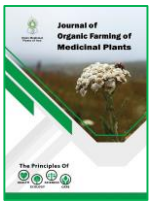
Journal of Organic Farming of Medicinal Plants: September 2024; 3 (1): 1-6
Available online at http://www.jofmp.areeo.ac.ir



Original Article

Free Radical Scavenging Activity and Total Phenol Content of Some Lamiaceae Family Species

Marzieh Babashpour-Asl[a], Elnaz Farajzadeh-Memari-Tabrizi[b], Afsaneh Yousefpour-Dokhanieh[c]

[a] *Department of Horticultural Science, Maragheh Branch, Islamic Azad University, Maragheh, Iran*
[b] *Department of Agronomy, Malakan Branch, Islamic Azad University, Malekan, Iran*
[c] *Department of Horticultural science, Tabriz Branch, Islamic Azad University, Tabriz, Iran*

ARTICLE INFO

Corressponding Author:
Marzieh Babashpour-Asl
babashpour@iau.ac.ir,
babashpour@gmail.com

Received: 22 December 2024
Accepted: 12 January 2025

Keywords:
Antioxidant
Peppermint
Horsemint
Desert rod
Thyme

ABSTRACT

This study investigated the antioxidant activity and total phenol content of six species from the Lamiaceae family. We assessed the antioxidant capacity using two distinct methods: DPPH radical scavenging and ferric reducing antioxidant power (FRAP) assays. Peppermint (*Mentha piperita* L.) and Horsemint (*Mentha longifolia* (L.) Huds.) exhibited the strongest DPPH scavenging activity, with IC_{50} values of 100.7 ± 50.1 µg DW/mL and 80.9 ± 39.1 µg DW/mL, respectively. These species also demonstrated high FRAP values (49.9 ± 0.8 µM/g DW and 40.7 ± 1.8 µM/g DW, respectively), further confirming their robust reducing power. In contrast, Desert rod (*Eremostachys laciniata* (L.) Bunge), Thyme (*Thymus deanensis* Celak), and White horehound (*Marrubium vulgare* L.) displayed considerably lower antioxidant activity in both assays, indicating weaker radical scavenging and reducing capabilities. Salvia/Sage (*Salvia hydrangea* DC.) showed moderate antioxidant activity, falling between the highly active mint species and the less active group. Total phenol content, quantified as mg of catechin equivalents per g of dry weight (mg CE/g DW), was also determined. Notably, Horsemint possessed the highest total phenol content (20.9 ± 1.8 mg CE/g DW), aligning with its strong antioxidant activity. While a general trend was observed between higher total phenol content and increased antioxidant activity, this correlation was not absolute, suggesting the involvement of other antioxidant compounds or mechanisms. This comparative analysis highlights the diverse antioxidant potential within the Lamiaceae family and identifies Peppermint and Horsemint as promising sources of natural antioxidants, potentially valuable for applications in food, pharmaceutical, and cosmetic industries.

1. Introduction

The Lamiaceae family, widely recognized for its aromatic herbs, plays a significant role in both culinary and medicinal applications. This family includes well-known species such as *Ocimum basilicum* (basil), *Salvia officinalis* (sage), and *Mentha* spp. (mint), which are celebrated not only for their flavor but also for their health-promoting properties. A key aspect of these plants is their rich content of phenolic compounds, which are known to exhibit potent antioxidant activities. These compounds are crucial in combating oxidative stress caused by free radicals, which are implicated in various chronic diseases, including cancer and cardiovascular disorders (Jena *et al.*, 2023). The increasing interest in natural antioxidants has led to extensive research on the Lamiaceae family, known for its rich diversity of phytochemicals, particularly phenolic compounds. This family includes a variety of species that exhibit significant free radical scavenging activities, which are crucial for mitigating oxidative stress and its associated health risks. Phenolic compounds, such as rosmarinic acid, flavonoids, and hydroxycinnamic acids, are recognized for their potent antioxidant properties and play a vital role in the biological activities of these plants, including anti-inflammatory, antibacterial, and anticancer effects (Emam *et al.*, 2024; Moshari-Nasirkandi *et al.*, 2023). Recent studies have demonstrated that extracts from various Lamiaceae species possess varying levels of total phenolic content (TPC) and antioxidant capacity. For instance, the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay has been widely employed to evaluate the free radical scavenging ability of these extracts, revealing that some species, such as *Salvia multicaulis* and *Thymus vulgaris*, exhibit particularly high scavenging activities (Matkowski &

