LGU Journal of
LIFE SCIENCES

LGU Society of Life Sciences

Research Article
Vol 6 Issue 1 January - March 2022

LGU J. Life. Sci
ISSN 2519-9404
eISSN 2521-0130

DPPH Assay and Reducing Power Activity of Water Extract of (*Mentha longifolia*) Mint

Muhammad Khalid Saeed, Naseem Zahra, Khurram Shahzad, Shamma Firdous, Ijaz Ahmad, Muhammad Ashraf, Syed Hussain Imam Abidi and Quratulain Syed*

Food and Biotechnology Research Centre, PCSIR Laboratories Complex, Lahore-54600, Pakistan

***Corresponding Author's Email:** drnaseemzahra@gmail.com

ABSTRACT: *Antioxidative properties of plants may be associated with oxidative stress defence in different human diseases. Oxidative stress and free radicals can be neutralized by antioxidants which are of great significance in preventing the expansion of these diseases. Many studies have investigated the toxic effect of synthetic antioxidants, thus to avoid these toxic effects new antioxidants of natural origin have been deliberated in recent years. Mint (Mentha longifolia) is extensively used as medicine, spice, food and flavouring. In the current work, an aqueous extract of mint leaves was determined using the DPPH assay and reducing power activity. Strapping and sound antioxidant effects were observed in 2,2-diphenyl-1-picrylhydrazyl radicals at concentrations of 0.1-0.5 mg/ml, ranging from 20.32 ± 0.35 % to 65.75 ± 1.5 %, while standard antioxidant BHT possess % Inhibition (DPPH) 30.41 ± 0.65 % to 83.50 ± 2.3 % at same concentration. A similar effect was found in the reducing power assay, which exhibited absorbance of M. longifolia water extract ranging from 0.1501 ± 0.010 to 0.5845 ± 0.042 and BHT exhibited 0.3221 ± 0.026 to 0.8197 ± 0.124 at 700 nm. The conclusion recommends that M. longifolia has confirmed vital benefits due to high concentration of antioxidants and has vast impending for claim in the preparation of useful food entities.*

Keyword: *M. longifolia, water extract, antioxidant activity, DPPH assay, RPA assay*

INTRODUCTION

More recently, dietary supplements made from various fruits, vegetables and herbs have emerged as a natural therapeutic and protective strategy to prevent disease and poor health. In developing countries, there is insufficient information on the nutritional value of several native plants, which may be beneficial for different

purposes. Herbal plants and their natural extracts can offer promising treatments for different diseases (Karimian et al., 2013; Kozłowska et al., 2021). Mint (*Mentha longifolia*) belongs to the family *Lamiaceae* and in temperate regions of the world widely grown (Fig. 1) (Hussain et al., 2010). Mints have traditionally been used for centuries as flavorings, condiments, herbal teas, fresh vegetables, infusions, decoctions and distillates (Dzamic et al., 2010; Krzyzanowska et al., 2011). They are also used as carminative, breath fresheners, anti-infective, choleretic, anti-inflammatory, antispasmodic, anticatarrhal, antiallergic laxative, diuretics, gastric tonics and antinociceptive (Zeinali et al., 2005; Naghibi et al., 2010). Therefore dried and fresh mints are used widely used in herbal medicine to treat a variety of health problems and discomforts (Younis et al., 2004; Sharopov et al., 2012).

Fig. 1. *M. longifolia* leaves and powder

(https://www.indiamart.com/proddetail/mint-leaves-powder-21944690991.html)

It is thought that free radicals trigger oxidation, which contributes to number of diseases in human being (Ścibisz et al., 2021). Antioxidants are a group of compounds that protect cells from free radicals and can help reduce different diseases such as aging and cancer (Spréa et al., 2020). Plant antioxidants are important because their presence in the human diet can help the body neutralize free radicals and reduce oxidative stress damage. In contrast, synthetic antioxidants may have carcinogenic effects (Suhaj, 2006; Tajner-Czopek et al., 2020). Various studies depicted that mint extracts have potent antioxidant properties (Gulluce et al., 2007; Mkaddem et al., 2009; Kadhim et al., 2020).

