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Determination and Evaluation of Total Antioxidant Capacity of methanolic extracts of *Quercus brantii*, *Thymbra spicata*, *Citrullus colocynthis*

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Abstract

Background: Flock medicine has become a very important part of people's lives, however, it is not known by countries' rules in some cases. The usage of flock medicine has considerably increased in developed regions, as well, because of the effectiveness problems and the diverse side effects of artificial drugs. This research evaluated the antioxidant activities of *Quercus brantii*, *Thymbra spicata*, and *Citrullus colocynthis* native to Ilam province that nurtures within the west of Iran.

Methods: The total antioxidant capacity of essential oils was measured via ELISA Reader at 570 nm.

Results: The total antioxidant capacities of oak, thyme, and watermelon were 4.1, 2.35, and 0.46 (mmol Fe²⁺/L), respectively.

Conclusion: The investigated traditional Iranian edible herbs are rich sources of natural antioxidant compounds. As a result, *T. spicata* and *Q. brantii* could be used in pharmaceutical and food industries as natural antioxidants and food products.

Introduction

Flock medicine has become an important part of human beings' life, however, it isn't always recognized by countries' rules in several cases. The majority of people particularly in less developed countries normally use herbal remedies. In past years, the using flock medicine has notably increased in developed nations, as well, due to the fact of effectiveness problems and the diverse side effects of artificial drugs [1,2]. Nowadays, the use of medicinal plants has developed as a commercial industry without a profound understating of their composition. Therefore, understanding the composition of plants from distinct areas could be very fundamental. Herbs are getting used as rich sources of medicine for ailment prevention and protection of human health. Iran from a long term in the past has an extensive medical tradition and traditional knowledge of plant remedies [3]. Different herbs with antimicrobial and antioxidant properties have been studied worldwide as rich sources of new bioactive substances with the capability to promote food preservation [4-8]. These natural agents are commonly derived from plant parts such as flowers, herbs, buds, leaves, fruits, skins, and seeds [9, 10]. Plant extracts and essential oils are commonly used in oral antiseptic solutions, cosmetics, hair and body care products, toothpaste, household cleaning products, air fresheners, and the food industry [11]. The antioxidant and antibacterial properties of herbs have attracted the attention of studies. A plant has shown to be a rich source of antioxidant components and composed a lot of secondary metabolites such as phenolic compounds, flavonoids and flavonoids, glycosides, and alkaloids [12-16].

Various factors including environmental pollutants, toxins, radiations, pathological incidents, aging, and other reasons are unavoidable incidents that cause oxidative stress [17]. Therefore, maintaining a balanced state of oxidant and antioxidant lead to oxidative stress prevention and also is an essential part of maintaining good health [18]. The only way to remove free radicals that cause oxidative stress is with the aid of antioxidants. Antioxidants are each exogenous or endogenous, can be effective in preventing the formation of free radicals via scavenging them and also, promoting their decomposition [19-23]. Recently, there's a great eagerness for herbal antioxidants of natural resources [24-26]. This reduces the reduction potential of the nutrient medium that restricts the growth of several microorganisms [5, 27, 28]. According to epidemiologic and in vitro research on healthful plants, it has been confirmed that antioxidant compounds of the plants are capable of exerting protecting effects against oxidative stress in biological systems [29]. Plants contain numerous phytochemicals that are important exogenous antioxidants [30], and

phenolic and flavonoid components are reputable antioxidants that are derived from plants [31, 32]. Typical phenols with high antioxidant activity can play a remarkable role in the adsorption and neutralization of free radicals [33] and are recognized to be mainly phenolic acids and flavonoids. These phytochemicals have been known as scavengers for various reactive oxygen species like hydroxyl and peroxy radicals. These compounds have strong biological activities [34] as antioxidant, antibacterial, anticancer, antiviral, and anti-inflammatory [35]. Flavonoids are secondary metabolites, including approximately 4,500, well-known compounds [36]. Flavonoids are valuable components because of their several beneficial and therapeutic applications. These beneficial properties of flavonoids on health have been proved by their antioxidant [37], antiviral, antitumor, antiallergic, and anti-inflammatory activity [38].

