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Determination of Antioxidant Properties of *Mentha longifolia, Pistacia khinjuk* and *Eucalyptus globulus*

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ABSTRACT

There is an increasing attention to using medicinal plants due to their beneficial effect on human health. The antioxidant properties of medicinal plants have prompted investigators to use them in the food and pharmaceutical industries. Hence, in this study we sought to evaluate antioxidant properties of *Mentha longifolia*, *Pistacia khinjuk* and *Eucalyptus globulus*. Aerial parts of these plants were dried and ground. Then, plant samples were prepared using homogenizing plant powders in methanol solution. Finally, the total antioxidant capacity of the plants was assessed by ferric iron reducing antioxidant power (FRAP) assay. The results showed that the total antioxidant capacity was found as 2.21, 0.78 and 7 mmol Fe^{2+} L^{-1} for *P. khinjuk* and *E. globulus*, respectively. Based on our findings, these plants showed a potent antioxidant activity. It is recommended that utilization of *M. longifolia, P. khinjuk* and *E. globulus* in food and pharmaceutical industries could possibly possess beneficial health effects.

Keywords: Antioxidant activity, Medicinal plants, *Mentha longifolia, Pistacia khinjuk, Eucalyptus globulus.*
Article type: Research Article.

INTRODUCTION

Human beings have relied on medicinal plants for as long as they have existed to treat various diseases (Delfani *et al*. 2017; Jamshidi-Kia *et al*. 2018; Altememy *et al*. 2022; AL- Ethawi *et al*. 2022; Eftakhari *et al*. 2022; khademian amiri *et al*. 2022; Haraira *et al*. 2022; Ibrahim *et al*. 2022). The World Health Organization (WHO) has estimated that medicinal plants play a vital role in fulfilment of the primary healthcare needs of 80% of the population in developing countries. Statistics have shown that medicinal plants participate in the production of at least 25% of pharmacological drugs (Sevindik *et al*. 2017). The long history of treatment with medicinal plants can be related to the presence of organic compounds with vast and diverse pharmacological properties (Ghamari *et al*. 2017; Beyranvand *et al*. 2019). Medicinal plants are valuable sources of secondary metabolites, since these compounds widely used in medicine, pharmaceutical and food industries (Bagheri *et al*. 2019; Mahadeva Rao *et al*. 2022). Alkaloids, tannins, flavonoids, terpenoids, saponins and phenolic compounds are the most well-known and widely used secondary metabolites (Kumar *et al*. 2022). Investigators have recently taken a positive approach to these compounds due to their therapeutic properties and low side effects (Mohammadrezaei Khorramabadi *et*

al. 2020). Many of these compounds have exerted antioxidant properties via scavenging reactive oxygen species (ROS; Sarabi *et al*. 2019; Hormozi *et al*. 2020). The increase of ROS in living organisms causes oxidative stress and consequently several health problems such as cancer, diabetes, heart diseases and neurological disorders (Nori-Garavand *et al*. 2020). So far, a variety of medicinal plants have been identified as sources of antioxidants that can act as protectors against oxidative stress (Ahmadvand *et al*. 2014). One of the most valuable medicinal plants with antioxidant activity is *Mentha longifolia* belonging to Lamiaceae (Labiatae) family and the common name of wild mint or horse mint. The habitat of *M. longifolia* is different geographical areas including temperate regions of Central and Southern Europe, and Southwestern Asia such as Iran (Farzaei *et al*. 2017). The wild mint has been used traditionally for the treatment of infectious and chronic diseases (Altememy *et al*. 2022; AL- Ethawi *et al*. 2022; Eftakhari *et al*. 2022; Khademian amiri *et al*. 2022; Haraira *et al*. 2022). The presence of flavonoids, monoterpenes and phenolic compounds play a role in the healing properties of *M. longifolia* (Bahadori *et al.* 2018; Shahsavari *et al*. 2022). *P. khinjuk* is another interesting plant belonging to family of Anacardiaceae which generally grows in the Mediterranean and Middle East countries. Different adjuncts of *P. khinjuk* are used as traditional remedy for treatment of various health problems including indigestion, nausea, vomiting and toothache. It has been shown that *P. khinjuk* could propose as antioxidant, antitumor, antiasthmatic and antimicrobial agent (Mahmoudvand *et al*. 2018; Manouchehri *et al*. 2022). Phellandrene and α-Pinene are the most important components of *P. khinjuk* which exhibit antioxidant property (Pirbalouti *et al*. 2021). *E. globulus* is a tree from myrtle family (Myrtaceae). Due to the presence of natural compounds including 1, 8-cineole, α-pinene, p-cymene, cryptone and spathulenol, *E. glabrous* has been proposed as an antioxidant, anti-inflammatory and anti-microbial agent (Shala *et al.* 2021). Hence, in this study we sought to evaluate antioxidant properties of *M. longifolia*, *P. khinjuk* and *E. globulus*.