Therefore, this study was conducted to appraise the antioxidant potential of aqueous extract of *Mentha*

longifolia (Mint) leaves. DPPH assay and reducing power of activity were performed to check the antioxidants potential in water extract Mint leaves powder.

MATERIALS AND METHODS

Plant Material and Pretreatment

The leaves of *M. longifolia* had been collected from the local market. In order to remove all traces of dust and insects, the leaves were rinsed under tap water and cleaned. Afterword; the plants Leaves were squeezed, dehydrated at 50-60°C in dehydrator for one day. Then it was ground in grinder mill to convert it in to a powder form with a size of 80 meshes. The dried *Mentha longifolia* leaves were kept in airtight bottles to be used for extraction (Mohammed et al., 2020).

Chemicals and Reagents

2,2′-diphenyl-1-picrylhydrazyl, $NaHPO_4$, NaH_2PO_4, $FeCl_3$, $K_3Fe(CN)_6$ and TCA were purchased from Sigma Chemical Co. In this study all other chemicals were analytical grade.

Preparation of extracts

The ethanol extract of the aerial parts of was obtained using maceration method. An amount of 20 g *M. longifolia* powder was extracted using 200 mL of water. The extraction was performed by shaking at room temperature during 12 h. Finally, the extract was passed through a paper filter and the filtrated solution was concentrated by a rotary evaporator at 40°C.

DPPH Assay

The water extract of *M. longifolia* was subjected for dogged of antioxidant activity based on free radical scavenging activity using DPPH assay as described by Brand-Williams 1995 with some modifications (Saeed et al., 2021). Mix 0.1 mL aliquots of extraction solution (1-5 mg/ml) with 3.0 ml DPPH (0.004% in methanol). The mixture was shaked vigorously and incubated for 1/2 hour at 25°C and water used as a control. By using a spectrophotometer UV-Vis (1700, Shimazdu, Japan) the absorbance at 517 nm of water extract of *M. longifolia* was deliberated. Results were expressed as % inhibition and calculated by following formula.

% Inhibition = $A_{control}–A_{sample}/A_{control} \times 100$

Reducing power activity

Oyaizu (1986) method was used for the determination of the reducing power activity of water extract of *M. longifolia* with minor modifications (Liu et al., 2012). 1 ml of water extract of *M. longifolia* at 1, 2, 3 ,4 and 5 mg/ml

concentrations was mix with phosphate buffer (pH 6.6) of 2.5 ml (0.2 mol/l) and 2.5 ml of 1% $K_3Fe(CN)_6$. Incubated it for 20 min at 50 °C and stopped the reaction by addition of 10% tri-chloro-acetic acid (2.5 ml) and for 10 min the mixture was centrifuged at 6000 g. Finally mix the upper layer 2.5 ml with 2.5 ml H_2O and 0.5 ml (0.1% $FeCl_3$) and absorbance was measured at 700 nm by using spectrophotometer (UV-Vis: 1700, Shimazdu, Japan).

Statistical analysis

Data were statistically analyzed using SPSS v.16.0 and is presented as mean ± standard deviation (SD). All experiments were performed in triplicate.

RESULTS AND DISCUSSION

Plant extracts, which contain different classes of compounds such as polyphenol, flavonoids, anthocyanins, are very important source of antioxidants for the food industry. Therefore, in this study we investigated antioxidant properties of *M. longifolia* water extracts as a source of natural antioxidants. Lamiaceae family plants are rich in antioxidant compounds and a large number of which, like sage, oregano, thyme, basil and mint exhibit strong antioxidant activity (Ozgen et al., 2006; Zeljkovic et al., 2021).

