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Free Radical Scavenging, Metal chelating and Antiperoxidative Activities of *M. communis* Berries Methanol extract and its Fractions

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ABSTRACT

Oxidative stress resulted from free radicals and reactive oxygen species (ROS) are associated with many diseases. Phytotherapy has known a great evolution all the world and some medicinal plants are important remedies of some diseases. Myrtle (*Mrytus communis* L.) is a plant of Myrtaceae family which is common in the Mediterranean region, with flowering, always green leaves and fruit. This study aims to investigate *in vitro* antioxidant capacity of *M. communis* berries methanol extract and its three fractions using five assays: ABTS scavenging radicals, metal chelating, hydrogen peroxide and inhibition of lipid peroxidation assays. EAE extract possessed the highest antioxidant activity in ABTS (EAE (IC_{50}=2.5 µg/mL) and lipid peroxidation models (90.17%). Whereas, AqE is the most active extract in metal chelating activity (IC_{50}=0.73±0.03 mg/mL) and H_2O_2 assay. These results support the traditional use of this plant in healthcare and it could be a new source of antioxidant natural drugs.

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Introduction

Free radicals are part of normal metabolites for many organisms, and a complex system of endogenous and exogenous antioxidant sources in the body are employed to mitigate the potential damage from free radicals (Sevindik et al., 2017; Mohammed et al., 2021). When the body is in a state of aging or stress, these highly reactive chemical species are produced excessively, and structural abnormalities and dysfunction of the cell and mitochondrial membranes can arise (Shang et al., 2018; Korkmaz et al., 2021; Akgül et al., 2022).

The role of free radicals in many disease conditions has been well established. Several biochemical reactions in our body generate reactive oxygen species and these are capable of damaging crucial bio-molecules (Kına et al., 2021). If they are not effectively scavenged by cellular constituents, they lead to disease conditions (Halliwell et al. 1995). The harmful action of free radicals can be blocked by antioxidant substances, which scavenge the free radicals and detoxify the organism (Uysal et al., 2021).

The search for newer natural antioxidants, especially of plant origin, has been increasing ever since. Plants have been a constant source of drugs and recently, much emphasis has been placed on finding new therapeutic agents from medicinal plants. Today many people prefer to use medicinal plants rather than chemical drugs (Mohammed et al., 2020).

Myrtle (*Mrytus communis* L.) is a plant of Myrtaceae family which is common in the Mediterranean region, with flowering, always green leaves and fruit. MC leaves contains phenolic acids (gallic acid,ferulic acid, caffeic acid, syringic acid and vanillic acid), flavonoids, hydrosable tannins (gallotannins), proanthocyanidins, essential oil (a-pinene, myrtenyl acetate, 1,8-cineole, limonene and linalool) as the main compounds (Ozcan et al., 2019).

M. communis, or myrtle, is an evergreen shrub typical of the Mediterranean flora. Both the leaves and the flowers are delicately and pleasantly scented. It is a plant that has long been known for its medicinal properties. It is also used in the production of liqueurs, for which the berries or a

mixture of leaves and berries is macerated in alcohol for a prescribed amount of time (Pereira et al., 2017). Fruit has carminative properties and have been used traditionally for the treatment of diarrhoea (Benchikh et al., 2016 a,b), hemorrhoids (Mahboubi et al., 2017; Malekuti et al., 2019), inflammation (Amira et al., 2012; Soomro et al., 2019), hepatotoxicity (Kumar et al., 2011; Hanaa et al., 2020), burns (Ozcan et al., 2019), pancreatitis (Ozbeyli et al., 2019) and skin diseases (Baharvand-Ahmadi et al., 2015).

Polyphenolic composition of the berries was characterized by high concentrations of flavonol glycosides, flavonols and flavanols. The major fatty acids of berries were reported as linoleic, palmitic, oleic and stearic acids (Aidi Wannes et al., 2010; Barboni et al., 2010).

Antioxidant activity of plant extracts cannot be evaluated by a single method. Therefore, commonly accepted assays were used to assess the antioxidative effect of *M. communis* different extracts. In a preliminary study, the antioxidant activity of the different extractives was evaluated employing ABTS scavenging radicals, metal chelating, hydrogen peroxide and inhibition of lipid peroxidation assays.