Thymbra spicata (generally known as Mediterranean thyme) is an aromatic plant endemic belonging to the Lamiaceae family and with wide industrial applications. *Spicata* L is well-known as "zahter, karakekik, karabas thyme" names, and is widely utilized as a flavoring agent in many foods and also an antimicrobial agent in different industries such as liquor and perfumery line productions [39, 40]. Carvacrol, γ -terpinene, pcimen, and thymol are phenolic compounds derived from *Spicata* that have antimicrobial activity against a range of microorganisms [40, 41]. Therefore, it is commonly used in traditional medicine and the cure of several diseases [41, 42]. In traditional Lebanese medicine, *T. spicata* is often utilized to cure arteriosclerosis, blood toxicity, cough, hypoglycemia, ophthalmia, insomnia, toothache, and sterility which showed cardiotoxic and oxytocic effects [43, 44]. Cholesterol reduction, antioxidant activity, and liver-protective properties were informed by Akkol et al [45]. According to in vitro testing, essential oils, and their chemical constituents, for instance, carvacrol and thymol, have been informed to show antimicrobial and antioxidant activities [46, 47].

Quercus brantii (QB) well-known as Oak of the Fagaceae family, is largely grown in the Zagros mountains of western and southwestern Iran [48, 49]. QB extract is a good source of polyphenols such as ellagic acid, gallic, and tannin, which show antioxidant and anti-inflammatory activity [50]. According to the results of the previous surveys, the hydroalcoholic extract of QB has antinociceptive, anti-inflammatory, and homeostatic stabilizing properties. The *Q. brantii* gall can have antioxidative and anti-inflammatory activity on the pathological and biochemical factors of colitis [51].

Citrullus colocynthis plant belongs to the watermelon family, although it differs from the common watermelon in that it is smaller and firmer in the body. The

Cucurbitaceae (the gourd family) is mostly frost-sensitive and drought-tolerant [52]. This family contains about 965 species of plants; the well-known members of this family is consisting of the cucumber, watermelon, squash, gourd, and the bitter apple (*Citrullus colocynthis*). *C. colocynthis* (L.) Schrad is known as a valuable cucurbit plant that commonly grew in the Arabian Peninsula, Africa, India, and tropical districts around the world [53]. Its skin is a good source of natural antioxidants, including a high level of lycopene, citrulline, cucurbitacin, and different polyphenols. It also has a spicy and astringent taste and shows a soothing effect. The plant demonstrates several medicinal properties such as antimicrobial, anti-inflammatory, anticancer, and antidiabetic activities [54, 55]. Polyphenols are natural components that show antioxidant and free radical scavenging properties. The antioxidant and free radical scavenging properties of *C. colocynthis* extracts are related to phytochemicals, like polyphenols. Some investigations have measured the polyphenol amount and antioxidant activity in different parts of the fruit of *C. colocynthis* [56].

Methods

Plant preparation:

In April 2022, medicinal plants of oregano, and pistachio are prepared from Dehloran and Ilam cities in Ilam province located in western Iran. The plant was identified and approved using the morphological keys of the book of plant flora of Ilam province in the BMPCRC of IUMS, Iran. Collected plants are cleaned and dried in the open air in the shade of the sun. The dried plant was pulverized by a plant mixer and used for antioxidant testing. The characteristics of the mentioned medicinal plant used in this study are specified in Table 1.

Antioxidant method:

The antioxidant activity of essential oil Nowadays, for eliminating or reducing chemical and synthetic compounds in food, much research has been carried out to replace chemicals with naturals. In this regard, several efforts have been done to find natural antioxidants from the source of plants.

