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Research Article

Antioxidant Activity and Phenolic Content of the Fractions from Some Iranian Antidiabetic Plants

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Abstract

Teucrium polium, *Rheum turkestanicum*, *Portulaca oleracea*, *Morus alba*, *Cinnamomum zeylanicum* and *Trigonella Foenum-graecum* are antidiabetic plants in Iranian traditional and folk medicine. Some researches demonstrated direct correlation between diabetes mellitus and oxidative stress. The aim of this research is to investigate antioxidant potency of different fractions of the mentioned plants and determination of their total phenolic contents (TPC). Antioxidant activities of the fractions were determined by inhibition of ABTS radical cation. In addition, determination of their total phenolic contents (TPC) was carried out using Folin method. According to the results, methanolic fraction of *C. zeylanicum* (84.8%), ethyl acetate and methanolic fraction of *R. turkestanicum* (90.0, 82.4%, respectively) and methanolic fraction of *T. polium* (70%) exhibited the most potent antioxidant activities, at concentration of 250 μg/mL. While, trolox, as a standard antioxidant showed antioxidant potency with 92.8%. On the other hand, ethyl acetate and methanolic fractions of *C. zeylanicum* (0.829, 1.092 $mg_{\text{gallic acid}}/g_{\text{fraction}}$, respectively), ethyl acetate and methanolic fractions of *R. turkestanicum* (0.612, 0.785 $mg_{\text{gallic acid}}/g_{\text{fraction}}$, respectively) and methanolic fraction of *T. polium* (0.625 $mg_{\text{gallic acid}}/g_{\text{fraction}}$) have the most contents of phenolic compounds. Therefore, these plants also reduce oxidative stress while reducing blood glucose levels. According to the results of the Pearson correlation analysis, there was a direct relation between the antioxidant activity of the fractions and their phenolic contents ($r = 0.711$, $p < 0.001$). Therefore, the antioxidant activity of plants can be attributed to their phenolic compounds.

Keywords: Antioxidant; ABTS; Fraction; Folin; Diabetes

Abbreviations

ABTS: 2,2'-Azinobis-(3-Ethylbenzothiazoline-6-Sulfonic Acid); Hex: n-Hexane; DCM: Dichloromethane: Chl: Chloroform; EA: Ethyl Acetate; Met: Methanol.

Introduction

Free radicals are atoms or molecules that contain unconjugated and active electrons, and hence are capable of rapidly reacting through chain reactions and converting other molecules into free radicals (oxidation). Free radicals play a major role as the source of diseases such as arthritis, cancer, arteriosclerosis and diabetes. The relationship between free radicals and disease can be explained by the concept of oxidative stress. Whenever the body is being exposed to a physical, chemical and environmental impairment such as air pollution, cigarette, ultraviolet, radiation, and toxic chemicals the oxidative stress occurs. The body use antioxidants to increases free radicals [1].

Antioxidants are widely used as food additives to provide protection against oxidative degradation of foods by free radicals. Currently, synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and tertiary butylhydroquinone (TBHQ) are used in various pharmaceutical, food and cosmetic industries. However, in spite of their high efficiency and stability as well as their relative low price, in recent years there are several works in order to replace them with natural antioxidants. Natural antioxidants are found in all parts of the plant. These compounds include carotenoids, vitamins, phenols, flavonoids, and other metabolites [2].

In recent decades, there has been great interest in screening essential oils and various plant extracts for natural antioxidants because of their good antioxidant properties.

Some recent studies have shown that high levels of blood glucose may lead to self-oxidation of glucose and eventually increasement of oxidative stress [3]. On the other hand, oxidative stress also increases side effects in diabetic patients [4]. Therefore, antioxidants can reduce oxidative stress in diabetic patients and reduce their adverse effects on various organs of the body [5].

In Iranian traditional medicine, many plants have been cited as antidiabetic agents. *Portulaca oleracea, Morus alba, Cinnamomum zeylanicum* and *Trigonella Foenum-graecum* are the most common diabetes plants in Iranian traditional medicine [6-10]. In addition, people in the Northern Khorasan region (Iran) use *Teucrium polium* and *Rheum turkestanicum* as antidiabetic herbs [6,11,12].

The purpose of the present study is to investigate the antioxidant effect of some extracts of these plants using the ABTS method and also to measure the amount of their phenolic contents (TPCs). Finally, the relationship between the antioxidant effect of the components and the amount of phenolic contents will be investigated. The results of this study may lead to the introduction of herbs that are not only having anti-diabetic effects, but also having appropriate antioxidant effects. This helps to control of oxidative stress caused by high blood glucose concentrations in addition to controlling blood sugar in diabetic patients, and even reduces the risk of diabetes and its side effects by reducing oxidative stress.