Materials and Methods

Plant Material

The fresh berries of *M. communis* were collected from Jijel (North-East of Algeria) in November, 2018. The taxonomic identity of the plant was done by Professor Hocine Laouer, Department of Plant Biology and Ecology, University Setif 1, Algeria. A voucher number 52 MB 07/12/18 JiJ/SA/, was deposed at the laboratory of Phytotherapy Applied to Chronic Diseases.

Extraction and Fractionation

The extraction procedure was conducted as described in our previous study (Benabdallah et al. 2014) with slight modification. This method has two major steps: the first is with methanol to dissolve the flavonoids and the second is with chloroform and ethyl acetate to separate aglycones and glycosylated fractions of flavonoids. The dried powder of *M. communis* berries was extracted with methanol (85%) at room temperature for 3 days. The resulting suspension was then filtered and concentrated by evaporation at low pressure at 40°C. The filtrate was freed of waxes, fats and chlorophyll by successive washings with n-hexane to give an aqueous phase. To separate aglycones flavonoids and glycosylated flavonoids, the aqueous phase was mixed with chloroform to obtain an organic phase containing the aglycones flavonoid and methoxylated aglycones. The remaining aqueous phase underwent a series of extractions with ethyl acetate to recover the organic phase which contained some aglycones flavonoid, but especially mono- and diglycosides flavonoids. The remaining aqueous phase contained more polar glycosylated flavonoids such as di-, tri- and tetraglycosides flavonoids. In this study, four extracts were used: methanol (ME), chloroform (CHE), ethyl acetate (EAE) and aqueous (AqE) extracts. The collected fractions were submitted to a concentration at low pressure at 40°C and then dried and stored at 4°C until use.

Determination of in vitro Antioxidant Activity

ABTS radical cation decolorization assay

The radical scavenging assay against ABTS was measured using the method of Re 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:

$$\% \text{ inhibition} = [(AC–ATS)/AC] \times 100.$$

AC : Absorbance of control
ATS : Absorbance of test sample

The antioxidant activity of plant extracts was expressed as IC_{50}, which is defined as the concentration of extracts (in µg/mL) required to scavenge 50% of ABTS radicals. IC_{50} values were estimated by a nonlinear regression. A lower IC_{50} value indicates higher antioxidant activity.

Hydrogen peroxide-scavenging activity

The ability of *M. communis* extracts to scavenge hydrogen peroxide ($H_2O_{2)}$ was determined according to the method of Ruch et al. 1(989). 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:

$$SE\ (\%) = (1 – AS\ 230\ nm/\ AC\ 230\ nm) \times 100$$

SE : Scavenging effect
AS : Abs of sample
AC : Abs of control

Ferrous ion chelating activity

The chelating effect of the extracts was determined according to the method of Decker and Welch (1990) (which is based on the inhibition of the formation of Fe^{2+}-ferrozine complex after treatment of samples with Fe^{2+} ions. Briefly, 250 µL of test material or EDTA at different concentration were added to 50 µL of $FeCl_2$ (0.6 mM in distilled water) and 450 µL of methanol. After 5 min of incubation, the reaction was initiated by the addition of 5 mM ferrozine (50 µL), the mixture was stirred and allowed to react at room temperature for 10 min. The control contained all the reaction reagents except the extract and EDTA. The absorbance of the Fe^{2+}-ferrozine complex was measured at 562 nm. The chelating activity was expressed as a percentage using the following equation:

$$CA\ (\%) = [(AC–ATS)/AC] \times 100$$

CA : Chelating activity
AC : Abs of control
ATS : Abs of test sample

To determine the IC_{50} values, a dose response curve was plotted. IC_{50} is defined as the effective concentration of the test material that is required to chelate 50% of iron ions.