Preparation of plant samples

After drying the plant, homogenize 1 gram of dry powder of the desired plant using 100 mL of methanol solution and let it shake in the same solution for 6 hours. The resulting solution was then poured into a plastic falcon and centrifuged at 6000 rpm over 10 minutes. The centrifuged solution was used as a sample.

Work solution preparation

2.2 mL of 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 vortexing, 5 times its volume was added to R1 solution. The resulting solution is the stock solution of an antioxidant kit.

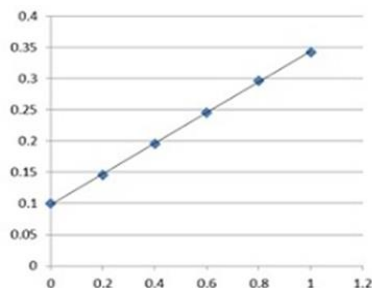


Figure 1: Linear equation obtained from different concentrations of standard solution.

Preparation of standard solution

Standard solution with concentrations of 0, 0.2, 0.4, 0.6, 0.8 and 1 was also prepared (Figure1). First, 5µl of the prepared plant solution was added to each well, and then 250 µL of the working solution was added to each well containing the plant solution. The microplate was then incubated for 30 minutes at 35 to 50 ° C and finally read at 570 nm with the Eliza Reader.

Results

As shown in Table-1, the results revealed that the total antioxidant capacity was found as 4.1, 2.35 and 0.46 mmol Fe²⁺/L for *Quercus brantii*, *Thymbra spicata* and *Citrullus colocynthis*, respectively (Table 2).

Name	Scientific name	Family	collection area	Geographical coordinates
Balout (Jaft balout)	<i>Quercus brantii</i>	Fagaceae	Dehloran	32° 41' 28" North, 47° 15' 58" East
Avishan zoufaei	<i>Tymbra spicata</i>	Lamiaceae	Ilam	32° 41' 28" North, 47° 15' 58" East
Hendavaneh abojahl	<i>Citrullus colocynthis</i>	Cucurbitaceae	Dehloran	32° 41' 28" North, 47° 15' 58" East

Table 1: Medicinal plant details of the present study

Samples	Total antioxidant capacity
Oak (<i>Quercus brantii</i>)	mmol Fe ²⁺ /L 4.1
Thyme (<i>Tymbra spicata</i>)	mmol Fe ²⁺ /L 2.35
Watermelon (<i>Citrullus colocynthis</i>)	mmol Fe ²⁺ /L 0.46

Table 2: Total antioxidant capacity of different plant extracts

Discussion

Many chronic and non-chronic, infectious and non-infectious diseases occur in humans. Free radicals are the cause of many diseases. Oxidative damage to vital molecules ultimately leads to chronic diseases such as heart disease, cancer, diabetes, Alzheimer's, Parkinson's, arthritis and infertility [57-69].

The antioxidant activity of *Q. brantii*L was determined at about 4.1 mmol Fe²⁺/L (the ferric reducing antioxidant power), which was higher than *T. spicata* and *C. colocynthis*, respectively. Alebrahim-Dehkordy et al (2019) [70] displayed that the total phenolic content

of *Q. brantii* L. fruit extract was 3.010 mg GAE/g DW and flavonol and flavonoid levels were 1.813 and 0.654 mg/g, respectively. Flavonoids are considered well-known phenolic compounds with high antioxidant capacity. Also, antioxidant activity was evaluated at 7.43 % via 2,2-diphenyl-1-picrylhydrazyl (DPPH). Antioxidants can deactivate free radicals and reduce the rate of cell damage or cell death. The high potency of *Q. brantii* may be because of high phenolic acids (gallic and ellagic) and flavonoids (catechin, quercetin, naringin) components. Gallic, ellagic acid, and quercetin are the most predominant antioxidant compounds in *Q. brantii* reducing macromolecules damage (lipids, proteins) and modestly increase in the total antioxidant capacity of plasma in humans [71]. In addition, antioxidant effects of *Q. brantii* extract in a dose-dependent manner were proved by Alizade Naini et al, 2021 [72]. In detail, treating rats with *Q. brantii* resulted in growth in superoxide dismutase (SOD) activity.