Antioxidant activity by DPPH assay

Diphynyl-picryl-hydrazine is a stable free radical and has been extensively acknowledged as a tool to appraise the antioxidant activity of extracts. It is a simple, rapid, reliable and a widely used assay which is sensitive to some Lewis bases and requires organic solvents and non-physiological radicals (Sirivibulkovit et al., 2018). The ability of *M. longifolia* water extract to donate proton to DPPH free radical and change its color from violet to yellow is accessed in this assay (Benkhaira et al., 2022). In Fig. 2, mint water extract of 0.1-0.5 mg/ml were prepared to evaluate the antioxidant activity. The method relies on the reduction of violet to yellow diphenyl-picryl-hydrazine and the scavenging DPPH (Katalinic et al., 2006; Ghafoor et al., 2010). The respective scavenging capacities were ranged from 20.32± 0.35% to 65.75 ± 1.5 %. A dose-dependent approach was pragmatic in the antioxidant activity of mint water extract. The highest % inhibition was 65.75 ± 1.5% at 0.5 mg/ml, indicating its considerable scavenging capacity. These findings are in agreement with previous works on the genus *Mentha* (Ahmad et al., 2012; Naqvi et al., 2018). According to relatively high amounts of phenolic compounds in *M. longifolia* and their

antioxidant potential which were confirmed using several *in vitro* assays, this species could be considered for possible applications in food industries (Sun et al., 2014; Abbood et al., 2020).

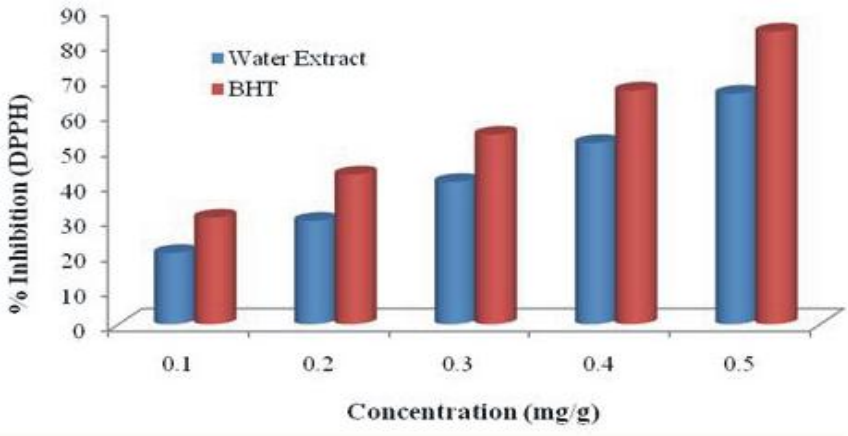

Fig. 2. % inhibition of water extract of *M. longifolia*

Reducing power assay

This assay used to measure the transferring capacity of Fe^{3+} to Fe^{2+}, which then reacts with $FeCl_3$ to form blue colored $(Fe^{3+})_4$ $[Fe^{2+}$ $(CN^{-})_6]^3$ complex that has an absorption maximum at 700 nm. The reducing power was correlated with electron transfer ability of the sample. Increased absorbance of the reaction mixture indicated increased reducing power of the plant extract (Mohamed et al., 2022). Reducibility is usually related to the occurrence of reducing agents. The reducing power effect of reducing agents is based on infringement free radical chains by donating a H°. Figure 3 depicted the reducing power of water extract of mint and it exhibits absorbance ranging from 0.1501 ± 0.010 to 0.5845 ± 0.042 and BHT exhibited 0.3221 ± 0.026 to 0.8197 ± 0.124 at 700 nm (Table 1). This reducing power activity of mint water extract was also depended upon the dose, higher the dose or concentration higher the absorbance (antioxidant activity). These finding suggested that *M. longifolia* water extract possesses significant reducing power due to the presence of secondary metabolites. These results are consistent with prior research on *Mentha* species (Nickavar et al., 2010; Tahseen et al., 2013; Wafa and Sofiane, 2020).