The "bioassay guided fractionation" method is one of the ways to target compounds with specific bioactivities. Paul Erlich use this method partially in the basic concepts of his book (drugs do not apply unless they are limited). Based on this principle, bioactive molecules should be isolated using separation methods to achieve a fraction or a pure compound with specific activities [13]. In the present study, "sequential extraction" method was applied for separation of plant compounds based on their polarities to achieve more effective fractions.

Materials and Methods

Plant materials

Teucrium polium aerial parts, *Rheum turkestanicum* roots, *Portulaca oleracea* seeds, *Morus alba* leaves, *Cinnamomum zeylanicum* peels and *Trigonella Foenum-graecum* seeds were collected and identified by botanical experts. The plant materials were dried at room temperature and grounded to fine powder.

Sequential extraction

In this study, a "sequential extraction" method was used to obtain n-hexane (polarity index: 0.0), dichloromethane (polarity index: 1.3), chloroform (polarity index: 1.4), ethyl acetate (index Polarity: 4.4) and methanol (polarity index: 1.5) fractions of the plants. Based on this method, 30 g of dried plant powder were mixed with 100 ml Hex and stirred for 12 hours. Then, the resulting extract was filtered and concentrated by a rotary evaporator at reduced pressure. In the second step, 100 ml of dichloromethane was added to the remaining dried plant powder. In the third stage, 100 ml of chloroform solvent was added to the remaining plant from the previous stage and the chloroformed extract was obtained. The fourth step of extraction was accomplished by ethyl acetate solvent and finally, the fifth step was extracted by methanol solvent. Finally, five fractional extracts, Hex, DCM, Chl, EA and Met, were obtained from each of the six plants and completely dried by freeze dryer [9].

Antioxidant assay

The radical cationic solution, ABTS, was prepared by mixing 5 ml of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid), 7 mM, with 88 µl of potassium persulfate (140 mM) and keeping it in darkness at room temperature for 14 hours. The solution was diluted with ethanol until it reached 0.74 ± 0.02 absorption at 734 nm. By adding 150 µl of ABTS solution to 50 µl of each extraction (with a final concentration of 250 µg/mL), absorption change was recorded at 734 nm after 6 minutes. According to the following equation, the results have been reported in the percentage of inhibition of $ABTS^{\bullet +}$ in concentration of 250 µg/ml for each extract [14].

$$\text{ABTS inhibition\%} = \frac{[\text{control absorption-sample absorption}]}{\text{control absorption}} \times 100$$

Phenolic content assay

The Folin method was used to measure the total amount of phenolic compounds [15]. 2.5 µl of the samples with a concentration of 2 mg/ml were mixed with 195 µl distilled water and 12.5 µl of Folin-Ciocalteu identifier. After 3 minutes, 50 µl of sodium carbonate 7% was added to the solutions and they were stirred at room temperature for 2 hours. Then, their adsorption was read at 765 nm. The amount of adsorption was fitted to the calcification curve of gallic acid to obtain the equivalent of gallic acid. The test was repeated at least 3 times for each sample. In order to obtain the calibration curve of gallic acid, 500, 250, 125, 62.5, and 31.55 μg/ml of gallic acid in DMSO were prepared and their absorbance was measured at 765 nm. Total phenolic compounds were calculated and reported as equivalent weight of gallic acid per weight of dry extract.

Statistical analysis

The relationship between antioxidation activities and the amount of phenolic contents in various plant extracts were investigated by Pearson Correlation Test in IBM SPSS Statistics 25 software.

Results and Discussion

In the present study, "sequential extraction" method was used to obtain Hex, DCM, Chl, EA and Met fractional extracts of *T. polium* aerial parts, *P. oleracea* seeds, *T. Foenum-graecum* seeds, *M. alba* leaves, *R. turkestanicum* roots and *C. zeylanicum* peels. The extraction sequence is from non-polar fraction (Hex, polarity index: 0.0) to very polar reaction (Met, polarity index: 1.5). After each extraction step, a more polar solvent was added to the remaining plant from the previous step, in order to fractionate plants components according to their polarity.

The antioxidant activities of the obtained fractions from the plants were determined by ABTS analysis. Results were expressed in terms of the percentage of ABTS inhibition in the concentration of 250 μg/mL (Table 1).

According to the results, Met fraction of *C. zeylanicum* (with 84.8%), EA and Met fractions of *R. turkestanicum* (with 90.0 and 82.4%, respectively) and Met fraction of *T. polium* (70.0%) showed the most antioxidant activities against ABTS radical cation. On the other hand, trolox (an analogue of vitamin E), as a standard antioxidant, showed an inhibitory strength of 92.8%. By calculating the average percentages of inhibition of components of each plant, showed that *C. zeylanicum, R. turkestanicum* and *P. oleracea* (with average percentages of 55.9, 56.7 and 51.4%, respectively) had an antioxidant effect higher than other herbs.