Piotrowska, 2006; Oalđe *et al.*, 2020). The correlation between TPC and antioxidant activity underscores the importance of these compounds in enhancing the health benefits associated with Lamiaceae plants (Babashpour-Asl & Piryaei, 2021; Mehrnia *et al.*, 2017; Sonboli *et al.*, 2010). This paper aims to explore the free radical scavenging activity and total phenol content of selected Lamiaceae species. By employing standardized assays such as DPPH and Folin-Ciocalteu methods, we will provide a comprehensive analysis of the antioxidant potential of these plants. The findings are expected to contribute to a deeper understanding of the health-promoting properties of Lamiaceae species and their potential applications in functional foods and nutraceuticals.

2. Materials and Methods

2.1 Plant Material

The plant specimens were gathered from various locations within the East Azerbaijan province of Iran during the period of March to June. They were then identified by the herbarium of the Faculty of Pharmacy (Tabriz University of Medical Sciences, Tabriz, Iran). The aboveground portions of the plants were air-dried, finely ground, and kept in a dark environment.

2.2 Chemicals and Reagents

Acetic acid glacial, ferrous sulfate heptahydrate, ferric chloride, Folin-Ciocalteu reagent, methanol, sodium acetate, sodium carbonate, and 2,4,6-tripyridyl-s-triazine (TPTZ) were obtained from Merck (Darmstadt). Catechin hydrate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 32% hydrochloric acid were acquired from Sigma-Aldrich (St. Louis). Trolox and quercetin were purchased from Acros Organics (Geel, Belgium).

2.3 Extraction

The plant materials were extracted using a methanol/water (90/10) solvent mixture. Specifically, 7.5 g of the dried and defatted plant matter was macerated in 200 mL of the solvent mixture for 2 days, with the solvent being replaced after the first day. The extract was then filtered and concentrated using a rotary evaporator in less than 10 minutes. Afterward, the extracts were freeze-dried overnight in a lyophilizer. The resulting powdered extracts were weighed to determine the yield and then stored at -20°C until needed for analysis. Prior to each experiment, the lyophilized powder was dissolved in methanol at the required concentration and tested for antioxidant activity and total phenolic content (Babashpour-Asl and Piryaei, 2022).

2.4 DPPH free radical assay

The DPPH radical scavenging activity of the plant extracts was measured using a modified version of a previously described method (Brand-Williams *et al.*, 1995; Hwang *et al.*, 2001). Briefly, different concentrations of the plant extracts dissolved in methanol were incubated with a methanolic solution of DPPH (100 μM) in 96-well microplates. The specific concentrations used for each plant extract were carefully selected to produce an appropriate dose-response curve, typically ranging from 1.6 to 100 μg/mL. After 30 minutes of incubation in the dark at room temperature, the absorbance at 490 nm was measured using a microplate reader (Bio-Rad, model 680). The percentage inhibition (%I) for each concentration was calculated using the formula:

$$\%I = [(ADPPH - AS)/ADPPH] \times 100$$

where ADPPH and AS are the absorbance values of the DPPH solution containing methanol and the sample extract, respectively.

The dose-response curve was plotted using R software version 4.4.2, and the IC_{50} values for the extracts were calculated. These IC_{50} values were then divided by the extract yield (%) to determine the IC_{50} value for the dry plant material.

2.5 Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was performed following a previously described method (Benzie & Strain, 1996; Firuzi *et al.*, 2006). The FRAP solution was freshly prepared by mixing 10 mL of 300 mM acetate buffer (pH 3.6), 1 mL of 20 mM ferric chloride hexahydrate dissolved in distilled water, and 1 mL of 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) dissolved in 40 mM HCl. The plant extract dissolved in methanol (40 μL) at a concentration of 1 mg/mL was then mixed with 4 mL of the FRAP solution. The absorbance was measured at 595 nm after 6 minutes of incubation at room temperature using a spectrophotometer. Quercetin, tested at a final concentration of 10 μM, was used as the reference compound. The FRAP values, expressed as μM quercetin equivalents per g of dry weight of the plant (DW), were calculated using the following formula:

$$\text{FRAP value} = (\Delta AP / \Delta AQ) \times Y \times 1000$$

Where ΔAP and ΔAQ are the absorbance changes of the FRAP solution in the presence of the plant extract and quercetin, respectively, and Y is the extract yield.