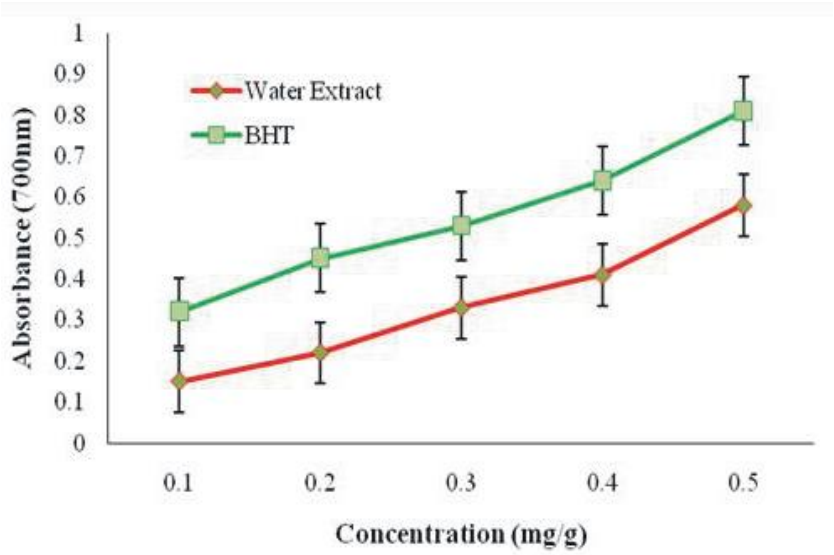

Fig. 3. RPA of water extract of *M. longifolia*

Table 1: Reducing Power Activity of water extract of *M. longifolia*

Sr. No	Concentration (mg/g)	Absorbance (700nm) H_2O Extract of *M. longifolia*	Absorbance (700nm) BHT
1	0.1	0.1503 ± 0. 010	0.3221 ± 0. 026
2	0.2	0.2211 ± 0. 012	0.4526 ± 0. 032
3	0.3	0.3350 ± 0. 015	0.5307 ± 0. 038
4	0.4	0.41233 ± 0. 218	0.6439 ± 0.105
5	0.5	0.5845 ± 0.042	0.8197 ± 0. 124

Data are represented± standard deviation

CONCLUSION

Mentha longifolia is drawn to traditional herbal medicine for health promotion and disease prevention. The main purpose of this study was to assess the antioxidant activity of an aqueous extract of *M. longifolia*. The results of this investigation indicated that *M. longifolia* water extract provides considerable antioxidant activities in these both DPPH & reducing power assays. Moreover, they may be used in pharmaceutical and natural therapies for treatment of oxidative stress.

REFERENCES

1. Abbood SM, Khalid F, Al-Rawi H, Qaddoori T, Mustafa TM Kadhim SM (2020). Antioxidant activity and acute oral toxicity of the methanol extract from *Mentha Longifolia L. ssp.* in Iraq. Sys. Rev. Pharm. 11(12): 743-746.
2. Ahmad N, Fazal H, Ahmad I, Abbasi BH (2012). Free Radical Scavenging (DPPH) Potential in Nine *Mentha* Species. Toxicol. Indus. Health. 28(1): 83–89.
3. Bahadori MB, Zengin G, Bahadori S, Dinparast L, Movahhedin N (2018). Phenolic composition and functional properties of wild mint (*Mentha longifolia* var. *calliantha* (Stapf) Briq.). Int. J. Food Prop. 21(1): 1-5.
4. Benkhaira N, Koraichi SI, Fikri-Benbrahim K (2022). In vitro methods to study antioxidant and some biological activities of essential oils: A Review. Biointerface Res. Appl. Chem. 12(3): 3332 – 3347.
5. Brand-Williams W, Cuvelier ME, Berset C (1995). Use of free radical method to evaluate antioxidant activity. LWT Food Sci. Technol. 28(1): 25-30.
6. Dzamic AM, Sokovic MD, Ristic MS, Novakovic M, Grujic-Jovanovic S, Tesevic V. Marin PD (2010). Antifungal and Antioxidant Activity of *Mentha Longifolia* (L.) Hudson (*Lamiaceae*) Essential Oil. Bot. Serbica. 34(1): 57–61.
7. Ghafoor K, Park J, Choi YH (2010). Optimization of supercritical carbon dioxide extraction of bioactive compounds from grape peel (*Vitis labrusca B.*) by using response surface methodology. Innov. Food Sci. Emerg. Technol. 11(1): 485-490.
8. Gulluce M, Sahin F, Sokmen MÜ, Ozer H, Daferera D, Sokmen AT, Polissiou M, Adiguzel AY, Ozkan Hİ (2007). Antimicrobial and antioxidant properties of the essential oils and methanol extract from *Mentha longifolia L. ssp.* longifolia. Food chem. 103(4): 1449-56.
9. Hussain AI, Anwar F, Nigam PS, Ashraf MA. Gilani H (2010). Seasonal variation in content, chemical composition and antimicrobial and cytotoxic activities of essential oils from four *Mentha* species. J. Sci. Food Agric. 90(11): 1827–1836.
10. Kadhim SM, Mohammed MT, Abbood SM (2020). Biochemical

