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Separation of free radical scavenging compounds from *Platanus orientalis* leaves

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Abstract

Cancer causes mortality in millions of humans around the world, and due to its predominance, the discovery of anticancer compounds is interesting. Free radical scavenging (FRS) compounds are bioactive compounds that are widely present in nature and significantly delay or prevent cancers, chronic cardiovascular diseases, and aging by inhibiting free radicals. Considering the adverse effects of synthetic antioxidants on human health there is a growing interest to introduce new and potential sources of bioactive compounds. The current study studied the effect of various solvents including ethanol, methanol and *n*-hexane on bioactive compounds recovery from *Platanus orientalis* leaves. The extracts' anti-radical capacity (in terms of percentage inhibition of DPPH and HO free radicals) and total phenolic compounds were measured using spectroscopy methods. Moreover, the main phenolic compounds obtained were quanti/qualitatively measured using high-performance liquid chromatography. From the results, ethanolic extracts showed the highest anti-radical capacity (%$DPPH_{sc}$ of 22.11 ± 0.13 and %HO_{sc} of 16.40 ± 0.14) and TPC (22.11 ± 0.18). Furthermore, catechin was the dominant compound determined in the extracts. Therefore, it could be stated that *P. orientalis* leaves are a potential source of valuable phytochemicals containing FRS compounds for further application in pharmacy, food, and cosmetic industries.

Keywords: Free radical, Cancer, Phenolic compounds, *Platanus orientalis* leaves, Chromatography.

1- Introduction

Cancer is a complex interaction among numerous signaling pathways involving a variety of target compounds. Cancer causes mortality in millions of individuals around the world, and due to its predominance, the discovery of novel anticancer molecules is critically required. Nature is a main source anticancer substance, and many of the cytotoxic

medicines are derived from natural sources. Reactive oxygen species (ROS) induce a variety of human cancers, and scavengers or antioxidants are consumed to control them [1]. A free radical could be explained as any molecule containing an unpaired electron. The presence of an unpaired electron leads to certain properties of free radicals, which are highly unstable and reactive substances. They can donate or accept an electron, thus acting as oxidant or reductant agents. Free radicals are able to attack the body's macromolecules such as lipids, nucleic acids and proteins, which ultimately lead to cell damage and abnormal functioning. Free radicals are produced by natural essential metabolic pathways in the human body or from external sources such as exposure to X-rays, ozone, air pollutants, smoking, and industrial waste [2,3].

Many diseases such as various cancers or heart and brain diseases, or even the aging process, are caused by the action of free radicals. Oxidative stress is used to explain conditions of oxidative damage that result from a critical imbalance between the formation of free radicals and antioxidants. The initiation and progression of cancer are associated with an imbalance between free radicals and the defense system of human body. Considering the serious harmful impacts of free radicals on human health, it is necessary to scavenge free radicals [4]. Bioactive compounds are chemical compounds existed in small amounts in different plants and foods such as vegetables, fruits, nuts, and grains. These compounds possess activities in the body that improve good health. Bioactive compounds are being investigated in the prevention of heart disease, cancer, and other diseases. Some major bioactive compounds are phenolic compounds, resveratrol, lycopene, tannins, lignan, and indoles [5]. Certainly, one of the efficient solutions to control and prevent the risk of cancer is to consume enough fruits and vegetables, which possess high biological activity. Various researches have indicated that there is a positive relationship between the consumption of fruits and vegetables and a reduced risk of cancer, especially breast, prostate, lung, and neck cancers [6]. Phenolic compounds are a major class of bioactive compounds which are presented in natural sources. These compounds are able to scavenge free radicals and prevent the initiation of various diseases caused by free radicals. Furthermore, it has been stated that synthetic antioxidant compounds showed adverse effects on human health. Sammar et al., 2019 presented a comprehensive study regarding the correlation between cytotoxicity in cancer cells and free radical scavenging activity of 57 medicinal and edible plant extracts [1]. Therefore, there is growing interest to introduce natural potential sources of bioactive compounds for further consumption in food and pharmacy products.