Ferric thiocyanate (FTC) assay

The FTC method was used to determine the amount of peroxide at the initial stage of lipid peroxidation using the method by Yen et al. (2003) with slight modifications. Linoleic acid emulsion (0.02 M) was prepared with linoleic acid (155 µL) and Tween 20 (155 µL) in phosphate buffer (50 mL, 0.02 M, pH 7.4). A reaction solution, containing extracts with different concentrations (0.5 mL), linoleic acid emulsion (2.5 mL), and phosphate buffer (2 mL, 0.02 M, pH 7.0) was placed in a glass vial with a screw cap and mixed with a vortex mixer. The mixture was incubated at 40°C in the dark. To 0.1 mL of reaction mixture, 4.7 mL of 75% ethanol and 0.1 mL of 30% ammonium thiocyanate were added. After 3 min of the addition of 0.1 mL of 0.02 M $FeCl_2$ in 3.5% HCl, the peroxide value was determined by recording the absorbance at 500 nm every 24 hours until the absorbance of the control reached a maximum. The positive and negative controls were subjected to the same procedures as the sample, except for the negative control, in which only the solvent was added, and for the positive control in which the sample was replaced with BHT and Vitamin C. The inhibition percentage of linoleic acid peroxidation was calculated as:

Inhibition% = (1 –AS at 500 nm/AC at 500 nm) × 100

AS : Abs of sample
AC : Abs of control

Thiobarbituric acid (TBA) assay

The TBA test was conducted on the final day of FTC according to the method described by Kikuzaki and Nakatani (1993) to determine the malonaldehyde (MDA) formation from linoleic acid peroxidation. The same sample preparation method as described in the FTC method was used. To 1 mL of sample solution, 20% trichloroacetic acid (2 mL) and thiobarbituric acid solution (2 mL) were added. The mixture was placed in a boiling water bath for 10 minutes. After cooling, it was then centrifuged at 3000 rpm for 20 minutes. Absorbance of the supernatant was measured at 532 nm. Antioxidant activity was recorded based on the absorbance of the final day of the FTC assay using the following equation:

% inhibition = 100 – [(S/C) × 100]

S : Abs sample
C : Abs control

Where Abs control and Abs sample are the absorbances of the control (without sample) and the experimental (with sample) reactions, respectively.

Statistical Data Analysis

Results were expressed as means ± standard deviation (SD) and were analyzed by one way analysis of variance (ANOVA) followed by Dunnet's test. The *P* Values of $P<0.05$ were considered significantly different using Graph Pad Prism Version 6.0 (GraphPad Software, Inc, La Jolla, CA, USA).

Results

In vitro Antioxidant Activities of M. communis Leaves Extracts

ABTS radical scavenging activity of M. communis leaves extracts

The ability of *M. communis* berries extracts to scavenge the radical ABTS are shown in Figure 1. All extracts exhibited high antioxidant activity and in the following order: EAE (IC_{50}=2.5 µg/mL)>ME (IC_{50}=13.6 µg/mL)>CHE (IC_{50}=16 µg/mL)>AqE (IC_{50}=25 µg/mL). EAE presented stronger antioxidant activity than Trolox (3 µg/mL), the reference drug used in this assay.

Ferrous ion chelating activity of M. communis berries (MBE) extracts

A decrease in absorbance indicates a higher chelating power of the extract. All the extracts demonstrated an ability to chelate ferric iron (II) ions. The chelating abilities on ferrous ions were in descending order: AqE (IC_{50}=0.73±0.03 mg/mL)>ME (IC_{50}=0.82±0.03 mg/mL)>EAE (IC_{50}=1.14±0.07 mg/mL)>CHE (IC_{50}=1.43±0.12 mg/mL). None of the extracts appeared to be better chelators of ferric iron (II) ions than the positive control EDTA (IC_{50}=0.02±00 mg/mL) in this assay system (Figure 2). In this assay, all extracts and EDTA interfered with the formation of ferrous and ferrozine complex, suggesting that it has chelating activity and captures ferrous ion before ferrozine.

Hydrogen peroxide scavenging activity of M. communis berries (MBE) extracts

The scavenging effect of the extracts on hydrogen peroxide decreased in the following order: AqE>EAE> ME >CHE (Figure 3). Aqueous extract displayed very strong H_2O_2 scavenging activity (IC_{50}=2.6 µg/mL). whereas chloroform extract exhibited an IC_{50} of 14 µg/mL.

Antioxidant activity of M. communis berries (MBE) extracts determined by FTC assay

As shown in figure 4, all plant extracts showed good antioxidant potential with percent inhibition ranging from (82.76±0.48%) to (90.17±1.21%) as compared with BHT as positive control. The results indicated that EAE exerted marked effects on inhibition of linoleic acid oxidation, which was as strong (90.17±1.21) as BHT (90.82±1.05%).

