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ANTIOXIDANT ACTIVITY OF METHANOLIC AND WATER WORMWOOD EXTRACTS MEASURED BY ESR SPECTROSCOPY

Sonja M. DJILAS, Jasna M. ČANADANOVIĆ-BRUNET,
Gordana S. ĆETKOVIĆ, Vesna T. TUMBAS

UNIVERSITY OF NOVI SAD,
FACULTY OF TECHNOLOGY, SERBIA

ABSTRACT
In this paper, the antioxidant activity of methanolic and water extracts of wormwood (*Artemisia absinthium* L.) on reactive hydroxyl radical formed in the Fenton reaction was investigated by ESR spectroscopy.
Wormwood (*Artemisia absinthium* L.) is an aromatic-bitter herb, which has been used as anthelmintic, choleretic, antiseptic, balsamic, depurative, digestive, diuretic, emmenagogue and in treating leukemia and sclerosis.

KEYWORDS
antioxidant activity, wormwood, aromatic-bitter herb

1. INTRODUCTION

In the past few years, there has been growing interest in involvement of reactive oxygen species in several pathological situations [1]. The presence of phytochemicals in herbs has been recently considered of crucial nutritional importance in the prevention of chronic deseases, such as cancer, cardiovascular disease and diabetes. For that reason, systematic search for useful bioactivities from medicinal plants is now considered to be a rational approach in nutraceutical and drug research [2].

Wormwood (*Artemisia absinthium* L.) is an aromatic-bitter herb, which has been used as anthelmintic, choleretic, antiseptic, balsamic, depurative, digestive, diuretic, emmenagogue and in treating leukemia and sclerosis.

In this paper, the antioxidant activity of methanolic and water extracts of wormwood (*Artemisia absinthium* L.) on reactive hydroxyl radical formed in the Fenton reaction was investigated by ESR spectroscopy.

2. MATERIAL AND METHODS

All chemicals were of analytical reagent grade. Plant was purchased from local herbal drugstore.

Extracts preparation. Dried areal parts of wormwood (5 g) were extracted with 70% methanol or distilled water (250 ml) in a shaker incubator at 25°C for 24 h. The extracts were filtered and obtained filtrates were concentrated under the reduced pressure to dryness. The yields (g), averages of triplicate analysis, of obtained extracts were: methanolic extract 1.2945 ± 0.0095 and water extract 1.3846 ± 0.0099.

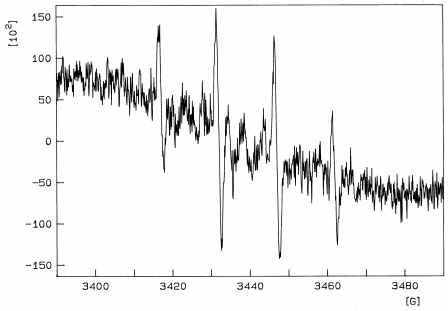
Total phenolic compounds and flavonoids were determined spectrophotometrically according to Folin-Ciocalteu and Markham, respectively [2].

Hydroxyl radicals generation and detection. Hydroxyl radicals were obtained by the Fenton reaction in the system: 0.2 ml 10mM H_2O_2, 0.2 ml 10 mM $FeCl_2x4H_2O$ and 0.2 ml 0.3 M DMPO as spin trap (blank). The influence of methanolic and water extracts on the formation and transformation of hydroxyl radicals was investigated by adding the extracts to the Fenton reaction system in the range of concentrations 0.08-0.80 mg/ml.

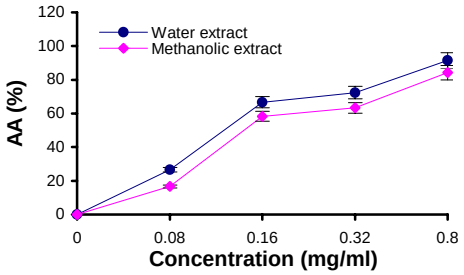
3. RESULT AND DISCUSSION

The contents of total phenolic compounds and flavonoids in methanolic extract are 12.49±0.34 and 4.86±0.31 mg/g, and in water extract are 13.57±0.97 and 5.62±0.77.

As shown in Fig. 1(A), the reaction of Fe^{2+} and H_2O_2 in the presence of spin trapping agent DMPO generated a 1:2:2:1 quartet of lines in the ESR spectrum with the hyperfine coupling parameters (a_N and a_H=14.9 G).



(A)



(B)

Fig. 1. ESR spectra of DMPO-OH spin adducts –blank (A), and antioxidant activity of water and methanolic extracts of wormwood (B). The antioxidant activity (AA) of the extracts was defined as: $AA=100\cdot(h_o-h_x)/h_o$ [%], where h_o and h_x are the height of the second peak in the ESR spectrum of DMPO-OH spin adduct of the blank and the probe, respectively.

4. CONCLUSIONS

The addition of the methanolic and water extracts of wormwood to the reaction system (from 0.08 to 0.80 mg/ml) resulted in a dose-dependent inhibition of the ESR signal intensity of DMPO-OH spin adduct.

As can be seen from Fig. 1(B), both extracts showed significantly high AA ($p<0.05$) which is in correlation with the contents of total phenolic compounds and flavonoids. AA is mainly due to capacity of phenolics to act as free radical scavengers and/or as metal chelators.

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