2.6 Total Phenol Content

The total phenolic content was determined using the Folin-Ciocalteu reagent, following a modified version of the method described by Singleton et al. (1999). A 0.25 mL aliquot of the extracts, appropriately diluted, was mixed with 0.25 mL of the Folin-Ciocalteu reagent and 2 mL of distilled water. After 3 minutes at room temperature, 0.25 mL of a saturated sodium carbonate (Na_2CO_3) solution was added, and the mixture was incubated at 37°C in a water bath for 30 minutes. The

absorbance was then measured at 750 nm using UV/visible spectrophotometer. Catechin was used as the reference standard, and the results were expressed as mg of catechin equivalents per g DW.

2.7 Statistical Analysis

All measurements were taken in triplicate, and the results are presented as arithmetic mean ± standard deviation (SD). The means comparison among the different species of the Lamiaceae family was conducted using Duncan's multiple range test. In order to evaluate the strength of the relations between variables, Pearson's correlation coefficients were calculated and regression curves were drawn with using R software ver. 4.4.2.

3. Results

Based on the Table 1 *Mentha piperita* and *Mentha longifolia* exhibit the highest antioxidant activity: They have the lowest IC_{50} values for DPPH scavenging (100.7 ± 50.1 and 80.9 ± 39.1 µg DW/mL, respectively) and high FRAP values (49.9 ± 0.8 and 40.7 ± 1.8 µM/g DW, respectively). This suggests that these mint species are potent sources of antioxidants. *Mentha longifolia* shows the highest total phenol content: It has a total phenol content of 20.9 ± 1.8 mg CE/g DW, significantly higher than other species in the table. This aligns with the understanding that phenolic compounds contribute to antioxidant activity. *Eremostachys laciniata***,** *Thymus deanensis*, and *Marrubium vulgare* display relatively lower antioxidant activity: They have higher IC_{50} values for DPPH scavenging and lower FRAP values compared to *Mentha piperita* and *Mentha longifolia*. Their total phenol content is also lower. *Salvia hydrangea* shows moderate antioxidant activity and total phenol content: It falls between the high-activity mint species and the lower-activity species. There is a general trend of higher antioxidant activity correlating with higher total phenol content, particularly evident when comparing horsemint with the other species. However, this correlation is not always perfect. For instance, peppermint has a similar total phenol content to desert rod but much higher antioxidant activity. This suggests that other factors, such as the specific types of phenolic compounds present and the presence of other non-phenolic antioxidants, also contribute to the overall antioxidant capacity.

Table 2 presents Pearson's correlation coefficient values, which measure the linear relationship between variables. A strong negative correlation exists between IC_{50}/DPPH and FRAP. As IC_{50}/DPPH increases, FRAP decreases significantly. This suggests that higher antioxidant activity (lower IC_{50}) is strongly associated with increased FRAP values. A moderate negative correlation exists between IC_{50}/DPPH and TPC (Total Phenolic Content). Higher antioxidant activity (lower IC_{50}) tends to correspond to higher phenolic content, but the relationship is not as strong as with FRAP. This is expected as phenolics often contribute to antioxidant activity. A moderate negative correlation is observed between IC_{50}/DPPH and extract yield. This indicates that higher antioxidant activity (lower IC_{50}) tends to be associated with higher extract yield. A weak positive correlation exists between FRAP and TPC. Higher phenolic content slightly corresponds to higher FRAP values, but the relationship is not strong. This could imply that factors other than phenolic content contribute to the FRAP values. A moderate positive correlation exists between FRAP and extract yield. Higher extract yields are associated with higher FRAP values, suggesting that the quantity of extract influences its antioxidant capacity. There is almost no correlation between TPC and extract yield. This indicates that phenolic content is not directly related to the amount of extract obtained (Fig. 1-3). This analysis underscores the importance of phenolic compounds and extract yield in influencing antioxidant activity and reducing power, though the strength of these relationships varies