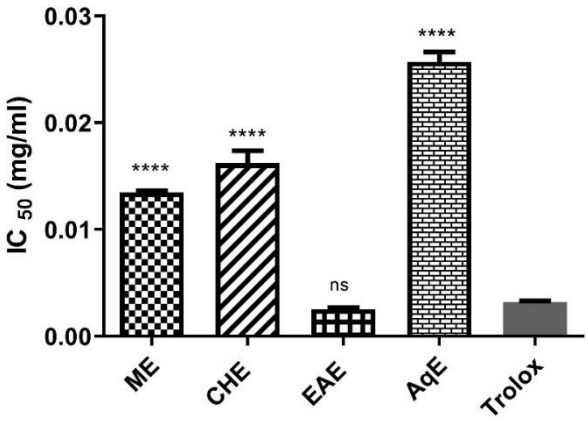

Figure 1. ABTS radical scavenging activity of *M. communis* berries extracts (MBE).
ME: M methanol extract, CHE: chloroform extract, EAE: ethyl acetate extract; AqE: aqueous extract. Data were presented as IC_{50} means±SD (n=3) (****P≤ 0.0001; ***P≤ 0.001; ns: not significant) vs Trolox as standard

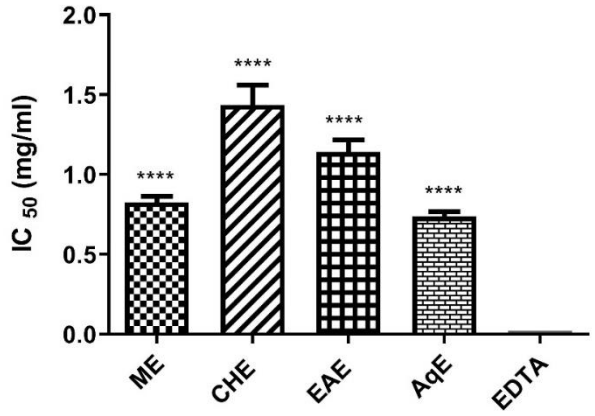

Figure 2. Ferrous ion chelating activity of M. communis berries extracts (MBE).
ME: methanol extract, CHE: chloroform extract, EAE: ethyl acetate extract; AqE: aqueous extract. Data were presented as IC_{50} means ± SD (n=3). Data were presented as IC_{50} means ± SD (n=3) (****P≤0.0001) vs EDTA as standard.

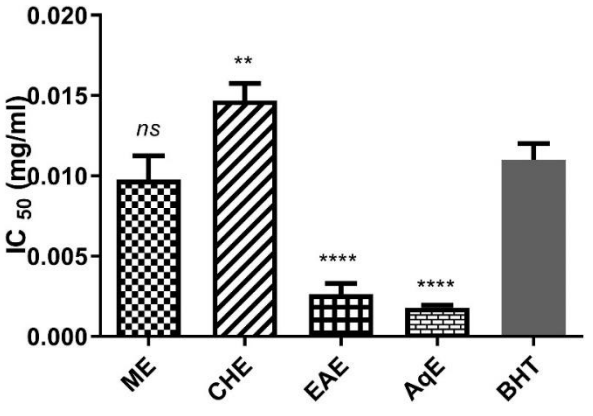

Figure 3. Hydrogen peroxide scavenging activity of M. communis berries extracts (MBE).
ME: M methanol extract, CHE: chloroform extract, EAE: ethyl acetate extract; AqE: aqueous extract. Data were presented as IC_{50} means ± SD (n=3) (****P ≤ 0.0001; **P ≤ 0.01; ns: not significant) vs BHT as standard.

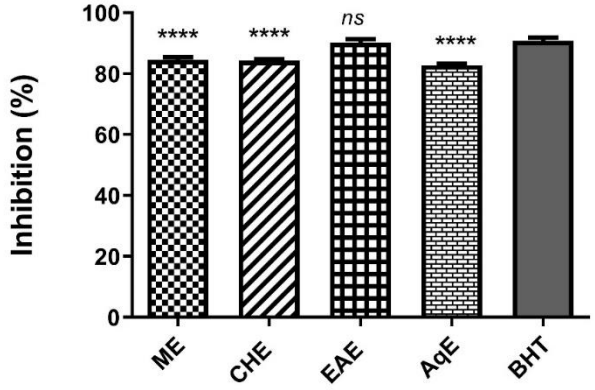

Figure 1. Antioxidant activity of M. communis berries extracts (MBE). (2 mg/mL at 96 h of incubation) measured by FTC method.
ME: M methanol extract, CHE: chloroform extract, EAE: ethyl acetate extract; AqE: aqueous extract. Data were presented as IC_{50} means±SD (n=3) (****P≤0.0001; ***P≤0.001; ns: not significant) vs BHT as standard.