MATERIALS AND METHODS

Collection of Plant Materials

The aerial parts of *Mentha longifolia*, *Pistacia khinjuk* and *Eucalyptus globulus* were collected from Ilam and Dehloran Counties, Ilam Province, Southwest of Iran, in March 2022. The plants were identified according to the morphological features of the Ilam Province Plant Flora at the Biotechnology and Medicinal Plants Research Centre, Ilam University of Medical Sciences, Ilam, Iran. Collected plants were air dried in the shade and then were ground and used for antioxidant evaluation. The characteristics of the abovementioned medicinal plants are shown in Table 1.

Table 1. The characteristics of the used medicinal plant.

Scientific name	Persian name	Family	Collection area	Geographic coordinate
Mentha longifolia	Pouneh kouhi	Lamiaceae	Dehloran County	32° 41' 28" North, 47° 15' 58" East
Pistacia khinjuk	Pesteh kouhi	Anacardiaceae	Ilam County	32° 41' 28" North, 47° 15' 58" East
Eucalyptus globulus	Okaliptous	Myrtaceae	Dehloran County	32° 41' 28" North, 47° 15' 58" East

Plant sample preparation

After drying the plants, 1 g of their dry powder was homogenized using 100 mL methanol solution and was shaken in the same solution for 6 h. The resulting solution was then poured into a plastic falcon and centrifuged at 6000 rpm for 10 min. The resulting solution was used as a sample.

Determination of Antioxidant Activity

The total antioxidant capacity of the plants was assessed by ferric iron reducing antioxidant power (FRAP) assay.

Stock Solution Preparation

2.2 mL R2b solution was added to the parent bottle R2a and vortexed until complete dissolution and R2 solution was obtained. Then, the R2 solution was mixed in a ratio of 1: 1 and after vortex, 5 times its volume was added to R1 solution. The resulting solution was the stock solution of an antioxidant kit.

Standard Solution Preparation

Standard solution at 0, 0.2, 0.4, 0.6, 0.8 and 1 was also prepared. The linear equation obtained from the different concentrations of the standard solution is illustrated in Fig. 1.

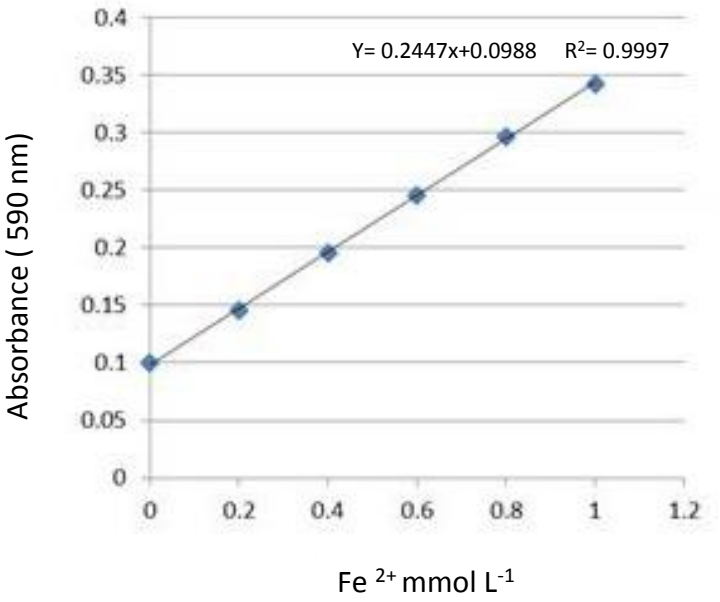

Fig. 1. Linear equation obtained from different concentrations of standard solution.

Procedure

At first, 5 µL of the prepared plant solution was added to each well and then 250 µL of the prepared working solution was added to each well containing the plant solution. The microplate was then incubated at 35-50 °C for 30 min and finally read at 570 nm with the ELISA reader (Parit *et al*. 2018).

RESULTS

The FRAP assay is a direct method for measurement of antioxidant activity. The principle of this test is based on the reduction of ferric ion Fe^{3+} to ferrous Fe^{2+}. As shown in Table 2, the results revealed that the total antioxidant capacities were 2.21, 0.78 and 7 mmol Fe^{2+} L^{-1} for *M. longifolia*, *P. khinjuk* and *E. globulus*, respectively.

Table 2. Total antioxidant capacity of *Mentha longifolia*, *Pistacia khinjuk* and *Eucalyptus globulus*.