studies of Ginkgo biloba extract on oxidative stress-induced myocardial injuries. Drug Invent. Today. 14(6): 817-820.

11. Karimian P, Kavoosi G, Amirghofran Z (2013). Antiinflammatory effect of *Mentha longifolia* in lipopolysaccharide-stimulated macrophages: reduction of nitric oxide production through inhibition of inducible nitric oxide synthase. J. Immunotox. 10(4): 393-399.
12. Katalinic V, Milos M, Kulisic T, Jukic M (2006). Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chem. 94: 550-557.
13. Kozłowska M, Ścibisz I, Przybył JL, Małgorzata Z, Żbikowska A. Majewska E (2021). Phenolic Contents and Antioxidant Activity of Extracts of Selected Fresh and Dried Herbal Materials. Pol. J. Food Nutr. Sci. 71: 269–278.
14. Krzyzanowska J, Janda B, Pecio L, Stochmal A, Oleszek W, Czubacka A, Przybys M, Doros-zewska T (2011). Determination of Polyphenols in *Mentha Longifolia* and *M. Piperita* Field-Grown and *in Vitro* Plant Samples Using UPLC-TQ-MS. J. AOAC Int. 94(1): 43–50.
15. Liu X, Sun Z, Zhang M, Meng X, Xia X, Yuan W, Xue F, Liu C (2012) Antioxidant and antihyperlipidemic activities of polysaccharides from sea cucumber *Apostichopus japonicus*. Carbohydr. Polym. 90(1): 1664–1670.
16. Mkaddem M, Bouajila J, Ennajar M, Lebrihi A, Mathieu F, Romdhane M (2009). Chemical composition and antimicrobi- al and antioxidant activities of Mentha (*longifolia* L. and viridis) essential oils. J. Food Sci. 74(7): M358- M363.
17. Mohamed TS, Refahy LA, Romeih MH, Hamed MM, Ahmed HO, El-Hashash MA (2022). Chromatographic Isolation and Characterization of Certain Bioactive Chemical Ingredients of *Phyllanthus emblica* Extracts and Assessment of Their Potentials as Antiviral and Anticancer Agents. Egypt. J. Chem. 65(10): 179 – 192.
18. Mohammed MT, Kadhim, SM, Abbood SM (2020). Study of some Crataegus leaves component and effect of their aqueous extract on oxidative stress during ischemia/reperfusion brain damage. Drug Invent. Today. 14(5): 718-721.

