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Extended Abstract

Study on the Phenolic Compounds and Antioxidant Activity of Gum Extract of *Astragalus fasciculifolius* Boiss

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Abstract

According to consumers trend for using natural conservator compounds, the efforts for finding new herbal resources replaced with synthetic antioxidant has increased. Anzarut is a medicinal plant with *Astragalus fasciculifolius* Boiss and belongs to the Astragalus genus and Fabaceae family. This research aimed to study the effect of two methods of extraction (supercritical fluid and maceration by methanol as solvent) on impressive composition in *Astragalus fasciculifolius* Boiss, also examining antioxidant activities and finding correlation coefficient between phenol and flavonoid content and capacity of antioxidant. The result showed phenol and flavonoid content in gum extract by supercritical fluid was more than extracts by maceration, and this difference was significant (P>0.05). Most of the antioxidant activity in all methods include 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2 Azino-bis (3-ethylbenzothiazloine-6-sulfonic acid) (ABST), Cupric ion reducing activity (CUPRAC), Phospho molybdenum (PMB), ferric ion reduction capacity (FRAP) was observed in gum extract by supercritical fluid, but only the increase in the DPPH method was found significant. Correlation findings that resulted in coefficients were significant (P≤0.05), and there was a good relation between antioxidant capacity and phenolic compounds. Citing our findings, using supercritical fluid at 100Mp, 35 °C, and 30 min did not significantly affect antioxidant activity compared with maceration. According to the high cost of extraction by supercritical fluid, this method must be optimized.

Keywords: Antioxidant activity, *Astragalus fasciculifolius* Boiss, Supercritical fluid

Introduction

Anzarut medicinal plant with the scientific name of *Astragalus fasciculifolius*. It belongs to the genus Astragalus and the legume family (Fabaceae) (Nosrati, Fakheri, Solouki, Mahdi Nezhad, & Valizadeh, 2019). More than 140 chemical compounds have been identified in the genus Astragalus, such as cyclovartan, triterpene glycosides, phenolic compounds, flavonoids, and various polysaccharides (Li *et al*., 2014). Today, it uses modern extraction methods to increase extraction efficiency, reduce extraction time, reduce solvent consumption, enhance the quality of essential oils and extracts, and prevent environmental pollution (Wang & Weller, 2006). Due to the importance of new plant sources for use in food, pharmaceutical, and health industries, the purpose of this study was to investigate the phenol content, antioxidant properties and evaluate the correlation between phenol content and antioxidant activity of Anzarut gum extract extracted by supercritical fluid method and soaking. Pearson correlation test is used to find a significant correlation between two variables. In line with the

studies conducted up to the time of writing this article; so far, no study has been done on Enzoot gum extract and its antioxidant activities and correlation with their phenolic compounds, so the results of this study can be of great value in the Anzarut gum research literature.

Materials and methods

Extraction of Anzarut gum extract by the supercritical fluid method

In this method, 20 g of Anzarut gum powder was combined with 100 mL of ethanol solvent as a modifier; then extraction was performed by supercritical CO_2 (Suprex MPS/225 Multipurpose) at 35 °C and pressure 100 times for 30 min (Delfanian, Esmaeilzadeh Kenari, & Sahari, 2015).

Measurement of total phenol compounds

To calculate the total phenol composition in the extract samples, the Folin-Cocaltive method was used.

Measurement of total flavonoids

To investigate the flavonoid content, 0.5 mL of the extract solution was mixed with 3 mL of methanol, 0.2 mL of 10% aluminum chloride (w/v), 0.2 mL of 1 M potassium acetate, and 5 mL of distilled water. Samples were stored at room temperature for 30 min. The absorbance of each sample was measured at 415 nm (Singh, Sharma, & Sharma, 2015).

Evaluation of antioxidant activity by free radicals 1, 1-diphenyl-2-picryl hydrazyl

1 mg/mL methanol solution was prepared and transferred to a test tube, then 1 mL of DPPH methanolic solution was added, a spectrophotometer defined the optical absorption at 517 nm. The percentage of DPPH radical inhibition of each extract is then calculated using Equation (1) below:

DPPH percentage of inhibition (%) = ("(A-B") / A) × 100

Here, A and B are the light absorption of the control sample and the light absorption of the sample, respectively (Jaradat *et al*., 2017).

