F.; Keshavarz, K.; Sorouri, S.; Khorasani, R.; Kazemi, A. Antioxidant, antidiabetic, antihyperlipidemic, reproduction stimulatory properties and safety of essential oil of *Satureja khuzistanica* in rat *in vivo*: A toxicopharmacological study. Med. Sci. Monit. 2003, 9, 331–5. https://pubmed.ncbi.nlm.nih.gov/12960922/
7. Tzakou, O.; Skaltsa, H. Composition and antibacterial activity of the essential oil of *Satureja parnassica* subsp. *Parnassica*, Planta Med. 2003, 69, 282–284. https://doi.org/10.1055/s-2003-38487
8. Gören, A.C.; Topçu, G.; Bilsel, G.; Bilsel, M.; Wilkinson, J.M.; Cavanagh, H.M. Analysis of essential oil of *Satureja thymbra* by hydrodistillation, thermal desorber and headspace GC/MS techniques and its antimicrobial activity. Nat. Prod. Res. 2004, 18, 189-95. https://doi.org/10.1080/14786410310001608145
9. Ghazanfari, G.; Minaie, B.; Yasa, N.; Nakhai, L.A.; Mohammadirad, A.; Nikfar, S.; Dehghan, G.; Boushehri, V.S.; Jamshidi, H.; Khorasani, R.; Salehnia, A. Biochemical and histopathological evidences for beneficial effects of *Satureja khuzistanica* Jamzad essential oil on the mouse model of inflammatory bowel diseases, Toxicol. Mech. Methods. 2006, 16, 365–72. https://doi.org/10.1080/15376520600620125
10. Eftekhar, F.; Raei, F.; Yousefzadi, M.; Ebrahimi, S.N.; Hadian, J. Antibacterial activity and essential oil composition of *Satureja spicigera* from Iran, Zeitschrift fu¨r Naturforschung. 2009, 64, 20-24. https://doi.org/10.1515/znc-2009-1-204
11. Santini, A.; Cicero, N. Development of food chemistry, natural products, and nutrition research: Targeting new frontiers, Foods. 2020, 9, 1-482. https://doi.org/10.3390/foods9040482
12. Nakajima, J.; Tanaka, I.; Seo, S.; Yamazaki, M.; Saito, K. LC/PDA/ESI-MS profiling and radical scavenging activity of anthocyanins in various berries. J. Biotechnol. Biomed. 2004, 5, 241-247. https://doi.org/10.1155/s1110724304404045
13. Žugić, A.; Đorđević, S.; Arsić, I.; Marković, G.; Živković, J.; Jovanović, S.; Tadić, V. Antioxidant activity and phenolic compounds in 10 selected herbs from Vrujci Spa, Serbia. Ind. Crops Prod. 2014, 52, 519-527. https://doi.org/10.1016/j.indcrop.2013.11.027
14. Singleton, V.L.; Orthofer, R.; Lamuela-Raventós, R.M.; Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent, Meth. Enzymol. 1999, 152-178. https://doi.org/10.1016/s0076-6879(99)99017-1
15. Chang, Q.; Zuo, Z.; Harrison, F.; Chow, M.S.S. Hawthorn. Clin. J. Pharmacol. 2002, 42, 605-612. https://doi.org/10.1177/00970002042006003
16. Lichtenthaler, H.K. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Meth. Enzymol. 1987, 148, 350-382. https://doi.org/10.1016/0076-6879(87)48036-1
17. Vallejo, M.J.; Salazar, L.; Grijalva, M. Oxidative stress modulation and ROS-mediated toxicity in cancer: A review on in vitro models for plant-derived compounds. Oxidat. Med. 2017. https://doi.org/10.1155/2017/4586068
18. Hayyan, M.; Hashim, M.A.; Al Nashef, I.M. Superoxide ion: generation and chemical implications. Chem. Rev. 2016, 116, 3029-3085. https://doi.org/10.1021/acs.chemrev.5b00407
19. Dabelstein, W.; Reglitzky, A.; Reders, K. Automotive Fuels, Ullmann's encycl. ind. chem. 7th Edition 2007, 1-36. https://doi.org/10.1002/14356007.a16_719.pub2
20. Tabatabaei, R.A.; Khalighi, A.; Kashi, A.; Asnaashari, S.; Bamdad, M.S.; Delazar, A.; Antioxidant activity and chemical compositions of essential oil of aerial parts of *Satureja sahendica* Bornm, Pharm. Sci. 2007, 3, 1–6.
21. Szőllősi, R.; Varga, I.S.I. Total antioxidant power in some species of Labiatae: Adaptation of FRAP method,

https://doi.org/10.58985/jpam.2024.v02i01.20

Acta Biol. Szeged. 2002, 46, 125-127.

