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STUDY OF SOME ANTIOXIDANT COMPOUNDS IN SOME CITRUS

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ABSTRACT

The present study was conducted to determine antioxidant activity, total phenolic and flavonoid content from four different varieties of citrus species peels, (*Citrus sinensis*, *Citrus reticulate*, *Citrus aurantium* and *Citrus aurantifolia*). The peels were separated from fruits, shade dried, powdered and extracted using two solvents methanol and methanol with water. The phenolic compounds was conducted by the use of high performance liquid chromatography (HPLC). The antioxidant activity was screened for their free radical scavenging properties using ascorbic acid as a standard antioxidant. Free radical scavenging activity was evaluated using 2-2diphenyl-2 – picrylhydrazyl free radical. The chemical analysis of the C*itrus* species using HPLC technology showed that the *Citrus* species contained the following phenolic and flavonoid compounds (Eriocitrin, Diosmin, Hespertin, Naringenin, Tangertin, Rutin) and it was found that the phenolic content in alcohol with water extract higher than alcohol extract, except for *Citrus reticulate*. In DPPH assay the results showed that the *Citrus* species had at the 1500 µg/ml highest antioxidant activity in alcohol extract attained by *Citrus sinensis*, *Citrus reticulate*, *Citrus aurantifolia* (100%). While *Citrus aurantium* variety showed the lowest level of antioxidant activity (98.63%). In alcohol with water extracts the highest antioxidant activity at the 1500 µg/ml was attained by *Citrus sinensis*, *Citrus reticulate*, *Citrus aurantifolia* (100%). While *Citrus aurantium* variety showed the lowest level of antioxidant activity (97.33%). Thus, it could be concluded that, citrus species contain bioactive substances such as phenolic and flavonoid compounds and seems to be a very good source of dietary antioxidants possessing high antioxidant activity.

KEYWORDS: Antioxidant activity; phenolic content; flavonoids content; citrus; peels.

INTRODUCTION

During the past years, reactive oxygen species (ROS) have been implicated in the oxidative deterioration of food products as well as in the pathogenesis of several human diseases such as atherosclerosis, chronic inflammation, neurodegenerative disorders and cancer.[1] Natural antioxidants become the target of a big number of research studies in finding the sources of potentially safe, effective and cheap antioxidants. Herbal drugs containing free radical scavengers are known for their therapeutic activity.[2] Plant derived antioxidants are now getting a special interest. Phenolic compounds present in a large number of vegetable food, such as fruits and nuts, have been reported to possess good antioxidant properties. Plant phenolic includes a wide range of compounds with antioxidant activity, that is phenolic acids, flavonoids, tannins and the less common stilbenes and lignans.[3] And the most important of which are flavonoids. Numerous studies have suggested that flavonoids show biological activities, including antiallergenic, antiviral, inhibition of lipid peroxidation and anti- inflammatory actions. However, most of the attention has been focused on the antioxidant activity of flavonoids because of their ability to reduce free radical formation and to scavenge free radicals.[4, 5, 6] Currently, increased attention in finding naturally occurring antioxidants for use in food or pharmaceutical applications, which can protect the human body from free radicals and retard the progress of many chronical diseases as well as retard lipid oxidative rancidity in food.[7] So plants containing high level of polyphenol have a great importance as natural antioxidants. Citrus (*Citrus* L. from Rutaceae) is one of the most important world fruit crops and is consumed mostly as fresh produce or juice because of its nutritional value and special flavor and is native to tropical and subtropical areas in southeast Asia.[8] Citrus peel, which represents roughly one half of the fruit mass, is a rich source of bioactive compounds including natural antioxidants such as phenolic acids and flavonoids.[9] Therefore, the present study was focused on four different varieties of local citrus Iraqi plants that are widespread in Iraq to determine the content of phenolic compounds and total antioxidant activity.

MATERIALS AND METHODS

Plant material and extraction

The plant materials were collected and washed under running water, cut into pieces, air dried and pulverized into fine powder in a grinding machine. 10 gm of dried powdered fruit peels were macerated with either by 100 ml of methanol (99%**)** in alcohol extract or 100 ml of (methanol / water 4/1: v/v) in alcohol with water extract at room

temperature for 24 hour under shaking condition. The extract was then filtered using Whatman filter paper No.1. Then the supernatant was concentrated under reduced pressure at 40°C for 3 hours using a rotary evaporator. The extracts were placed in dark bottles and stored in refrigerator at 4°C until use.

Chemical analysis of the plant extracts

Test for Glycosides

The test was performed by adding 2ml of Benedict's reagent to 1ml of plant extract in a test tube. The mixture was then shakes well and transferred into a water bath for 5min., and then left to cool. Presence of red sediment indicates a positive result.

