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In vitro evaluation of the amount of flavonoids, flavanols, and total phenolic compounds in 10 medicinal plants used in Iran; focus on diabetes and kidney diseases

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Abstract

Introduction: Free radicals and reactive oxygen species (ROS) as oxidants are important concerns in medicine due to their adversative effects and risk of carcinogenicity. Antioxidants can neutralize free radicals and ROS thus possesses protective effect on the body. Plants are rich sources of natural antioxidants and phenolic compounds are among the most important antioxidants in the plants; therefore, many studies have investigated their protective effects.

Objectives: This study was conducted to evaluate and compare the number of flavonoids, flavonols, and total phenolic compounds in 10 commonly used medicinal plants in Iran.

Materials and Methods: Extraction was performed using 70% ethanol solvent. The extracts were concentrated by rotary evaporator at 40°C. Folin-Ciocalteu method was used to measure the amount of phenol. The amount of flavonol was measured using 2% aluminum chloride and 5% sodium acetate. In addition, to determine the flavonoid compounds, 2% aluminum chloride and 5% potassium acetate was used.

Results: The outcomes of this study disclosed that the studied plants had reasonably high quantities of flavonoid and phenolic compounds. The uppermost amount of phenolic compounds was detected in the myrtle plant (62.7 mg/g), and the lowermost amounts were detected in fig leaves and lemon balm. Moreover, the highest amount of flavonoid compounds was detected in ginger, myrobalan, and myrtle; the measured amounts of flavonoid compounds in the mentioned plants were 42.49, 32.15, and 34.38 mg/g, respectively. The highest amount of phenolic compound was detected in hydroalcoholic extract of barberry.

Conclusion: Hydroalcoholic extract of ginger, barberry, fig leaves, rhubarb, myrobalan, walnut diaphragm, pomegranate peel, lemon balm, cardamom, and myrtle had great quantities of phenolic, flavonol, and flavonoid compounds. As a result, these herbal plants can be considered and used as an important source of natural antioxidants.

Keywords: Flavonoid, Flavonol, Total phenol, Medicinal plants

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Introduction

Free radicals and reactive oxygen species (ROS) can lead to the development of diseases such as diabetes, neurological disorders, rheumatologic disease, heart disease, Parkinson's disease, and Alzheimer's disease (1,2). Therefore, the administration of herbs with high antioxidant compounds can protect the body from oxidative damage. Researches have exposed that natural antioxidants increase the level of antioxidants and reduce the incidence of some diseases like diabetes (3,4), heart diseases (5), and stroke (6). Phenols are the largest secondary metabolites of plants that generated in response to the environmental stresses. Phenols with their hydroxyl groups can act as an electron or hydrogen donor and are able to neutralize free radicals. Among different types of the compounds, flavonoids are one of the most important polyphenolic compounds that are commonly found in fruits, vegetables, leaves, seeds, roots, and other parts of the plant (7). Flavonoids, as the most phenolic compounds, have high antioxidant activities and exerted protective effects on biological systems (8). These compounds have anti-inflammatory, and anti-tumor effects and prevent the accumulation of platelets (9). So far, more than 4000 flavonoid compounds have been detected in herbal sources. These compounds mainly exist as natural colors that cause color variations in the autumn and are the origin of yellow, red, and orange colors in flowers and foods. Often, flavonoid compounds are formed by two hexacarbonyl aromatic rings (A and B) and three biosynthesis pathways of shikimic acid, malonic acid, and acetate (10). Nowadays, the use of medicinal plants for the treatment and prevention of diseases are increasing. The most important factors

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Implication for health policy/practice/research/ medical education

Hydroalcoholic extract of ginger, barberry, fig leaves, rhubarb, myrobalan, walnut diaphragm, pomegranate peel, lemon balm, cardamom, and myrtle had great quantities of phenolic, flavonol, and flavonoid compounds. As a result, these herbal plants can be considered and used as an important source of natural antioxidants.