Sengun et al, 2021 studied antioxidant capacity and the total phenolic content of the extract and essential oil of *Thymbra spicata* L. from Turkey [73]. The highest number of components of the plant extract included carvacrol with 88.75% and acetic acid with 11.25%, while the major components of the essential oil consisted of carvacrol with 56.03%, trans caryophyllene with 10.41%, p-cymene with 9.61% and γ -terpinene with 6.87%, respectively. The quantified total phenolic content of the extract and essential oil was 1350 and 3440 μ g GAE g⁻¹, respectively. The antioxidant activities were measured via DPPH and ABTS⁺ radical scavenger activities in samples. The author showed that the antioxidant activity of the extract and essential oil by the percentage of 50.51–98.28% is extremely associated with the number of phenolic compounds. These outcomes are consistent with results of our study. Therefore, *T. spicata* L. could be utilized in the food industries and pharmaceutical applications as a natural source of antioxidants.

In the study conducted by Kizil et al., the antioxidant activity of thyme species has been studied and it has been reported that the inhibition percentage of the DPPH radical by the essential oil of *T. spicata* L. was about 92.34% [74]. Also, Iriti et al., reported that essential oils inhibited DPPH radical scavenging activity by 90.9% [75]. According to Erturk et al, the total polyphenolic content of the *T. spicata* L. extracted by methanol was measured at around 9.63 mg GAE g⁻¹ in Amasya [76]. But, in the investigations performed by Gumus et al. and Ozcan et al., total phenol levels were reported in extracts of *T. spicata* L. between 55.58–75.19 and 74.52–163.10 mg GAE g⁻¹, respectively [77, 78].

A study informed that the total phenol content of *Thymbra spicata* (zahter) obtained by methanol was 13.14 mg GAE/g for dry weight and the total flavonoid

content was 4.36 mg QUE/g DW [79]. Additionally, the free radical scavenging capacity of thyme essential oil was detected at around 35.28 ± 0.45 μ g/mL. The results of the iron-reducing antioxidant power (FRAP) of methanol extract was about 14.99 μ M Fe²⁺/g. Hydrodistillation of *Thymbra spicata* (HD: 1.642 μ M Fe²⁺/g) essential oils had considerably higher FRAP index than microwave-assisted extraction (MAE: 1.641 μ M Fe²⁺/g). However, the *Thymbra spicata* essential oils shown in Table 2 have a different FRAP value than the *Thymbra spicata* essential oil in this study. Ballester-Costa et al. (2017) informed Trolox-equivalent FRAP amounts of various *Thymus* essential oils [80]. The type of species is important to factor in reporting different results. They reported that *T. capitatus* showed the maximum FRAP level, and after that *T. zygis*, *T. mastichina*, and *T. Vulgaris* have high FRAP values, respectively. The test conditions such as the type of buffer used also affect the final result [81].

In the present study total, the antioxidant capacity of *C. colocynthis* was assessed by the Eliza technique. The literature does not provide FRAP (Ferric Reduction Antioxidant Power) values for *C. colocynthis* essential oil, making our findings the first to the best of our knowledge. The FRAP value was measured for *C. colocynthis* extract (mmol Fe²⁺/L 0.46). Several investigations have measured the antioxidant activity and phenolic content in whole parts of *C. colocynthis* fruits. Total phenolic content (TPC) and antioxidant activity of various parts of *C. colocynthis* including pulps, rinds, and seeds were measured. The outcomes indicated that the TPC and the DPPH free radical scavenging power differ between accessions, seasons, and the parts of the fruit. The phenolic content and antioxidant activity in different parts of the fruit depend on both the environment and genetics. The highest phenolic content was assessed in rinds; however, the maximum antioxidant activities were reported in the seeds in summer [82].