Test for Phenolic compounds

Detection of phenols was performed by using ferric chloride solution. It was prepared by dissolving ferric chloride salt in distilled water 1%. Color change to green or blue was an indication of the presence of phenolic compounds in the plant extract.

Test for Flavonoids

The test was performed by adding 4 ml of ethyl alcohol (95%), to 1ml of plant extract in a test tube. The mixture was then shakes well and transferred into a water bath for 25-30 minutes, and then take it out of the a water bath, then add a few drops of potassium hydroxide solution 0.5 mm. Formation of dark color precipitate indicates the presence of flavonoids.

Separation and identification of phenolic compounds by HPLC

High performance liquid chromatography was applied for the separation and identification of phenolic and flavonoid compounds, in citrus species extracts. The HPLC system (Shimadzu LC-10 A, Japan) is equipped with dual pump LC-10AT binary system, the eluted peaks were monitored by UV-Vis 10A-SPD Spectrophotometer., C18 column (i.d. 4.6 mm × 100 mm). The mobile phase consisted of (A) 0.1% trifluoro acetic acid in water and (B) 0.1% acetonitrile using linear gradients from 0-100% B in 12 minutes. The amount of phenolic compounds was calculated by comparison of peak area (280 nm) of the individual phenolic compounds with that of standards. Known quantities of phenolic compound standards such as eriocitrin, diosmin, hespertin, naringenin, tangertin, rutin and quercetin used for the identification and quantification of phenolic compounds present in the extract of citrus species.

DPPH Radical Scavenging Activity (DPPH) Assay

Antioxidant assay was carried out using 2,2-diphenyl-1-picryl hydrazyl (DPPH) spectrophotometric method explained by[10] With some modifications. About 1ml of (0.02) DPPH solution in methanol was added to 2ml of sample solutions, and let stand for 30 min at room temperature before the absorbance was measured at 517 nm using a spectrophotometer. Ascorbic acid in methanol was used as standard and absolute methanol was used as blank. The difference in absorbance between the test sample and control (DPPH) expressed as percentage inhibition is taken as antioxidant activity was determined as follows.

Inhibition (%) = A blank – A sample / A blank × 100

Where, A is the absorbance. All determinations are performed in triplicate.

Statistical analysis

The Statistical analysis System- SAS[11] was used to effect of different actors in study parameters. The LSD test or T-test the comparative between means in this study.

RESULTS DESCUSSION

Preliminary Phytochemical assay

The results of phytochemical screening shown in Table: 1 which reveals phenolics, flavonoids and glycosides are present in methanolic and methanolic with water peel extract of citrus species.

Table 1: Phytochemical analysis of Citrus peel extracts.

Component	Alcohol extracts	Alcohol with water extracts
Phenol	+	+
Flavonoid	+	+
Glycoside	+	+

Key: + = present

The result in table (2) shows the comparison in phenolic compounds concentration between the four different varieties of *Citrus* species extraction by alcohol with water. *C. aurantium* variety was the major phenolic constituents among other varieties while *C. reticulate* variety was the minor.

Table (2) phenolic compounds of four different varieties of Citrus species extraction by alcohol with water (mg/100gm).

Flavonoids compounds	Citrus species				LSD value
	Citrus sinensis	*Citrus reticulate*	*Citrus aurantium*	*Citrus aurantifolia*	
Eriocitrin	2.17b	0.77c	0.58c	3.67a	1.04 *
Diosmin	15.73a	3.12b	1.78b	15.57a	3.95 *
Hespertin	12.62b	6.21b	18.97a	11.12b	5.68 *
Naringenin	3.48b	2.37b	7.27a	3.47b	2.09 *
Tangeritin	13.88a	3.22c	15.77a	8.78b	3.66 *
Rutin	5.39a	0.88b	7.42a	3.42a	2.37 *
Quercetin	4.62a	0.97b	6.62a	1.53b	2.61 *
* (P<0.05).					

The result in table (3) showed that comparison in phenolic compounds concentration between the four different varieties of Citrus species extraction by alcohol. *C. aurantium* variety was the major phenolic constituent among other varieties, while *C. aurantifolia* variety was the minor.

Table (3) Phenolic compounds of four varieties of Citrus species extraction by alcohol (mg/100gm).

Flavonoids compounds	Citrus species				LSD value
	Citrus sinensis	*Citrus reticulate*	*Citrus aurantium*	*Citrus aurantifolia*	
Eriocitrin	1.32b	0.41b	4.77a	0.77b	1.52 *
Diosmin	0.38c	6.30b	15.77a	4.47b	2.48 *
Hespertin	5.92b	10.07a	4.78b	1.92b	2.94 *
Naringenin	2.07b	4.31a	2.88b	0.48c	1.14 *
Tangertin	5.92a	5.58a	5.82a	0.66b	2.62 *
Rutin	0.52b	3.57b	6.92a	2.57b	2.19 *
Quercetin	0.08c	2.97a	3.32a	1.42b	0.972 *
* (P<0.05).					