Platanus orientalis which, belongs to Platanaceae family is widely planted in Iran for the beauty of the urban space. *P. orientalis* trees are tall with broad-leaves and their height reaches 30 to 50 meters. The palmate leaves are more deeply incised and usually have 5-7 sharply serrated lobes, the central lobe being longer than others [7]. Considering the availability and low economic cost of *P. orientalis* leaves, it is of great interest to evaluate its phytochemicals to replace synthetic antioxidants. In the current study, the effect of different solvents on bioactive compounds separation from *P. orientalis* leaves was studied. Furthermore, the radical scavenging capacity and total phenolic compounds of extracts was

measured using spectroscopy methods. Moreover, the main phenolic compounds obtained were analyzed using high performance liquid chromatography.

2- Materials and Methods

2-1- Sample preparation

P. orientalis leaves were obtained from an area located in Zanjan, Iran. After washing and removing excess water, the leaves were dried in a shaded area at ambient temperature to reach a constant weight. Dried leaves were powdered to obtain a certain particle size. The obtained powder was stored in impermeable polyethylene bags for further experiments.

2-2- Classical separation procedure

To perform the classical separation procedure, 3 g of the dried sample is transferred to the thimble which, was placed in the Soxhlet device. Different solvents including methanol, ethanol and *n*-hexane were applied considering proper sample to solvent ratio. The extraction temperature was equivalent to the boiling point of relevant solvent. After 6 hr extraction time, the sample was placed in a rotary vacuum evaporator (40 °C) to remove solvent and concentrate bioactive compounds. The bioactive compounds were kept at -18 °C for further measurements.

2-3- Determination of free radical scavenging capacity

2-3-1- DPPH assessment

The investigation of the free radical scavenging capacity of the bioactive compounds separated from *P. orientalis* leaves was carried out by measuring the free radical inhibition activity of 2 and 2-diphenyl-1-picrylhydrazyl (DPPH) according to the method of Zandi et al. [8]. In brief, 2 mL of the diluted extract were mixed with 2 mL of DPPH solution (0.1 mM), and the mixture was shaken well and kept in the dark for 30 minutes at room temperature. Then, the absorption was measured by a UV-vis spectrometer at a wavelength of 517 nm. The inhibition percentage of DPPH free radicals was calculated using Eq. 1.

$$\mathrm{DPPH}sc\ (\%) = \frac{A_c - A_s}{A_c} \times 100 \qquad (1)$$

where, A_c and A_s were absorbance of the control and the sample, respectively.

2-3-2- Peroxide hydrogen assessment

In order to evaluate the inhibition of peroxide hydrogen free radicals by extracts obtained from *P. orientalis* leaves, 1.5 mL of sample was combined with hydrogen peroxide solution (30% v/v). Then, the absorbance was measured at 530 nm wavelength using UV-vis spectrometer [9]. The inhibition percentage of HO free radicals was calculated using Eq. 2.

$$\mathrm{HOsc}\ (\%) = \frac{A_c - A_s}{A_c} \times 100 \qquad (2)$$

where, A_c and A_s were absorbance of the control and the sample, respectively.

2-4- Measurement of total phenolic compounds

The total phenolic compounds (TPC) of the bioactive compounds separated from *P. orientalis* leaves was measured by the Folin-Cicaltio method according to the method of

Singleton et al. (1999). Briefly, 0.5 mL of the sample was added to 2.5 mL of Folin-Ciocalteu reagent and it was placed for 5 minutes at room temperature in a dark room. Then, 2.5 mL of sodium carbonate (7.5%) was added and was kept in the dark room for 30 minutes. Finally, the absorbance was measured at the wavelength of 760 nm using a UV-vis spectrometer. The TPC was expressed as milligrams of gallic acid equivalents per gram of extract [10].

2-5- High performance liquid chromatography

The major phenolic compounds present in the extract obtained from the *P. orientalis* leaves was determined using an High performance liquid chromatography (HPLC) device equipped with a UV-vis detector using gradient mode. Deionized water and ethanol were applied as mobile phase. Retention time was adjusted at 45 min. All major phenolic compounds were identified according to the related retention time against the standard compounds. The quantification was performed according to the linear calibration curve prepared from the standard compound [11].