Plant	part	Fraction	ABTS inhibition (%)	Phenolic content ($g_{gallic\ acid}/g_{fraction}$)
C. zeylanicum	peels	Hex	36.4 ± 1.2	0.498 ± 0.081
		DCM	40.5 ± 0.9	0.339 ± 0.005
		Chl	-	-
		EA	61.9 ± 2.9	0.829 ± 0.002
		Met	84.8 ± 4.3	1.092 ± 0.018
M. alba	leaves	Hex	49.1 ± 1.1	0.150 ± 0.004
		DCM	56.8 ± 1.2	0.082 ± 0.003
		Chl	40.1 ± 0.9	0.139 ± 0.009
		EA	49.4 ± 1.5	0.498 ± 0.010
		Met	51.0 ± 1.0	0.451 ± 0.009
P. oleracea	seeds	Hex	38.1 ± 0.9	0.091 ± 0.002
		DCM	51.4 ± 1.5	0.072 ± 0.002
		Chl	43.0 ± 1.2	0.093 ± 0.002
		EA	61.8 ± 2.5	0.212 ± 0.003
		Met	62.7 ±1.5	0.232 ± 0.005
R. turkestanicum	roots	Hex	27.5 ± 1.5	0.040 ± 0.002
		DCM	33.7 ± 1.1	0.082 ± 0.001
		Chl	50.0 ± 1.3	0.139 ± 0.004
		EA	90.0 ± 3.1	0.612 ± 0.006
		Met	82.4 ± 2.7	0.785 ± 0.007
T. polium	Aerial parts	Hex	57.7 ± 2.6	0.140 ± 0.005
		DCM	34.7 ± 1.3	0.199 ± 0.007
		Chl	27.6 ± 1.6	0.160 ± 0.005
		EA	58.4 ± 3.5	0.591 ± 0.010
		Met	70.0 ± 4.1	0.625 ± 0.011
T. foenum-graecum	seeds	Hex	39.4 ± 2.8	0.180 ± 0.005
		DCM	33.6 ± 1.9	0.110 ± 0.004
		Chl	41.6 ± 3.4	0.156 ± 0.006
		EA	-	-
		Met	46.1 ± 2.5	0.403 ± 0.008

Table 1: Antioxidant activities and phenolic contents of the fractions of the plants.

Note: The fractions were tested at concentrations of 250 μg/mL for ABTS inhibitory activity.

As shown in Table 1, the highest number of phenolic compounds is related to polar fractions, especially Met fractions, followed by EA fractions. EA and Met fractions of *C. zeylanicum*

(0.829 and 1.092 g $_{\text{gallic acid}}$/g $_{\text{fraction}}$, respectively), followed by Met and EA fractions of *R. turkestanicum* (0.612 and 0.785 g $_{\text{gallic acid}}$/g $_{\text{fraction}}$, respectively), Met fraction of *T. polium* (0.625 g $_{\text{gallic acid}}$/g $_{\text{fraction}}$), have the highest amounts of phenolic compounds. By calculating the average total phenolic compounds of each plant, it was found that *C. zeylanicum*, with an average of 0.689 g $_{\text{gallic acid}}$/g $_{\text{fraction}}$, has the highest number of phenolic compounds. After *C. zeylanicum*, *T. polium* and *R. turkestanicum* showed the highest amounts of phenolic contents (0.343 and 0.331 g $_{\text{gallic acid}}$/g $_{\text{fraction}}$, respectively).

By comparing of the results, it has demonstrated that by increasing the polarity of the fractions, the number of phenolic compounds was increased, as well as antioxidant activity. Also, by comparing the antioxidant activities and the amount of the phenolic contents in different fractions by Pearson correlation test, it has been demonstrated that there was a direct relationship between the antioxidant activity of the fractions and their phenolic contents ($r = 0.711$, $p < 0.001$). Therefore, it can be concluded that the antioxidant activities of the plants can be attributed to their amount of phenolic contents.

Conclusion

In this study, a sequential extraction method was performed to separate ingredients of plants according their polarities. The results led to introduce Met fraction of *C. zeylanicum,* EA and Met fractions of *R. turkestanicum* and Met fraction of T. polium as strong antioxidants agents with high amounts of phenolic compounds. *C. zeylanicum* is a common spice used by different cultures around the world for several centuries. The main active ingredient of the bark of this taxon is cinnamaldehyde [16]. *R. turkestanicum* grows widely in central Asia and also in north-east of Iran. Traditionally, people use roots of this plant as an antidiabetic and antihypertensive as well as anticancer agent [17]. *T. polium* is one of the wild-growing found abundantly in Iran. This plant is used to prepare herbal tea and as traditional medicine. The tea of *T. polium* is used as an appetizer especially in children and also as a spice [18]. Due to anti-diabetic effects of these plants in Iranian traditional medicine, the results of this study could be useful for nutrition researchers as well as pharmacists. Further work is needed to compare antioxidant activities of these plants extracts and their biomarkers with their in-vitro and in-vivo (animal and clinical) antidiabetic properties.

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