

Phenolic Contents and Antioxidant Activity of Jojoba (*Simmondsia chinensis* (Link). Schindler

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Received: 16 February 2017 - Revised: 11 April 2017 - Accepted: 26 April 2017

Abstract: The ethanol and methanol extracts of Jojoba (*Simmondsia chinensis* (Link). Schnider) leaves and seeds were screened for antioxidant activity. The antioxidative potential of ethanol and methanol extracts of Jojoba (*Simmondsia chinensis*) were investigated for the first time using DPPH 2,2-diphenyl-1-picrylhydrazyl, total phenolic contents, antioxidant activity. Total phenolic substance amounts were calculated according to Folin-Ciocalteu method, substance concentrations in mg/GAE g, equivalent to gallic acid based on mg/ml gallic acid equivalent (GAE). The phenolic substance amounts in the leaves extracts (jojoba leaves: 313 mg/g GAE). Free radical clearance activities of the extracts were determined by using DPPH free radical. The Phenolic substances were calculated in highest jojoba leaves and lowest jojoba seeds. When DPPH radical clearance activity results were analyzed, it was seen that the highest activity was exhibited by jojoba leaf extract (% 43.20, 0.5 mg/ml concentration). The antioxidant activities of extracts were calculated in (nmol/g) via ascorbic acid system. When the activity scores are analyzed, higher scores were found in the ethanol extracts of jojoba leaves. The antioxidant activity was lower in the extracts with methanol.

Keywords: Phenolic contents, Antioxidant, Jojoba, leaves, seeds,

1. Introduction

Jojoba (*Simmondsia chinensis* (Link). Schnider) originates from the Sonoran desert and is grown in different place as a commercial crop [1]. There are about 720 ha of jojoba plantations in La Rioja reported information about methods for the elimination of appetite suppressant compounds constitute a good raw material for animal feed [2]. Due to the fact that these seeds have a high percentage of wax and proteins, they represent valuable raw material for various industries such as the jojoba wax producer and the animal food manufacturer, respectively [3]. The chemical quality parameters of damaged jojoba seeds were unknown. In this study, we followed phenolic contents and antioxidant properties of jojoba plant. Phenolic compounds are a complex, but important group of naturally occurring products in plants and are present in the pharmacological products which includes jojoba seed and leaves. Phenolic compounds are plant secondary metabolites, which play important roles in disease resistance [4], protection against pests and species dissemination. The interest on these compounds is related with their antioxidant activity and promotion of health benefits. Antioxidant activity of several plant materials has recently been described [5-8], and a number of plant products,

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including polyphenols, flavonoids and terpenes, exert an antioxidant action. Plant species belong to several botanical families, such as Labiatae, Compositae, Umbelliferae, Asteraceae, polygonaceae. Many species have been investigated for their antioxidant properties.

The purpose of this work was to study the main phenolic contents present in jojoba tree leaves, seeds obtained from jojoba of Turkey cultivars. The total antioxidant activities of these samples were evaluated by the extent of their capabilities.

2. Material and Methods

2.1. Plant Materials and Extracts preparations

The original jojoba *Simmondsia chinensis* (L.) Schneider seeds and leaves were brought from Arizona, USA, in 1991 and transplanted to Sarıcaşu town, Kumluca, Turkey in 1994. The jojoba seeds and leaves variety of Arizona A42 were used for experiments. The fresh underground and above ground parts of the plant materials were cleaned and dried in the shadow for extraction. Jojoba seeds and leaves were extracted through ethanol and methanol with the help of Soxhlet device (GFL TYP1042) and were added in the ratio of 1/10 and then mixture was filtered by a filter paper (Whatman No:1), and the solvents were evaporated in a rotary evaporator (IKA R 10) at 50 °C. Each process of this experiment was carried out with 4 replications and repeated twice. Extracts were obtained from leaves and seeds of *S. chinensis*. These extracts are: *Simmondsia* Seed-Methanol (SSM), *Simmondsia* Seed-Ethanol (SSE), *Simmondsia* Leaf-Methanol (SLM), *Simmondsia* Leaf Ethanol (SLE).