promoting the administration of herbal medicines are low-side effects (11). In recent years, several studies have been conducted to measure the antioxidant, flavonoid, and phenolic compounds of plants. Findings suggested the effectiveness of these compounds in inhibiting of free radicals (12-14). Ginger (*Zingiber officinale* Roscoe, family Zingiberaceae) is a plant with a rhizome that grows to a height of 90 cm; its underground swollen stem used as a medicinal plant. Ginger extensively been applied for the treatment of various diseases from many decades ago (15-17). The phytochemical compounds of this plant are included essential oils, phenolic compounds, carbohydrates, proteins, alkaloids, glycosides, steroids, and saponins, which plays an important role in the therapeutic properties of Ginger (18-20). Barberry (*Berberis vulgaris*, family Berberidaceae) is one of the plants that its roots, skin, stems, leaves, flowers, and fruits have various nutritional, pharmaceutical, and industrial uses (21). Barberry is a shrub with a height of 1 to 3 cm, covered with sharp thorns, that has duck-like leaves. Alkaloids which are presents in this plant are including an isoquinoline core such as berberine, oxycontin, berberine, and pulmotin (22, 23). Fig (*Ficus carica*, family Moraceae) an as Mediterranean plant that today is grown in most areas the world. It can be found in most of areas of Iran such as the northern forests, Azerbaijan, Isfahan, Fars, Khuzestan, and Khorasan (24,25). Fig leaves are used as a diuretic and a painkiller and for the removal of intestinal worms (26). Rhubarb (*Rheum officinale*, family Polygonaceae) is a durable plant with thick, red, and green leaves. Rhubarb stems have a laxative effect, and its root extract is used to treat diabetes, stomach pain, and liver disorders (27). Yellow myrobalan (*Terminalia chebula*, family Combretaceae) exists indigenously in India and Asia. It is traditionally used to treat various diseases. This plant contains tannin and phenolic compounds counting gallic acid, ellagic acid, flavonoids, and triterpene (28). Walnut (*Juglans regia*, family Juglandaceae) is a plant with 21 species which are deciduous and have an edible fruit. The walnut core is considered as a warm and dry medicine that used to produce blood, treat pulmonary diseases, and prevents the formation of kidney stones and gallstones (29). Pomegranate (*Punica granatum*, family Punicaceae) has a shrub with a height of 1.5 to 5 m, with more or less irregular branches that have thorns and shiny and crusty leaves (30). This plant contains various types of phenolic and flavonoid compounds, including cinnamic acid, luteolin, and naringenin (31). Lemon balm (*Melissa officinalis,* family Lamiaceae) is known as one the most commonly used herbs. Several studies have been proven the antioxidant, anti-inflammatory, and sedative properties of lemon. Lemon balm has several phenolic compounds such as rosmarinic acid, tannin, and flavonoids such as apigenin, and luteolin (32,33). Cardamom (*Elettaria cardamomum*, family Zingiberaceae) is recognized as the queen of spices. The main compounds of essential oils of cardamom are included limonene, cineole, and sabinene. Cardamom is commonly used to relieve indigestion, cough, digestive disorders, and oral infections (34(. Myrtle (*Myrtus communis*, family Myrtaceae) has 145 types and more than 5500 species. Myrtle is often grown in warm and tropical regions of Iran; it is rarely grown in areas with moderate temperate. Myrtle extract contains a range of biological compounds such as tannin, flavonoid, coumarin, vitamin C, and antioxidants (35-37).

Nowadays, herbal therapies are considered by peoples throughout the world thus it seems necessary to study medicinal plants and their effective ingredients more and more.

Objectives

The aim of this study was to measure and compare the amount of flavonoid, flavonol, and phenolic compounds in ten medicinal plants including ginger, barberry, fig leaves, rhubarb, yellow myrobalan, walnut diaphragm, pomegranate peel, lemon balm, cardamom, and myrtle.