2-6- Statistical analysis

All experiments were carried out considering three replications and the experimental data were expressed as mean ± standard deviation (SD). One-way analysis of variance (one-way ANOVA) was performed using Minitab version 17.0 (State College, PA, USA) statistical software and mean comparisons were made using Tukey's test at $p \leq 0.05$.

3- Results and Discussion

3-1- Effect of different solvents on antiradical capacity

In many studies, classical separation procedure has been used as a reference method to recover various bioactive compounds from different plant sources, which possess anticancer capacity. In the present study, the classical separation procedure was used to investigate the effect of different solvents on separation of the bioactive compounds from *P. orientalis* leaves. In this study, methanol, ethanol and *n*-hexane solvents were used and the results were reported in Figure 1. The antiradical capacity of extracts ranged from 4.47 ± 0.12 to 22.11 ± 0.13% applying different assessment of free radical scavenging assays, which showed the presence of FRS compounds in the *P. orientalis* leaves. Based on the results all extracts showed free radical scavenging activity however, the highest antiradical capacity ($\%DPPH_{sc}$ of 22.11 ± 0.13 and $\%HO_{sc}$ of 16.40 ± 0.14) was determined for the ethanolic sample. The lowest antiradical capacity was measured for the extracts obtained using *n*-hexan, which indicated the low solubility of target compounds in the *n*-hexane.

Solvent polarity is one of the important factors affecting the quality and quantity of bioactive compounds extraction. According to the obtained results, increasing the polarity of the solvent increases the antiradical capacity of compounds. Polar solvents have a great ability to recover bioactive compounds such as antioxidant compounds; while non-polar solvents have a high ability to recover non-polar compounds from cell matrix [12]. Our findings were in agreement with those reported by Ghasemzadeh et al. [13]. From the results, there is no significant difference between the data obtained from ethanol and methanol solvents, which suggesting the priority of the ethanol solvent due to the toxicity

of methanol solvent. In other studies, ethanol has been successfully used to extract effective compounds [14].

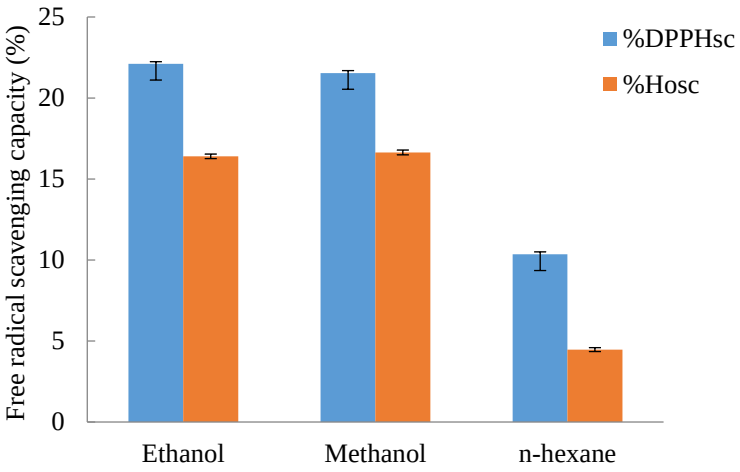

Figure 1: Effect of different solvents on antiradical capacity of bioactive compounds.

3-2- Effect of different solvents on TPC

The phenolic compounds are valuable secondary plants metabolites having a hydroxyl group and are known to be involved in various physiological activities. The free radical scavenging activities of these compounds have been demonstrated in various studies [15, 16]. From the results all extract obtained from *P. orientalis* leaves contained phenolic compounds with different contents. According to the results, extracts obtained using ethanol (22.11 ± 0.18 mg GAE/g) and methanol (21.54 ± 0.18 mg GAE/g) showed the highest values of TPC, while no phenolic compounds were determined in the extract obtained using *n*-hexane. These findings could be due to the higher solubility of phenolic compounds in polar solvents. Among solvents studied, *n*-hexane was non-polar solvent lead to non-efficient recovery of phenolic compounds from *P. orientalis* leaves. These findings were in accordance with those obtained for the effect of different solvents on antiradical capacity of extracts. Same findings were reported by Salih et al. [17]. In another studies, it has been stated that curcumin as a natural polyphenol compound obtained from turmeric rhizome and also quercetin possess potent free radical-scavenging and cytotoxic activity. These activities highlighted the importance of these valuable bioactive compounds for prevention of cancers [18, 19].