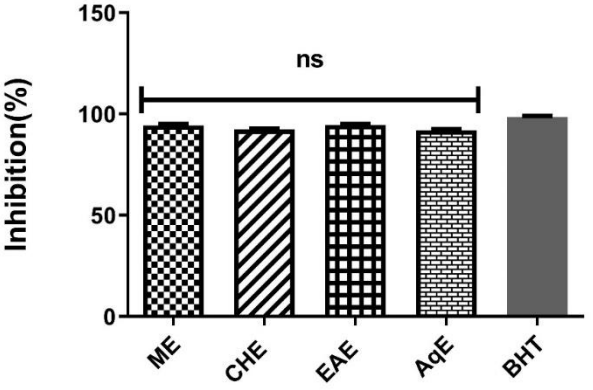

Figure 5. Antioxidant activity of M. communis berries extracts (MBE). (2 mg/mL at 96 h of incubation) measured by TBA assay.
ME: M methanol extract, CHE: chloroform extract, EAE: ethyl acetate extract; AqE: aqueous extract. Data were presented as IC_{50} means ± SD (n=3) (****P ≤ 0.0001) vs BHT as standard.

Antioxidant activity of M. communis berries extracts (MBE) determined by thiobarbutiric acid assay (TBA)

As shown in figure 5, all plant extracts strongly inhibited MDA formation in the following order: EAE > (94.61±0.4 %) > ME (94.28±0.76 %) > CHE (92.26±0.55%) > AqE (91.99±0.51%). The percentage of inhibition exhibited by EAE and CHE was comparable to BHT as positive control (98.47±0.55).

Discussion

The antioxidant properties of polyphenols are due to their redox properties, which allow them to act as reducing agents, hydrogen donators, metal chelators and single oxygen quenchers. Polyphenolics exhibit a wide range of biological effects including antibacterial, anti-inflammatory, antiallergic, hepato-protective antithrombotic, antiviral, anticarcinogenic and vasodilatory actions; many of these biological functions have been attributed to their free radical scavenging and antioxidant activity (Piluzza and Bullitta, 2011).

The ABTS free radical is formed by oxidation of ABTS with potassium persulfate which is reduced by hydrogen donating capacity of *Myrtus* extracts. The hydrogen-donating ability of different extracts of M. *communis* was evaluated by measuring the decrease in the absorbance spectrum in the blue-green ABTS radical reaction.

Extracts examined in this study (Figure 1) efficiently scavenged ABTS radicals generated by the reaction between 2,2'-azinobis (3-ethylbenzothiazolin-6-sulphonic acid) (ABTS) and ammonium persulfate, and showed a very good ABTS scavenging activity ranging from 2.5 to 25 µg/mL in the order of EAE>ME>CHE>AqE. EAE extract presented stronger antioxidant activity (IC_{50}=2.5 µg/mL). This value is close to that of the ethyl acetate fraction (Trolox), which means that use of synthetic antioxidants such as Trolox could be avoided by replacing them with the natural ones. These results were in

accordance with those of Aidi Wanness et al. (2016) who reported that myrtle seed, flower and leaf extracts showed stronger scavenging ability and that they were rich in hydrolysable tannins.

Metal ion chelating activity of an antioxidant molecule prevents oxyradical generation and the consequent oxidative damage. Metal ion chelating capacity plays a significant role in antioxidant mechanisms, since it reduces the concentration of the catalysing transition metal in lipid peroxidation (Jomova and Valk, 2011). In this assay, both extracts and EDTA interfered with the formation of ferrous and ferrozine complex, suggesting that it has chelating activity and captures ferrous ion before ferrozine.