2.2. Antioxidant capacities (activity)

In this article, antioxidant activities (capacity) was identified by measuring the conjugated diene methanol (MERCK, Germany) sodium methylate methyl hydroperoxides arising from linoleic acid oxidation [13-14]. A stock solution of linoleic acid was prepared as follows: A preparation step was necessary prior to introduction of the oil into the GC/MS (Shimadzu 17A-GC/MS Q P5050, Kyoto, JAPAN) and auto sampler (Shimadzu AOC 20 i, Kyoto, JAPAN) for the individual determination of linoleic acid ingredient FAME (Fatty acid methyl esters) s were obtained by transesterification with sodium methylate in methanol. 0.5 ml of a 0.5% (W/v) solution of sodium methylate in methanol and 100 µl oil were mixed [15]. Antioxidant capacity (AC) was measured in terms of successful bleaching of linoleic acid by using a slightly modified version of the formula from [16] and the absorbance was measured during.

2.3. Determination of total phenolic content

The total phenolic contents of ethanol, methanol seeds and leaves of *S. chinensis* were determined using Folin-Ciocalteu's (FC) reagent according to the method of Singleton et al. [17]. Crude ethanol and methanol extracts (40 µL) of plant materials (2 g mL⁻¹) were mixed with 200 µL FC reagent (Sigma Aldrich, Steinheim, Germany) and 760 µL of distilled water. After shaking, the mixture was incubated for 7.5 min. at room temperature. Then, 600 µL of 20% Na₂CO₃ solution was added. The mixture was shaken for 2 h at room temperature. The absorbance of the solution was measured at 765 nm against a blank. Gallic acid was used as a standard. The concentration of total phenolic compounds in *S. chinensis* was determined as a µg of gallic acid equivalents per 1 mg of extract using the following equation obtained from a standard gallic acid graph (R² = 0.9999).

Absorbance = 0.0024 x gallic acid (µg).

Spectrophotometric analysis was performed by using a five point calibration curve generated with pure gallic acid. Gallic acid was obtained from Sigma, Aldrich.

2.4. Free radical scavenging activity

Free radical scavenging activity of ethanol and methanol extracts of *S. chinensis* was measured by (1,1- DPPH diphenyl-2- picrylhydrazyl (DPPH; Sigma Aldrich, Steinheim, Germany) using the of Shimada et al (1992), Briefly, a 0.1 mM solution of DPPH in ethanol and methanol were prepared. Then, 1 mL of this solutions were incubated with varying concentrations of *S. chinensis* leaves and seeds (1-500 $\mu\text{g}/\text{mL}$). The reaction mixtures were then shaken well and incubated for 30 min. in dark at room temperature.

Statistical Analysis: All data presented are means of four replicates along with standart deviations. Correlation coefficients were determined between antioxidant capacity and phenolic constituents.

3. Results and Discussion

Phenolic compounds exhibit a wide range of physiological properties, such as anti-allergenic, antiarterogenic, antiinflammatory, antimicrobial, antioxidant effects [9-10]. These compounds including flavanoids and phenolic acids are known to be responsible for antioxidant capacities in fruits and the fruits with higher phenolic contents generally show stronger antioxidant capacities [11]. The *Hypericum* family seems to be a rich source of plant species containing large amounts of phenolic acids, so it is considered to be a promising source of natural antioxidants [12]. We found that, the phenolic content of the ethanolic extracts are higher than the methanolic extracts. The phenolic contents of the extracts as follow:

The absorbance of the *S. chinensis* plant extracts at 695 nm was evaluated using absorbance of the sample that was measured every 30 min. using UV spectrophotometer (Pelkin Elmer, Japan). Measurements were carried out in triplicate and the mean values of the measurements were calculated. Methanolic and ethanolic extracts of *S. chinensis* presented different absorbance values. The highest initial absorbance value is detected as 31.60 for SLE (*Simmonsia chinensis*, leaves, Ethanol) and the lowest initial absorbance value is detected as 19.93 for SSM (*Simmonsia chinensis*, Seed, Methanol). The antioxidant activities of the plant extracts were detected as follows: SLE ($50.43 \pm 1.2\%$) > SSE ($24.16 \pm 0.71\%$) > SLM ($40.32 \pm 0.92\%$) > SSM ($13.87 \pm 0.02\%$). The antioxidant activities of extracts were calculated in (nmol/g) via ascorbic acid system (Fig. 1).

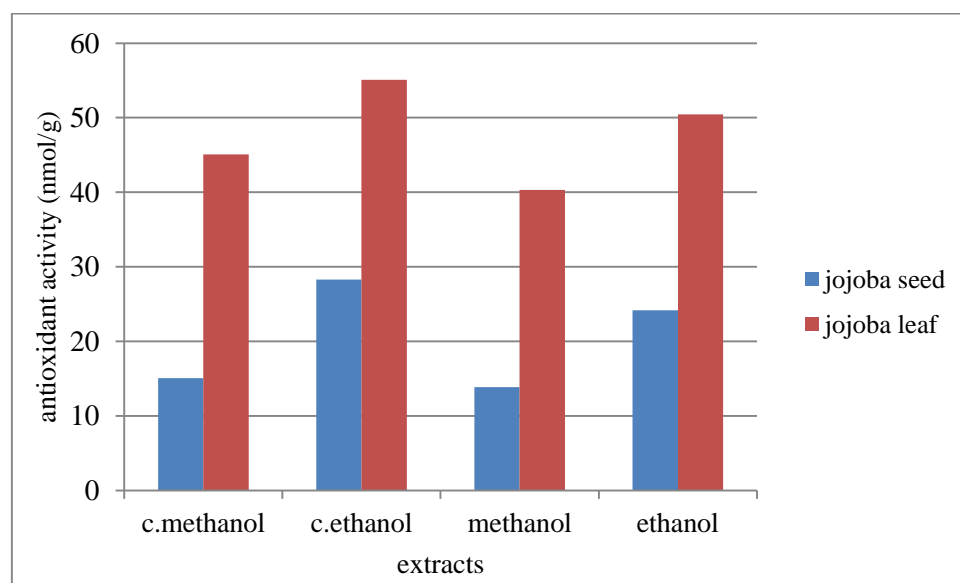


Fig. 1. The antioxidant activity in the methanol and ethanol extracts.

The free scavenging capacities of *S. chinensis* leaf and methanolic and ethanolic extracts of seeds were evaluated and determined as follows (Table-1). Results showed that lavender plant leaves has high phenolic contents. Because of that, it exhibited the high antioxidant and free radical scavenging activities [12].

Table 1. The free radical scavenging activity by DPPH method in *Simmondsia chinensis* leaf and seed.

Jojoba Seed				Jojoba Leaf			
Methanol		Ethanol		Methanol		Ethanol	
The sample quantity ($\mu\text{g/ml}$)	inhibition %	$\mu\text{g/ml}$	Inhibition %	The sample quantity ($\mu\text{g/ml}$)	inhibition %	$\mu\text{g/ml}$	Inhibition %
500	36.43 \pm 0.05	500	43.20 \pm 0.09	500	37.23 \pm 0.06	500	37.45 \pm 2.99
400	32.10 \pm 0.04	400	37.79 \pm 0.18	400	32.95 \pm 0.05	400	38.12 \pm 0.18
300	29.12 \pm 0.03	300	38.97 \pm 1.42	300	30.00 \pm 0.03	300	31.25 \pm 0.04
200	26.50 \pm 0.2	200	27.24 \pm 0.27	200	27.42 \pm 0.02	200	20.24 \pm 0.00
100	15.62 \pm 0.1	100	7.58 \pm 0.62	100	16.67 \pm 0.01	100	10.43 \pm 0.36