4. Discussion

The observed antioxidant activities can be attributed to the high TPC found in the extracts, suggesting a moderate correlation between phenolic content and free radical scavenging ability. These findings are consistent with recent literature, which emphasizes the role of phenolic compounds in mitigating oxidative stress and their implications for chronic disease prevention (Jena et al., 2023; Emam et al., 2024). This is also consistent with established theories that posit phenolic compounds as key contributors to antioxidant activity due to their ability to donate electrons and neutralize free radicals (Singleton et al., 1999). Notably, the high IC50 values calculated for some extracts indicate a potent capacity for inhibiting oxidative stress, which is particularly relevant given the increasing prevalence of oxidative stress-related diseases. Surprisingly, some extracts exhibited greater scavenging activity than anticipated based on their TPC levels. This could suggest the presence of synergistic effects among various phytochemicals within the extracts or the influence of non-phenolic compounds that enhance antioxidant activity (Babashpour-Asl & Piryaei, 2021; Firuzi et al., 2006). Further investigation into these interactions may provide deeper insights into the mechanisms underlying the antioxidant properties of Lamiaceae species. The correlation observed between total phenolic content and antioxidant activity aligns with previous studies, further validating the importance of these phytochemicals in promoting health (Matkowski & Piotrowska, 2006; Mehrnia et al., 2017). However, discrepancies in antioxidant capacities reported in other

Table 1. Antioxidant activity and total phenol content of some Lamiaceae family species

Common name	Scientific name	IC_{50} DPPH scavenging (µg DW/mL)	FRAP (µM/g DW)	Total phenol content (mg CE/g DW)	Extract yield (%)
Desert rod	*Eremostachys laciniata* (L.) Bunge	989.1 ± 14.8 [a]	11.9 ± 1.5 [e]	3.5 ± 0.5 [d]	8.5 [d]
Peppermint/Black mint	*Mentha piperita* L.	100.7 ± 50.1 [e]	49.9 ± 0.8 [a]	3.5 ± 1.2 [d]	12.1 [a]
Horsemint/Brookmint	*Mentha longifolia* (L.) Huds.	80.9 ± 39.1 [f]	40.7 ± 1.8 [b]	20.9 ± 1.8 [a]	10.9 [b]
Salvia/Sage	*Salvia hydrangea* DC.	300.1 ± 33.1 [d]	32.1 ± 0.5 [c]	11.1 ± 0.8 [b]	9.5 [c]
Thyme	*Thymus deanensis* Celak.	505.8 ± 37.8 [c]	24.9 ± 0.8 [d]	10.9 ± 1.5 [b]	7.9 [d]
White horehound /Common horehound	*Marrubium vulgare* L.	560 ± 40.1 [b]	21.9 ± 0.9 [d]	7.9 ± 0.8 [c]	11.6 [b]

Table 2. Pearson's correlation coefficient for the variables

	IC_{50}/DPPH	FRAP	TPC	Extract yield (%)
IC_{50}/DPPH	1.000			
FRAP	-0.948**	1.000		
TPC	-0.513*	0.272ns	1.000	
Extract yield (%)	-0.591*	0.642*	0.005ns	1.000

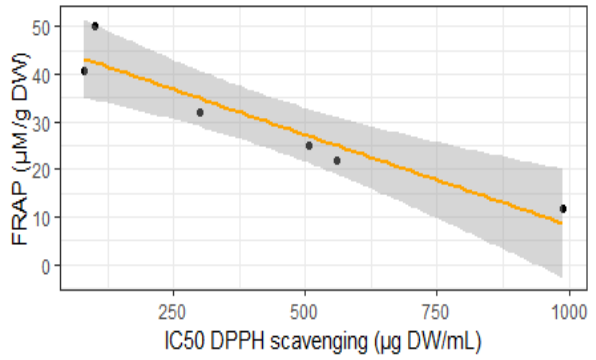


Fig. 1. Relationship between values of FRAP and IC50 DPPH scavenging for some species of Lamiaceae family extracts