As in the case of ABTS radical scavenging, AqE and ME extracts exhibited the highest metal chelating capacity. (IC_{50}= 0.73 and 0.82 mg/mL, respectively). This activity could be related to the richness of these extracts in tannins as proved in our previous study (Benchikh et al., 2018). EAE and CHE extracts showed weak chelating activity (IC_{50}=1.14 and 1.43 mg/mL, respectively). Although flavonoids contents in EAE and ME extracts are higher than that in AqE and CHE extract, they showed lower chelating activity. This could be explained that flavonoids are not good metal chelators. None of the extracts appeared to be better chelators of iron (II) ions than the positive control EDTA in this assay system (IC_{50}= 0.02 mg/mL).

Hydrogen peroxide is a weak oxidizing agent which inactivates enzymes by oxidation of the essential thiol (SH-) groups. It rapidly transverses cell membranes and once inside the cell interior, interacts with Fe_{2+} and Cu_{2+} to form hydroxyl radicals, which is harmful to the cell. It is therefore biologically advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate (Peng et al., 2011).

All extracts were capable of scavenging hydrogen peroxide in a concentration-dependent manner. AqE exhibited the highest scavenging capacity (2.6 µg/mL). This observed H_2O_2 scavenging activity may be attributed to the presence of phenolic components which can easily donate electrons to hydroxyl radicals

Lipid peroxidation is the mechanism by which lipids are attacked by ROS to form a carbon radical that reacts with oxygen, resulting in a peroxyl radical and thus generating lipid peroxides (Audin et al., 2014). Lipid peroxidation leads to oxidative degradation of unsaturated fatty acids and leads to the alteration of the structural integrity of membranes and their permeability. However, the conversion of Thiobarbituric Acid Reactive Substances (TBARS) equivalent of MDA is widely used to assess the importance of lipid peroxidation (Benabdallah et al., 2014). Flavonoids, phenolic acids and tannins inhibit mechanisms of enzymatic and non-enzymatic initiation of lipid peroxidation (Morton et al., 2000).

Two tests were used to study the effects of myrtle berries extracts on lipid peroxidation. The ferric thiocyanate method (FTC) measures the ability of antioxidants to scavenge peroxyl radicals produced during the initial stages of oxidation, which react with polyunsaturated fatty acids, through hydrogen donation (Huang et al., 2005).

In this study, after 48h of testing, the percentage inhibition of peroxidation in linoleic acid system by all MBE extracts was high. EAE showed the strongest activity (90.17±1.21 %). This value is similar to BHT as drug reference (90.82±1.05%). These results are in agreement with those of Kumar et al. *(*2011) who demonstrated that myrtle extracts are effective inhibitors of lipid peroxidation. Phenolic compounds and other chemical components present in the extract may suppress lipid peroxidation through different chemical mechanisms, including free radical quenching, electron transfer, radical addition or radical recombination (Galvez et al., 1995).

Conflict of Interest Statement

We declare that we have no conflict of interest.

Acknowledgements

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Conclusion

Extracts of *M. communis* berries contain high levels of total phenolic compounds, and they were able to scavenge free radicals and thereby terminate the radical chain reactions. This study indicates that MBE contains relevant antioxidant compounds responsible, at least in part, for its antioxidant and radicals scavenging activity. Further work is required to isolate and characterize the bioactive compounds that are responsible for this antioxidant activity.

References

Aidi Wannes W, Mhamdi B, Sriti J, Ben Jemia M, Ouchikh O, Hamdaoui G, Kchouk ME, Marzouk B. 2010. Antioxidant activities of the essential oils and methanol extracts from myrtle (*Myrtus communis* var. italica L.) leaf, stem and flower. Food and Chemical Toxicology; *48*: 1362-1370. doi: 10.1016/j.fct.2010.03.002.

Akgül H, Mohammed FS, 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.

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.

Baharvand-Ahmadi B, Bahmani M, Naghdi N, Saki K, Baharvand-Ahmadi S, Rafieian-Kopaei M. 2015. Review on phytochemistry, therapeutic and harmacological effects of myrtus (*Myrtus communis*). Der Pharmacia Lettre ; 7 (11) : 160-165.