Evaluation of antioxidant activity as IC_{50} (mg/L)

The amounts of antioxidants required for DPPH to reach 50% of the initial value (median inhibition concentration) or (IC_{50}) of *Astragalus fasciculifolius* Boiss extract according to Jaradat *et al*. (2017) and through linear regression obtained from the inhibition percentage curve in Different concentrations were calculated in milligrams per liter.

Evaluation of antioxidant activity by iron ion reduction capacity

For this purpose, Kocak *et al*. (2016) method was used with a slight modification. 1 mL of 20 mM FRAP solution was added to 1 mL of extract (mg/mL methanol) and placed at 37 °C for 5 min. The absorption rate was read at 593. The calibration curve was plotted using ferrous sulfate.

Evaluation of antioxidant activity by phosphomolybdenum method

0.2 mL of extract (mg/mL methanol solvent) was added to 2 mL of reagent solution (0.6 mg sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The sample was read at 695 nm after holding for 90 min at 95 °C.

Evaluation of radical inhibition activity of 2-2 azine-bis-3-ethyl benzodiazepine-6-sulfonic acid cations

The sample solution (1 mL) was added to the ABTS solution (2 mL) and homogenized by the vortex. The sample adsorption was read at 734 nm after incubation for 30 min at room temperature (Zengin *et al*., 2015).

Evaluation of antioxidant activity by copper ion reducing activity

0.5 mL of the extract was added to the reaction mixture containing copper chloride, neocoprotein, and ammonium acetate buffer. The samples were read at 450 nm (Apak, Güçlü, Özyürek, Esin Karademir, & Erçağ, 2006).

Results and discussion

The antioxidant activity of the extract extracted by the supercritical fluid method was higher in all test methods than the extract extracted by the maceration method. Results show that the inhibition of DPPH for the samples of the extract extracted by soaking and supercritical fluid was 36.6 and 59.9 (%), respectively. The inhibitory activity of DPPH of the extract extracted by the method. The supercritical fluid at a 95% confidence level was higher than the antioxidant activity of Anzarut extract extracted by soaking ($P \leq 0.05$). The amount of iron ion reduction (FRAP) of the extracts extracted by the supercritical fluid and soaking method was also investigated, and the results showed that the supercritical capacity of the supercritical fluid extract was high. 29 and 28.3 (mmol of iron per gram of extract), which has no statistically significant difference. Evaluation of antioxidant activity by azinobase radical or antioxidant potential of ethyl thiazoline sulfonic acid (ABTS +) equivalent to trolux (unit of antioxidant capacity based on ABTS +) also showed that the difference between the two extracts extracted by supernatant fluid in the supernatant 95% method was not significant ($P > 0.05$).

Pearson test was performed to investigate the correlation between the antioxidant capacities of the extract extracted by the supercritical fluid method and their phenol content. According to the results presented in Fig. (1), with increasing the concentration of the extract extracted by the supercritical fluid method, the amount of phenol compounds has increased, and the amount of antioxidant activities has also increased.