19. Naghibi F, Mosaddegh M, Motamed MM, Ghorbani A (2010). Labiatae Family in Folk Medicine in Iran: From Ethnobotany to Pharmacology. Iran. J. Pharm. Res. 4(2): 63–79.
20. Naqvi SA, Umar U, Hasnain A, Rehman A, Perveen R (2018). Effect of botanical extracts: A potential biocontrol agent for *Xanthomonas oryzae pv. oryzae*, causing bacterial leaf blight disease of rice. Pak. J. Agric. Sci. 32(1): 59-72.
21. Nickavar B, Alinaghi A, Kamalinejad M (2010). Evaluation of the Antioxidant Properties of Five *Mentha* Species. Iran. J. Pharm. Res. 10(2): 203–209.
22. Oyaizu M (1986). Studies on products of browning reactions: antioxidative activities of products of browning reaction prepared from glucosamine. Jap. J. Nut. 44(1): 307–315.
23. Ozgen U, Mavi A, Terzi Z, Yildirim A, Coskun M, Houghton PJ (2006). Antioxidant properties of some medicinal *Lamiaceae* (*Labiatae*) species. Pharm Biol. 44(1): 107-12.
24. Saeed MK, Nisa A, Ahmad I, Hina S, Zahra N, Kalim I, Masood S. Quratulain S (2021). Physico-chemical Analysis, Total Polyphenolic Content and Antioxidant Capacity of Yellow Dye Extracted from *Curcuma Longa*. Pak. J. Sci. Ind. Res. Ser. B: Biol. Sci. 64B (1): 25-29.
25. Ścibisz I, Jarosław LP, Ziarno M, Żbikowska A, Majewska E (2021). Phenolic Contents and Antioxidant Activity of Extracts of Selected Fresh and Dried Herbal Materials. Pol. J. Food Nutr. Sci. 71(3): 269–278.
26. Sharopov FS, Sulaimonova VA, Setzer WN (2012). Essential Oil Composition of *Mentha Longifolia* from wild populations growing in Tajikistan. J. Med. Active Plant. 1(2): 6-15.
27. Sirivibulkovit K, Nouanthavong S, Sameenoi Y (2018). Paper-based DPPH assay for antioxidant activity analysis. Anal. Sci. 34(1): 795–800.
28. Spréa RM, Fernandes Â, Calhelha RC, Pereira C, Pires TCSP, Alves MJ, Canan C, Barros L, Amaral JS, Ferreira ICFR. (2020). Chemical and bioactive characterization of the aromatic plant *Levisticum officinale* W.D.J. Koch: a comprehensive study. Food Functi. 11(2): 1292–1303.

29. Suhaj M (2006). Spice antioxidants isolation and their antiradical activity: a review. J. Food Comp. Analy. 19(1): 531–537.
30. Sun Z, Wang H, Wang J, Zhou L, Yang P (2014). Chemical composition and anti-inflammatory, cytotoxic and antioxidant activities of essential oil from leaves of *Mentha piperita* grown in China. PLoS ONE, 9(12): e114767.
31. Tahseen I, Hussain AI, Shahzad ASC, Ali SR (2013). Antioxidant activity and volatile and phenolic profiles of essential oil and different extracts of wild mint (*Mentha longifolia*) from the Pakistani flora. J. Analyt. Meth. Chem. 1(1): 1-5.
32. Tajner-Czopek A, Gertchen M, Rytel E, Kita A, Kucharska AZ, Sokół-Łętowska A (2020). Study of antioxidant activity of some medicinal plants having high content of caffeic acid derivatives. Antioxidant. 9(5): 412.
33. Wafa G, Sofiane S (2020). Antioxidant, anti-inflammatory and antimicrobial activities of aqueous and methanolic extract of *Rosmarinus eriocalyx* Jord. and Fourr. Int. J. Biol. Chem. Sci. 14(1): 254-262.
34. Younis, YM, Beshir SM (2004). Carvone-Rich Essential Oils from *Mentha Longifolia* (L.) Huds. sp. *Schimperi* Briq. And *Mentha Spicata* L. Grown in Sudan. J. Essent. Oil Res. 16(6): 539–541.
35. Zeinali H, Arzani A, Razmjoo K, Rezaee MB (2005). Evaluation of oil compositions of Iranian Mints (Mentha species). J. Essent. Oil Resear. 17(2): 156–159.
36. Zeljkovic CS, Šišková, J, Komzáková K, De Dieg, N, Kaffková K, Tarkowski P (2021). Phenolic compounds and biological activity of selected Mentha species. Plants. 10: 550.