Hussain et al, 2013 [56] quantified phenolic acids and flavonoid contents from the roots, leaves, and fruits of *Citrullus colocynthis* by the reversed-phase high-performance liquid chromatography method [56]. Analysis of extracts of *C. colocynthis* revealed that vanillic, ferulic, gallic, p-coumeric, p-hydroxybenzoic, and chlorogenic acids are present as the main phenolic acids and quercetin, catechin, and myricetin being the major flavonoids constituents. Total phenolic was about (3.07–18.6 mg GAE/ g DW) and total flavonoid (0.51–13.9 mg GAE/ g DW). Quercetin and ferulic acid were reported as the major compounds of *Citrullus colocynthis* extracts, and also among all extracts, ethanol extracts of roots and leave contained a high total phenolic and total flavonoid amount [56]. In addition, the levels of total phenols and flavonoids were

determined using gas chromatography-mass spectrometry (GC-MS). The highest level of total phenolic was identified in extracts from ethyl acetate (205 mg GAE/g) and methanol solvents (85 mg GAE/g), respectively. The antioxidant activities were assessed by DPPH and lipid peroxidation approaches. The results indicated that *C. colocynthis* extracts can be suggested as a natural source of antioxidants for human health [83].

Screening of total polyphenol and flavonoid contents of *C. colocynthis* seed extracts, with crude aqueous, defatted aqueous, hydroethanolic, ethyl acetate, and n-butanol extracts was studied by Benariba et al, 2013 [84]. As result, extracts by crude aqueous, hydroethanolic, and ethyl acetate were rich in Catechic tannins and flavonoids, whilst terpenoids in large quantities were observed in the crude aqueous and n-butanol extracts. Polyphenols, expressed as gallic acid equivalent were per 100 g of plant material, to 329, 1002, and 150 mg in ethyl acetate, hydroethanolic and crude aqueous extracts, respectively. Flavonoids, expressed as catechin equivalent, amounted to 620, 241, and 94 mg per 100 g of plant material in ethyl acetate, hydroethanolic and crude aqueous extracts, respectively. Quercetin, myricetin, and gallic acid were determined in the ethyl acetate and hydroethanolic extracts using thin layer chromatography, The antioxidative effect of these extracts was accomplished, when experiment was completed at a concentration of 2 000 µg/mL in a 1,1-diphenyl-2-picrylhydrazyl test, a percent reducer of 88.8% for ethyl acetate, 74.5% for hydroethanolic and 66.2% for crude aqueous extracts, and IC50 values of 350, 580 and 500 µg/mL as compared to 1.1 µg/mL for ascorbic acid [84]. If free radicals increase for any reason, the antioxidant properties decrease, so plant antioxidants increase the antioxidant capacity [85-90].

In conclusion, the present study is one of the surveys that determined the antioxidant properties of Iranian medicinal plants such as oak, thyme, and watermelon. The results showed that all samples exhibited antioxidant activity which can reveal that the samples were rich in total antioxidant capacity. In general, those extracts with a higher number of active components possessed more potent antioxidant activity. The species investigated could be valuable sources of natural antioxidants and appear to have applicability in both health medicine and the food industry. However, in vivo safety and identification of active ingredients must be thoroughly studied before use.

Competing Interest

All listed authors declare no conflict of interests in any capacity including competing and financial.

Author Contributions

Indrajit Patra, Fadhil Hussam, Huda Sabah Jabr: Data analysis

Nathera Hussin Alwan, Ashour H. Dawood: Monitoring of research

Nathera Hussin Alwan, Ashour H. Dawood, Samira Shokri, Mohammad Darvishi: Drafting

Dania Fouad Abdelamir, Doaa A. Hamad, Samira Shokri, Mohammad Darvishi: Manuscript scanning

Dania Fouad Abdelamir, Doaa A. Hamad, Samira Shokri, Mohammad Darvishi : Manuscript scanning

Samira Shokri, Mohammad Darvishi: Data evaluation

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