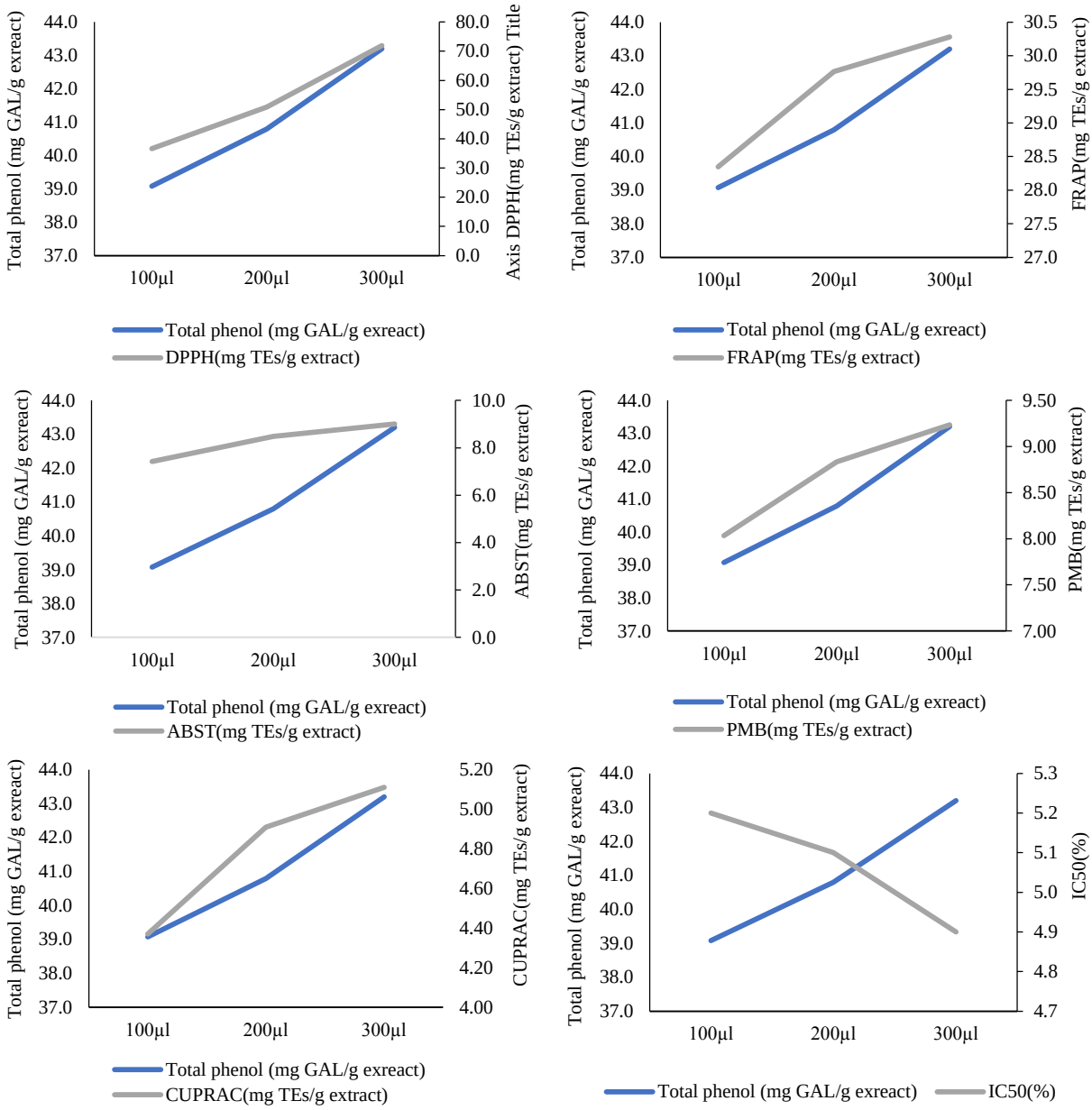

Fig. 1. Correlation between total phenol compounds and antioxidant activities of the extract extracted by the supercritical fluid method in volumes of 100, 200, and 300 μL of the extract (1 mg/mL)

Conclusions

The results of this study showed that the extraction of Anzarut extract by supercritical fluid method, at a pressure of 100 bar, 35 °C and an extraction time of 30 min, had a higher amount of phenol and flavonoid compounds than the extract extracted by soaking, which is a significant difference. However, the antioxidant activities of supercritical fluid extract were higher than soaking, which was significantly higher in the study of DPPH activity than the soaking extract. The extraction efficiency in the supercritical fluid method was 15.91% and for the extraction method was 26.31%, and the correlation between phenol compounds and antioxidant activities of DPPG, FRAP, ABST, PMB, CUPRUC, and IC_{50} was significant at 95 and 99% levels. Based on the results of this study, it can be stated that Anzarut gum is a rich combination of phenol and flavonoid compounds that has a significant effect on antioxidant activities and has been able to reduce oxidation. Due to the high cost of extraction of effective compounds by supercritical fluid method, the conditions for extraction of supercritical fluid in this study are not suitable in industrial scales, and the conditions need to be optimized.

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