Barboni T, Venturini N, Paolini J, Desjobert JM, Chiaramonti N, Costa J. 2010. Characterisation of volatiles and polyphenols for quality assessment of alcoholic beverages prepared from Corsican *Myrtus communis* berries. Food Chemistry; 122: 1304-1312. https://doi.org/10.1016/j.foodchem.2010.03.087

Benabdallah H, Gharzouli K, Khennouf, Amira S, Soufane S. 2014. Phytochemical analysis and anti-lipid peroxidation activity of *Tamarix africana* L. extracts. Global Journal of Research on Medicinal Plants & Indigenous Medicine; Koppa; 3: 278-285.

Benchikh F, Benabdallah H, Dahamna S, Khennouf S, Flamini G, Amira S. 2016a. Antimotility and Andidiarrhoel Activity of *Myrtus communis* L. Leaves Essential Oil in Mice. International Journal of Pharmacognosy and Phytochemical Research; 8(7): 1238-1244.

Benchikh F, Amira S, Benabdallah H, Bouaziz A et al. 2016b. Evaluation of the antidiarrhoel and antimotility activities of methanolic extract and fractions of *Myrtus communis* L. leaves in mice. Der Pharma Chemica; 8(14): 174-182.

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, 1-14. DOI: 10.9734/ARRB/2018/39217

Decker E A and Welch B. 1990. Role of ferritin as lipid oxidation catalyst in muscle food.Journal of Agricultural and Food Chemistry ; 36 : 674-677.Doi.org/10.1021/jf00093a019

Galvez J, De la Cruz JP, Zarzuelo A, Sanchez de la Casta F. 1995. Flavonoid inhibition of enzymic and nonenzymic lipid peroxidation in rat liver differs from its influence on the glutathione related enzymes. Pharmacology; 51 (2): 127133. DOI: 10.1159/000139325

Halliwell B, Aeschbach R, Lóliger J, Aruoma OI. 1995. The characterization of antioxidants. Food and Chemical Toxicology; 33: 601-17. https://doi.org/10.1016/0278-6915(95)00024-V

Hanaa A, Hassan HA, EL-Kholy WM, EL-Sawi MRF. 2020. Myrtle (*Myrtus communis*) leaf extract suppresses hepatotoxicity induced by monosodium glutamate and acrylamide through obstructing apoptosis, DNA fragmentation, and cell cycle arrest. Environmental Science and Pollution Research https://doi.org/10.1007/s11356-020-08780-7

Huang D, Ou B, Prior RL. 2005. The chemistry behind antioxidant capacity assays. *J Agric Food Chem*;53:1841-56.

Malekuti J, Mojgan J, fourvand M, Samadi K, Abbasalizadeh F, Khodaei L. 2019. Comparison of the effect of *Myrtus communis* herbal and anti-hemorrhoid ointments on the hemorrhoid symptoms and quality of life in postpartum women with grade I and II internal hemorrhoid: A triple-blinded randomized controlled clinical trial. Journal of Complementary and Integrative Medicine; 20180147. Doi: 10.1515/jcim-2018-0147

Jomova K and Valko M. 2011. Advances in metal-induced oxidative stress and human disease. Toxicology; 238: 65-87. DOI: 10.1016/j.tox.2011.03.001

Kına E, Uysal İ, Mohammed FS, Doğan M, Sevindik M. 2021. In-vitro antioxidant and oxidant properties of *Centaurea rigida*. Turkish Journal of Agriculture-Food Science and Technology, 9(10): 1905-1907. https://doi.org/10.24925/turjaf.v9i10.1905-1907.4603

Kikuzaki H and Nakatani N. 1993. Antioxidant effects of some ginger constituents. Journal of Food Science; 58(8):1407-1410. https://doi.org/10.1111/j.1365-2621.1993.tb06194.x

Korkmaz N, Dayangaç A, Sevindik M. 2021. Antioxidant, antimicrobial and antiproliferative activities of *Galium aparine*. Journal of Faculty of Pharmacy of Ankara University, 45(3): 554-564. https://doi.org/10.33483/jfpau.977776

Kumar MR, Phaneendra P, Bodhanapu S, Rahiman OMF, Niyas KM, Tamizmani T. 2011. Antioxidant and hepatoprotective activity of the aqueous extract of *Myrtus Communis* (Myrtle) Linn. Leaves. Pharmacologyonline; 1:1083-1090.