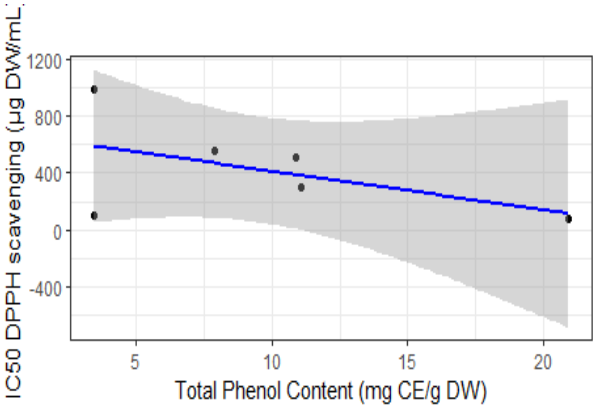


Fig. 2. Relationship between values of IC_{50} DPPH scavenging and FRAP for some species of Lamiaceae family extracts

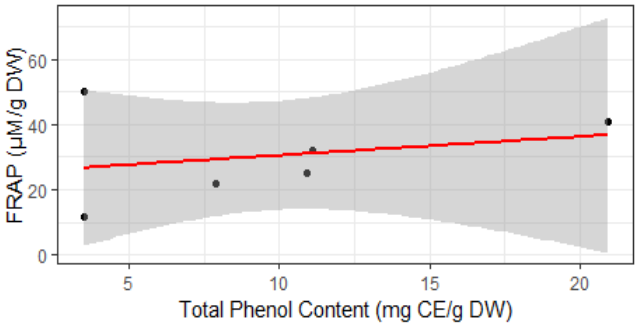


Fig. 3. Relationship between values of FRAP and total phenol content for some species of Lamiaceae family extracts

studies suggest that further research is warranted to explore the variability in phytochemical profiles across different species and environmental conditions (Moshari-Nasirkandi et al., 2023). Despite these promising findings, some limitations must be acknowledged. The sample size was relatively small and limited to specific regions in Iran, which may affect the generalizability of the results. Additionally, variations in environmental conditions during plant growth could influence phytochemical composition and antioxidant capacity (Mehrnia et al., 2017). Future studies should aim for larger sample sizes and include a wider range of environmental conditions to validate these findings. Furthermore, while we employed standardized assays such as DPPH and FRAP to assess antioxidant activity, additional methods could provide a more comprehensive evaluation of phytochemical profiles and their biological activities (Benzie & Strain, 1996).

5. Conclusion

In conclusion, this study underscores the significance of Lamiaceae species as valuable sources of natural antioxidants. The implications extend beyond academic interest; they suggest practical applications in dietary interventions aimed at reducing oxidative stress-related health risks. By promoting the consumption of these plants, we can leverage their health-promoting properties while contributing to a more sustainable approach to nutrition. This research not only enriches our understanding of plant-based antioxidants but also paves the way for future studies that could lead to innovative applications in health and wellness. To explain the variability in antioxidant activity and total phenol content among Lamiaceae species, future phytochemical analyses could include the following approaches:

Comprehensive Phytochemical Profiling: Utilize advanced techniques such as High-Performance Liquid Chromatography (HPLC) and Gas Chromatography-Mass Spectrometry (GC-MS) to identify and quantify a broader range of phytochemicals present in the extracts. This will help determine the specific compounds contributing to antioxidant activity beyond just phenolic content.

Isolation and Characterization of Bioactive Compounds: Isolate individual phytochemicals from the extracts, such as flavonoids, rosmarinic acid, and other phenolic acids, to evaluate their specific contributions to antioxidant properties. Characterizing these compounds will provide insights into their mechanisms of action.

Synergistic Effects Analysis: Investigate potential synergistic effects between different phytochemicals within the extracts. Conducting combination studies can reveal how various compounds interact to enhance overall antioxidant capacity.

In Vivo Studies: Complement *in vitro* findings with *in vivo* studies to assess the biological relevance of the antioxidant activities observed. This can include evaluating the effects of Lamiaceae extracts on oxidative stress markers in animal models.

By implementing these future phytochemical analyses, researchers can gain a deeper understanding of the factors contributing to variability in antioxidant activity among Lamiaceae species, ultimately leading to more effective applications in food, pharmaceuticals, and nutraceuticals.

References

Babashpour-Asl, M. & Piryaei, M. (2021). Free Radical Scavengering and Phenolic Compounds of Peel and Pulp of Quince. *International Journal of Horticultural Science and Technology*. *8*(1), 91-101, https://doi.org/10.22059/ijhst.2020.285851.308.