Patients and Methods

Study design

Different parts of the plants were purchased from an authorized medicinal plant store and their herbarium samples were registered after identification and approval by an expert in Medical Plant Research Center of Shahrekord University of Medical Sciences. Extraction was performed using a 70% ethanol solvent via maceration method. The extracts were concentrated by a rotary evaporator at 40°C. Total phenolic compounds were measured based on the technique approved by Sharafati-Chaleshtori et al (29). Then, 0.1 mL of diluted extract (0.01 g in 10 mL of 60% methanol) was added to 0.5 mL of 10% Folin-Ciocalteu solution. After 3-4 minutes, 0.4 mL of 7.5% sodium carbonate was added to the solution. In the next step, after 30 minutes of incubation period at the room temperature, sample adsorption in proportion to distilled water was measured at 765 nm. At the same time, different dilutions of gallic acid were prepared, and the above- mentioned steps were performed and the standard curve was set. The amount of total phenol was calculated based on the amount of gallic acid per mg (29). To measure the amount of flavonoid compounds, 0.5 mL of each extract (0.01 in 10 mL of 60% methanol) was assorted with 0.5 mL of 2% aluminum chloride and then 3 mL of 5% potassium acetate was added to the solution. Afterward 40 minutes, samples adsorptions in proportion to distilled water were

measured at 415 nm. The amount of flavonoid in each extract was intended in mg/g of dry extract. In this study, to measure the amount of flavonol compounds, 0.5 mL of each extract (0.01 g per 10 mL of 60% methanol) were assorted with 0.5 mL of 2% aluminum chloride and then 3 mL of 5% sodium acetate was added to the solution. After 2.5 hours, samples adsorptions in proportion to distilled water were measured at a wavelength of 440 nm. The amount of flavonol in each extract was calculated in mg/g of dry extract.

Statistical analysis

Each extract measured triplicate and average for each compound has been reported as milligram of compound per gram of extract.

Results

In this study, the amount of flavonoid, flavonol, and total phenolic compounds in 10 medicinal plants were assessed. The results gotten for each plant are presented in Table 1. According to the results, it was found that the studied plants had relatively high amounts of flavonoid and phenolic compounds. The highest amount of phenolic compounds was observed in the myrtle plant (62.7 mg/g), and the lowest amounts were observed in fig leaves and lemon balm. Furthermore, the uppermost amount of flavonoid compounds was detected in ginger, myrobalan, and myrtle; the measured amounts of flavonoid compounds in the mentioned plants were 42.49, 32.15, and 34.38 mg/g, respectively. The highest amount of phenolic compound was observed in hydroalcoholic extract of barberry.

Discussion

Phenolic and flavonoid compounds are amongst the best sources of natural antioxidants which play an important role in neutralizing free radicals. Flavonoids present in plants have a nutritional role in human diets. Many nutritionists recommend the use of herbs, fruits, and vegetables to maintain health and prevent some diseases (11). Numerous studies have been completed to investigate the amount of phenolic compounds in various species of barberry as well as in different parts of the barberry plant. Based on the results, total phenolic compounds in different species range from 657-2611 mg GA/100 g DM; in addition, the amount of total phenolic compounds in different parts of the barberry plant including root, branch, and skin are 10.34, 12.53, and 52.54 mg GAg^{-1}, respectively (38,39). The stated results are in line with our findings regarding the amount of phenolic compounds in the barberry plant. The difference between results of our study with other researches is related to the environmental and genetic factors, extraction method, and type of the solvent. Motalleb et al conducted a study to evaluate the amount of phenolic compounds and total antioxidant capacity of the barberry fruit extract. Based on their findings, the maximum rate of free radical inhibition was perceived in barberry aqueous extract. In addition, 80% methanolic extract of barberry had the highest amount of phenolic compound in compared to the aqueous extract. The results of this study confirmed the antioxidant capacity of barberry fruit (40). Gundogdu et al showed that the extract of barberry had high antioxidant capacity; the high antioxidant capacity of the plant was attributed to the presence of phenolic compounds (41). According to the study by Negi et al, the amount of total phenol in pomegranate peel (Ganesh species) in methanolic extract was 462 mg/g (42). Moreover, according to the study by Yasoubi et al, the amount of total phenol in pomegranate peel was 400 mg/g (43). As reported in a study by Zhang et al, the amount of total phenol in pomegranate peel in the aqueous, methanolic, acetonic, and ethyl acetate extracts was 431.9, 415.6, 537.1, and 327.3 mg/g, respectively. According to the researches, the difference in the amount of total phenol in different extracts are due to the differences in hydrolyzing tannins and punicalagin (44). Previously Tehranifar et al showed that the difference between various types of pomegranate in terms of the amount of total phenolic content is due to the difference in the biosynthesis of secondary metabolites of various cultivars (45). In several studies, it has been proposed that there is a straight association between antioxidant activity and the amount of phenolic and flavonoid compounds. Therefore, the plants with higher amounts of phenolic compounds, especially flavonoid and