Mahboubi M. 2017. Effectiveness of *Myrtus communis* in the treatment of hemorrhoids. Journal of Integrative Medicine; 15(5): 351-358. https://doi.org/10.1016/S2095-4964(17)60340-6

Mohammed FS, Kına E, Sevindik M, Dogan M, Pehlivan M. 2021. *Datura stramonium* (Solanaceae): Antioxidant and Antimicrobial Potentials. Turkish Journal of Agriculture-Food Science and Technology, 9(4): 818-821. https://doi.org/10.24925/turjaf.v9i4.818-821.4264

Mohammed FS, Şabik AE, 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

Morton LW, Abu-Amsha Caccetta R, Puddey IB, Croft KD. 2000. Chemistry and biological effects of dietary phenolic compounds: Relevance to cardiovascular disease. Clinical and experimental pharmacology & physiology.; 27: 152-159. Doi: 10.1046/j.1440-1681.2000.03214.x.

Ozbeyli D, Sen A, Cilingir Kaya OT et al. 2019. *Myrtus communis* leaf extract protects against cerulein-induced acute pancreatitis in rats. Journal of Food Biochemistry. 2019;00:e13130. https://doi.org/10.1111/jfbc.13130

Ozcan O, Ipekci H, Alev B et al. 2019. Protective effect of Myrtle (*Myrtus communis*) on burn induced skin injury. Burns; https://doi.org/ 10.1016/j.burns.2019.07.015

Peng XZ, Chen SS, Ning FL, Li ML. 2011. Evaluation of in vitro antioxidant and antitumour activities of *Astragalus membranaceus* aqueous extract. Journal of Medicinal Plants Research; 5(29): 6564-6570. DOI: 10.5897/JMPR11.1102

Pereira P, Cebola MJ, Conceic M, Oliveira O, Bernardo Gil MG. 2017. Antioxidant capacity and identification of bioactive compounds of *Myrtus communis* L. extract obtained by ultrasound-assisted extraction. Journal of Food Science and Technology; 54(13): 4362-4369. DOI 10.1007/s13197-017-2907-y

Piluzza G and Bullitta S. 2011. Correlations between phenolic content and antioxidant properties in twenty-four plant species of traditional ethnoveterinary use in the Mediterranean area. Pharmaceutical Biology; 49(3): 240–247 DOI: 10.3109/13880209.2010.501083

Re R. 1999. Antioxidant activity applying an improved ABTS radical cation decolourization assay. *Free Radical Bio Med*; 26:1231-1237.

Ruch RJ, Cheng SJ, Klaunig JE. 1989. Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis; 10:1003-1008. DOI: 10.1093/carcin/10.6.1003

Sevindik M, Akgul H, Pehlivan M, Selamoglu Z. 2017. Determination of therapeutic potential of *Mentha longifolia* ssp. *longifolia*. Fresen Environ Bull, 26(7): 4757-4763.

Shang HM, Zhou HZ, Yang JY et al. 2018. *In vitro* and *in vivo* antioxidant activities of inulin. *PLOS ONE* | https://doi.org/10.1371/journal.pone.0192273.

Soomro S, Mesaik MA, Shaheen F, Khan N, Halim SA, Ul-Haq Z, Ali Siddiqui R, Choudhary MI. 2019. Inhibitory effects of Myrtucommuacetalone 1 (MCA-1) from *Myrtus communis* on inflammatory response in mouse macrophages. molecules; 25: 13, doi:10.3390/molecules25010013.

Uddin N, Afrin R, Uddin J, Uddin J, Alam AHMK, Abdur Rahman A and Sadik G. 2015. *Vanda roxburghii* chloroform extract as a potential source of polyphenols with antioxidant and cholinesterase inhibitory activities: identification of a strong phenolic antioxidant. BMC Complementary and Alternative Medicine; 15:195 DOI 10.1186/s12906-015-0728-y.

Uysal İ, Mohammed FS, Şabik AE, Kına E, Sevindik M. 2021. Antioxidant and Oxidant status of medicinal plant *Echium italicum* collected from different regions. Turkish Journal of Agriculture-Food Science and Technology, 9(10): 1902-1904. https://doi.org/10.24925/turjaf.v9i10.1902-1904.4588

Yen GC, Chang YC, Su SW. 2003. Antioxidant activity and active compounds of rice koji fermented with *Aspergillus condidus.* Food Chemistry; 83:49-54.