3-3- Effect of different solvents on major phenolic compounds

Phenolic compounds are the main factor determining the amount of antiradical activity of bioactive compounds. The quanti/qualification measurements of the main phenolic

compounds presented in the extracts obtained from *P. orientalis* leaves were performed and the results were shown in Table 1. From the results, four major phenolic compounds including catechin, rutin, myricetin and gallic acid were identified, and the highest amount of these compounds was obtained from the ethanolic extract.

Table 1: Effect of Solvents on main phenolic compounds (mg/g) of *P. orientalis* leaves extracts.

Solvent type	Catechin	Rutin	Gallic acid	Myricetin
Ethanol	$3.11^{a} \pm 0.14$	$2.41^{a} \pm 0.12$	$1.05^{a} \pm 0.13$	$2.05^{a} \pm 0.12$
Methanol	$3.20^{a} \pm 0.11$	$2.20^{a} \pm 0.12$	$1.10^{a} \pm 0.12$	$2.07^{a} \pm 0.13$
n-hexane	tr*	-	tr	-

*tr: trace elements; Different lower case letters in each column represent significant difference ($p \leq 0.05$).

Among these compounds catechin (Fig. 2) was the most abundant compound obtained from *P. orientalis* leaves. Catechin is known to have different valuable biological activities such as reducing the growth of intestinal tumor cells and human breast cancer cells [20]. Furthermore, it exhibited radical scavenging activity. Based on the results, no considerable difference was observed between the phenolic compounds obtained from the ethanolic and methanolic extracts. On the other hand, no phenolic compound was observed in the extract obtained using *n*-hexane. These results can indicate the influence of the polarity of the solvent used in the extraction process on the release and recovery of phenolic compounds from the cell structure [17].

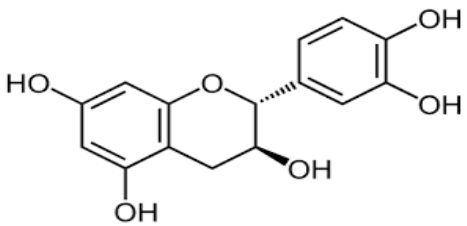

Figure 2: Chemical structure of catechin

The obtained results showed that ethanol is the most effective solvent for the recovery of phenolic compounds from the *P. orientalis* leaves, and by reducing the polarity of the extraction solvents, the TPC decreased. These findings could be related to the polar nature of phenolic compounds. Phenolic compounds have hydroxyl and carboxyl functional groups that can be easily recovered by polar solvents [21]. Phenolic compounds include a large group of secondary metabolites that have multiple roles in the plant, such as protecting the plant from insects and pathogens. In addition, this group of compounds contain significant antioxidant power [22]. As can be seen, the highest amount of anti-radical activity was also observed in the ethanolic extract, which can indicate the significant role of phenolic compounds in creating antioxidant power of the extracts. These

results were parallel to those obtained regarding the effect of solvents on the TPC and anti-radical of extracts from *P. orientalis* leaves.

4- Conclusion

The results of this study indicated that *P. orientalis* leaves is a potential source of phytochemicals such as rutin, myricetin, gallic acid and rutin. The extracts rich in valuable bioactive phenolic compounds showed free radical scavenging ability. The ethanolic extracts contained anti-radical capacity in terms of scavenging of free DPPH and HO free radicals. From the results, *P. orientalis* leaves could be considered as an economic and potential source of natural and valuable free radical scavengers for further utilization in food, pharmacy and cosmetic industries. Moreover, th effect of novel extraction methods on the qulity and quantity of bioactive compounds could be suggested for further studies.

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