Table 1. The amount of flavonoid, flavonol, and total phenolic compounds in 10 medicinal plants

Plant name	Flavonol (mg/g)	Flavonoid (mg/g)	Total phenol (mg gallic acid)
Ginger	29.6	42.49	116.44
Barberry	4.57	4.43	894.0
Fig leaves	21.67	16.7	103.4
Rhubarb	7.9	1.5	229.1
Myrobalan	15.36	32.15	326.82
Walnut diaphragm	16.8	11.23	326.86
Pomegranate peel	18.95	15.95	153
Lemon balm	19.8	10.29	77.02
Cardamom	15.36	18.28	19.27
Myrtle	62.7	34.38	11.36

flavonol, have higher antioxidant capacity (46). It can be concluded that the plants investigated in our study have a significant antioxidant compounds because of high amounts of phenolic compounds. Due to the antioxidant and anti-free radical properties of phenolic and flavonoid compounds in plants, it is suggested to use these plants as a source of natural antioxidants. Moreover, Jamshidi et al investigated the methanolic extracts of some indigenous plants of Mazandaran in aspect of flavonoid and phenol compounds and found a positive relationship between antioxidant activity and polyphenolic acid compounds; as they definite, high amounts of phenolic compounds are the major cause of antioxidant activity in some herbal extracts (47). Therefore, based on the results of this study that showed high amounts of phenolic compounds in ginger, barberry, fig leaves, rhubarb, myrobalan, walnut diaphragm, pomegranate peel, lemon balm, cardamom, and myrtle, it is proposed that these herbs can be used as natural antioxidants.

Conclusion

In conclusion, it can be concluded that hydroalcoholic extract of ginger, barberry, fig leaves, rhubarb, myrobalan, walnut diaphragm, pomegranate peel, lemon balm, cardamom, and myrtle have high amounts of phenolic, flavonol, and flavonoid compounds. As a result, these medicinal plants can be used as an important source of herbal antioxidants.

Limitations of the study

Perform a comparison among extracts is a possible limitation of this study.

Authors' contribution

Conceptualization: MRM.
Methodology: MRM and MR.
Validation: MRM.
Formal analysis: EB and RE.
Investigation: MR and MRM.
Resources: MB, MRM.
Data curation: MRM, EB and RE.
Writing—original draft preparation: MB, RE, EB, MRM and MR.
Writing—review and editing: MB, RE, EB, MRM and MR.
Visualization; MR.
Supervision: MR.
Project administration: MR.

Conflicts of interest

The authors declare that they have no competing interests.

Ethical issues

The Ethics Committee of the Shahrekord University of Medical Sciences approved the protocols of this study (IR.SKUMS.REC.1394.233). This study was extracted from Ph.D thesis of Mohammad Rahimi-Madiseh entitled" Evaluation of the phytochemical Effects of several pharmaceutical plants to formulate and develop an effective herbal drug for treatment of diabetes". Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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