DPPH radical scavenging assay

The results are presented in Table (4) showed the antioxidant activity of four different varieties of Citrus species and comparing it to control (Vitamin C) in both alcohol and alcohol with water extracts by using DPPH method. The highest antioxidant activity at the 1500 µg/ml in alcohol extract was attained by *Citrus sinensis*, *Citrus reticulate*, *Citrus aurantifolia* (100%). While *Citrus aurantium* variety showed the lowest level of antioxidant activity (98.63%). In alcohol with water extracts the highest antioxidant activity at the 1500 µg/ml was attained by *Citrus sinensis*, *Citrus reticulate*, *Citrus aurantifolia* (100%). While

Citrus aurantium variety showed the lowest level of antioxidant activity (97.33%). the methanol extract show higher antioxidant activity compared to methanol with water extract.

Table (4) DPPH. Scavenging activity of standard (ascorbic acid) and four different varieties of Citrus species.

Standard and Citrus species	Alcohol				LSD	Alcohol with water				LSD
	250 µg/ml	500 µg/ml	1000 µg/ml	1500 µg/ml		250 µg/ml	500 µg/ml	1000 µg/ml	1500 µg/ml	
Ascorbic acid (+ve)	87.13	94.40	98.26	100.00	7.43 *	--	--	--	--	--
Citrus sinensis	71.43	77.73	94.63	100.00	6.94 *	35.80	64.86	84.60	100.00	7.02 *
Citrus reticulate	64.06	79.00	97.13	100.00	7.02 *	47.40	36.96	)	0	9.35 *
Citrus aurantium	33.03	53.23	67.76	98.63	7.83 *	35.50	58.63	80.53	97.33	7.85 *
Citrus aurantifolia	47.13	75.67	88.40	100.00	8.14 *	47.43	64.96	83.90	100.00	7.92 *
LSD	0.465 *	0.406 *	0.409 *	0.093 *	---	0.348 *	0.401 *	0.338 *	0.393 *	---

*** (P<0.05).**

DESCUSSION

The results shows that the Citrus species had highly amount of total phenolic compound this is agreement with Ma *et al.*,(2008). Who studied the physical and chemical characteristics of Citrus peel and traced high amount of total phenolic compounds. In particular flavonoids, especially polymethoxy flavones and flavanones (hesperidin, narirutin and naringin) are identified in Citrus pulp and peel.[13] As well as the result obtained showed that alcohol with water extracts of Citrus contain higher total phenolic and flavonoid contents compared to alcohol extracts. Except for *Citrus reticulate* where alcohol extracts was contain higher total phenolic and flavonoid contents than alcohol with water, and that may be due as previously mentioned the nature of the separated compounds and to solvents polar used in the extraction.[14] Moreover, the polarity of the solvent is also one of interest in the processing and extraction of phenolic compound.[15]

The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples.[16] DPPH is a commercial oxidizing radical which can bereduced by antioxidants. In this assay, the violet color of DPPH was reduced to a pale yellow color due to the abstraction of hydrogen atom from antioxidant compound. The more antioxidants occurred in the extract, the more the DPPH reduction will occur. High reduction of DPPH is related to the high scavenging activity performed by

particular sample.[17] The high phenolic content of *Citrus species.* could be the main reason for its high antioxidant activity towards DPPH radicals. Jayaprakasha *et al*. (2008) have demonstrated the DPPH radical scavenging activities of citrus fractions is by their hydrogen donating ability.

CONCLUSION

The present study reported the antioxidant activity, total phenolic and flavonoid contents of four Citrus species (*Citrus sinensis*, *Citrus reticulate*, *Citrus aurantium* and *Citrus aurantifolia*). Eriocitrin, Diosmin, Hespertin, Naringenin, Tangertin, Rutin, Quercetin, phenolic compound that found in citrus species by using HPLC system. High phenolic content found in alcohol with water extracts for except *Citrus reticulate*. Therefore, Antioxidants were extracted from fruit peels of four different varieties of local Iraqi citrus species in this study could serves as a good of natural antioxidant. It can be concluded from the study that citrus peels due to its high antioxidant activity and phenolic content may prove to be a better substitute in place of synthetic antioxidants in extending the shelf life of food product by preventing the peroxide formation in the product containing fat . In addition natural antioxidants are safe and impart health benefit to the consumer.

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