The amount of total phenolics, measured by Folin-Ciocalteu method, varied widely in help materials and ranged from 0.00 to 0.00 mg GAE/g dry material. The highest level of phenolics was found in *Echinacea purpurea*, while the lowest was in *Carum carvi* [18]. The amount of total phenolics varied widely in plant materials and ranged from 0.59 to 313 mg GAE/g dry material (Fig. 2). The highest level of phenolics was found in *S. chinensis*, leaf, while the lowest was in *S. chinensis* seed. Total phenolic substance amounts were calculated according to Folin-Ciocalteu method, substance concentrations in mg/GAE g, equivalent to gallic acid based on mg/ml gallic acid equivalent (GAE). The phenolic substance amounts in the leaf extracts (jojoba leaf: 313 mg/g GAE). Nawar et. al. (1984) *Zizyphus spina Christi* (L.) Wild is a wild tree, with spiny branches and small, orange-yellow fruits, commonly found in Jordan, Israel and Egypt, known in Egypt, where it is used to treat the blisters, bruises, chest pains, dandruff, fractures, and headache. The fresh leaves are applied on swollen eye at night.

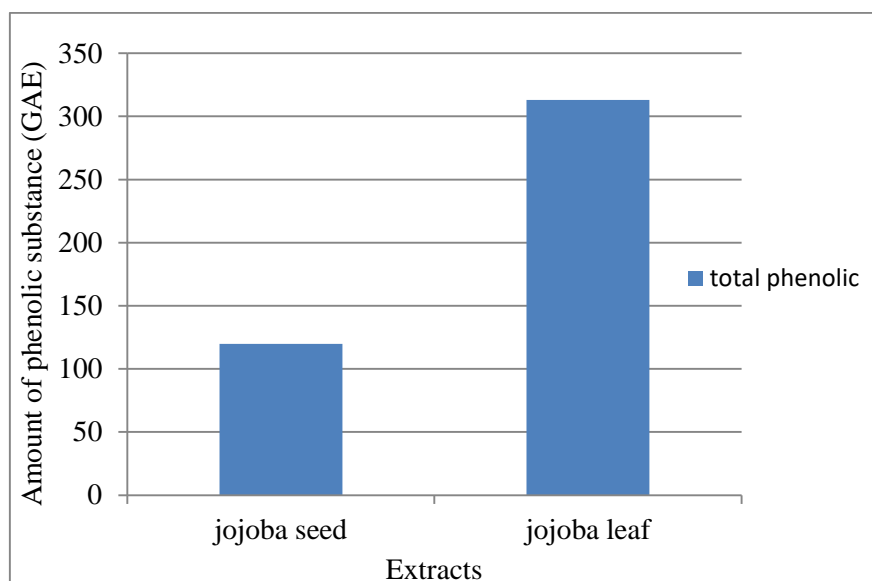


Fig. 2. Total phenolic substance amounts of the extracts

4. Conclusion

Our results demonstrated that all extracts of *S. chinensis* have efficient phenolic compounds. The methanol and ethanol extracts of *S. chinensis* leaves and seeds showed antioxidant activity base on scavenging. When the activity scores are analyzed, higher scores were found in the methanol extract of lavender seed. The antioxidant activity is lower in the extracts with hexane. Total phenolic substance amounts were calculated according to Folin-Ciocalteu method, substance concentrations in mg/GAE g, equivalent to gallic acid based on mg/ml gallic acid equivalent (GAE). The phenolic substance amounts in the leaf extracts (jojoba leaf: 313 mg/g GAE, lavender leaf: 314.4 mg/g GAE) were found more compared to seed extracts. When DPPH radical clearance activity results were analyzed, it was seen that the highest activity was exhibited by jojoba leaf extract (43.20%, 0.5 mg/ml concentration).

Acknowledgements

This study was supported by The Scientific Research Projects Coordination Department in Pamukkale University, Project No: 2014FBE010.

5. References

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