22. Hajimehdipoor, H.; Gohari, A.R.; Ajani, Y.; Saeidnia, S.; Comparative study of the total phenol content and antioxidant activity of some medicinal herbal extracts, Res. J. Pharmacogn. 2014, 21-25. https://doi.org/10.4103/0973-1296.93316
23. Alizadeh, Z.; Fattahi, M. Essential oil, total phenolic, flavonoids, anthocyanins, carotenoids and antioxidant activity of cultivated Damask Rose (*Rosa damascena*) from Iran: With chemotyping approach concerning morphology and composition. Sci. Hort. 2021, 288, 110341. https://doi.org/10.1016/j.scienta.2021.110341
24. Saeidnia, S.; Yassa, N.; Gohari, A.R.; Shafiee, A. Isolation and identification of flavonoid constituents from of *Achillea conferta* DC, J. Med. Plant Res. 2005, 4, 12-20. file:///C:/Users/Ghader/Downloads/jmpir-v4n14p12-en.pdf
25. Nantitanon, W.; Yotsawimonwat, S.; Okonogi, S. Factors influencing antioxidant activities and total phenolic content of guava leaf extract, LWT - Food Sci. Technol. 2010, 43, 1095–1103. https://doi.org/10.1016/j.lwt.2010.02.015
26. Rahimmalek, M.; Afshari, M.; Sarfaraz, D.; Miroliaei, M. Using HPLC and multivariate analyses to investigate variations in the polyphenolic compounds as well as antioxidant and antiglycative activities of some Lamiaceae species native to Iran. Ind. Crops Prod. 2020, 154, 112640. https://doi.org/10.1016/j.indcrop.2020.112640
27. Fattahpour, B.; Fattahi, M.; Hassani, A.; Essential oil composition, morphological characterization, phenolic content and antioxidant activity of Iranian populations of *Hymenocrater longiflorus* Benth. (Lamiaceae). Sci. Rep. 2024, 14(1), 7239. https://doi.org/10.1038/s41598-024-57826-0
28. Kumar, S.; Abedin, M.M.; Singh, A.K.; Das, S. Role of phenolic compounds in plant-defensive mechanisms. Plant Phenol. Sust. Agri. 2020, 1, 517-532. https://doi.org/10.1007/978-981-15-4890-1_22
29. Tanaka, Y.; Sasaki, N.; Ohmiya, A. Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids, Plant J. 2008, 54, 733-749. https://doi.org/10.1111/j.1365-313x.2008.03447.x
30. Masuda, T.; Tanaka, A.; Melis, A. Chlorophyll antenna size adjustments by irradiance in *Dunaliella salinainvolve* coordinate regulation of chlorophyll an oxygenase (CAO) and Lhcb gene expression, U.S. DOE. Hydrogen Program Review. 2002, 1-22. https://doi.org/10.1023/a:1022545118212
31. Alan-Blackburn, G. Hyperspectral remote sensing of plant pigments, J. Exp. Bot. 2007, 58, 855–867. https://doi.org/10.1093/jxb/erl123
32. Mlodzinska, E. Survey of plant pigments: molecular and environmental determinants of plant, Acta Biol. Crac. Ser. Bot. 2009, 51, 7–16. https://abcbot.pl/pdf/51_1/07_16_mlodzinska.pdf
33. Noviendri, D.; Hasrini, R. F.; Octavianti, F. Carotenoids: Sources, medicinal properties and their application in food and nutraceutical industry, J. Med. Plant Res. 2011, 5, 7119-7131. https://doi.org/10.5897/jmprx11.011

https://doi.org/10.58985/jpam.2024.v02i01.20