Plant	Total Antioxidant Capacity
Mentha longifolia	2.21 mmol Fe^{2+} L^{-1}
Pistacia khinjuk	0.78 mmol Fe^{2+} L^{-1}
Eucalyptus globulus	7 mmol Fe^{2+} L^{-1}

DISCUSSION

Nowadays, investigators are very interested in studying medicinal plants and extracting natural antioxidants from them to be used instead of synthetic antioxidants (Pourjabali *et al*. 2017). Natural antioxidants are healthier and have more benefits, as well as less harmful side effects (Ahmadvand *et al*. 2017). Medicinal plants are proposed as rich sources of natural antioxidants. Phenolic compounds are one of the most important secondary metabolites of medicinal plants. These compounds exhibit high antioxidant power and are effective in eliminating and preventing the formation of free radicals in various ways. These compounds eliminate free radicals and also cause the deposition of oxidant elements such as iron (Tungmunnithum *et al*. 2018). Thus, the present study was designed to evaluate total antioxidant capacity of *Mentha longifolia*, *Pistacia khinjuk* and *Eucalyptus globulus* using FRAP assay. The results of our study exhibited that the total antioxidant capacity of *M. longifolia* was obtained as 2.21 Fe^{2+} L^{-1}. The total antioxidant activity of a plant is directly related to the type and amount of antioxidant compounds such as carotenoids, phenol and ascorbic acid (Gorinstein *et al*. 2004). *M. longifolia* is a medicinal plant which exert a potent antioxidant activity. It is known as a rich sources of antioxidant compounds such as oxygenated monoterpenes, Pulegone, isomenthone, 1, 8-cineole, borneol, piperitenone oxide and thymol. The study of HO Elansary and the colleagues revealed that *M. longifolia* could exert significant antioxidant

activity. They understood that *M. longifolia* presented a higher antioxidant activities than another species, *M. piperita*. Similar to our study, they obtained the total antioxidant capacity of *M. longifolia* by FRAP assay equal to 12 mM TEAC/g Extract (Elansary *et al*. 2020). The difference in the values obtained from the antioxidant activity of *M. longifolia* in the study of HO Elansary and our study could be due to the difference in the preparation of plant samples and modifications in the method of measuring the antioxidant activity of *M. longifolia*. Bahadori *et al.* (2018) reported that *M. longifolia* exhibits a strong antioxidant effect. They found the value of FRAP assay as 346.20 and 239.87 mg TEs/g sample for the infusion and ethanol extract of *M. longifolia*, respectively. Despite the confirmation of high antioxidant activity in the above two studies, the different values of FRAP assay in these two studies could be due to differences in the steps of infusion preparation and water use in the abovementioned study and methanol in the present study. Asghari *et al.* (2018) reported reducing power of *M. longifolia* essential oil by obtaining the value of FRAP assay as 102 mmol TEs/g oil. The present study revealed that the total antioxidant capacity of *P. khinjuk* was 0.78 Fe^{2+}/L. It is a valuable plant and rich in bioactive compounds including α-Pinene, Myrcene, Limonene, β-Caryophyllene and α-Humulene which causes high antioxidant activity. Hatamnia *et al*. (2016) demonstrated the high total phenolic and flavonoid contents of *P. khinjuk*. They concluded that its high antioxidant activity could be related to its phenolic and flavonoid contents. They achieved the value of FRAP assay as 1 mg AEAC/g extract (Hatamnia *et al*. 2016). According to the another part of the present study, *E. globulus* exhibited a high antioxidant activity. Its antioxidant capacity was 7 Fe^{2+} L^{-1} displaying a great antioxidant activity. *E. globulus* is a plant with known antioxidant properties attributing to its phytochemical composition which is rich of bioactive compounds particularly polyphenols such as 1, 8-cineole and α-Pinene (Almas *et al*. 2021). Va´zquez *et al*. (2008) reported that the aqueous extract of *E. globulus* has a high value of antioxidant capacity equal to 912 nmol AAE/mg extract which could scavenge free radicals. In another similar study, Gonzalez-Burgos *et al*. (2018) established the high antioxidant activity of the *E. globulus* leaves in association with the phenolic and flavonoid compounds present in this plant. They obtained the value of the FRAP assay as 9.79 TE, mmol g^{-1} DW for its ethanol extracts (González-Burgos *et al*. 2018). The above studies in consistent with our study revealed the high antioxidant activity of *M. longifolia, P. khinjuk* and *E. globulus*.

CONCLUSION

The present study supported the opinion that the three chosen medicinal plants including *Mentha longifolia, Pistacia khinjuk* and *Eucalyptus globulus* could exert a promising antioxidant capacity. It was also revealed that the abovementioned medicinal plants are potent for application in the pharmaceutical industry. Based on our findings, it is recommended that the utilization of *M. longifolia, P. khinjuk* and *E. globulus* could possibly leads to the beneficial health effects.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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