Babashpour-Asl, M. & Piryaei, M. (2022). Antioxidant activities and several bioactive substances of different extracts of *Vitis vinifera* L. *Journal of Food Biosciences and Technology*. 12(2), 49-60, https://doi.org/10.30495/JFBT.2022.19335.

Benzie, I. F. F. & Strain, J. J. (1996). The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay. *Analytical Biochemistry.* *239*(1), 70-76, https://doi.org/10.1006/abio.1996.0292.

Brand-Williams, W., Cuvelier, 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.

Emam, M., El-Newary, S. A., Aati, H. Y., Wei, B., Seif, M. & Ibrahim, A. Y. (2024). Anti-Alzheimer's Potency of Rich Phenylethanoid Glycosides Extract from *Marrubium vulgare* L.: In Vitro and In Silico Studies. *Pharmaceuticals.* *17*(10), 1282, https://www.mdpi.com/1424-8247/17/10/1282.

Firuzi, O., Mladenka, P., Riccieri, V., Spadaro, A., Petrucci, R., Marrosu, G. & Saso, L. (2006). Parameters of oxidative stress status in healthy subjects: their correlations and stability after sample collection. *Journal of Clinical Laboratory Analysis*. *20*(4), 139-148, https://doi.org/10.1002/jcla.20122.

Hwang, B. Y., Kim, H. S., Lee, J. H., Hong, Y. S., Ro, J. S., Lee, K. S. & Lee, J. J. (2001). Antioxidant benzoylated flavan-3-ol glycoside from *Celastrus orbiculatus*. *Journal of Natural Products*. *64*(1), 82-84, https://doi.org/10.1021/np000251l.

Jena, B., Biswal, B., Sarangi, A., Giri, A. K., Bhattacharya, D. & Acharya, L. (2023). GC/MS Profiling and Evaluation of Leaf Essential Oil for Bactericidal Effect and Free Radical Scavenging Activity of Plectranthus amboinicus (Lour.) Spreng Collected from Odisha, India. *Chemistry & Biodiversity*. 20(3), e202200691, https://doi.org/10.1002/cbdv.202200691.

Matkowski, A. & Piotrowska, M., (2006). Antioxidant and free radical scavenging activities of some medicinal plants from the Lamiaceae. *Fitoterapia*. *77*(5), 346-353, https://doi.org/10.1016/j.fitote.2006.04.004.Mehrnia.

M. A., Bashti, A. & Majdi Nasab, H. (2017). Chemical composition and antioxidant properties of some species of Lamiaceae family from Iran. *Journal of Medicinal plants and By-Products*. 6(2), 125-130, https://doi.org/10.22092/jmpb.2017.113534.

Moshari-Nasirkandi, A., Alirezalu, A., Alipour, H. & Amato, J. (2023). Screening of 20 species from Lamiaceae family based on phytochemical analysis, antioxidant activity and HPLC profiling. *Scientific Reports*. *13*(1), 16987, https://doi.org/10.1038/s41598-023-44337-7.

Oalđe, M. M., Kolarević, S. M., Živković, J. C., Vuković-Gačić, B. S., Jovanović Marić, J. M., Kračun Kolarević, M. J., Đorđević, J. Z., Alimpić Aradski, A. Z., Marin, P. D., Šavikin, K. P. & Duletić-Laušević, S. N. (2020). The impact of different extracts of six Lamiaceae species on deleterious effects of oxidative stress assessed in acellular, prokaryotic and eukaryotic models in vitro. *Saudi Pharmaceutical Journal*. *28*(12), 1592-1604, https://doi.org/10.1016/j.jsps.2020.10.006.

Singleton, V. L., Orthofer, R. & Lamuela-Raventós, R. M. (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In *Methods in Enzymology*. (Vol. 299, pp. 152-178). Academic Press. https://doi.org/10.1016/S0076-6879(99)99017-1.

Sonboli, A., Mojarrad, M., Nejad Ebrahimi, S. & Enayat, S. (2010). Free Radical Scavenging Activity and Total Phenolic Content of Methanolic Extracts from Male Inflorescence of *Salix aegyptiaca* Grown in Iran. *Iranian Journal of Pharmaceutical Research*. *9*(3), 293-296, https://doi.org/10.22037/ijpr.2010.869.