Free radical scavenging activity of extracts from Bulgarian Veronica officinalis L. and GC-MS analysis of ethanol extract

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Citation

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Abstract

The antioxidant activities of MeOH and EtOH extracts and EtOAc fraction of EtOH extract from Veronica officinalis were carried out using 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) in vitro assays. The antioxidant potential measurements were expressed as Trolox equivalent antioxidant capacity (TEAC). The EtOAc fraction showed the highest radical scavenging activity in both $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ methods: 2.43 mmol/g and 1.69 mmol/g TEAC, respectively. Total phenolic content was also determined. It was the greatest for EtOAc fraction (318.83mg GAE/g extract) and a strong positive correlation was observed between phenolic content and antioxidant activity. In addition, the chemical composition of EtOH extract was examined by GC-MS analysis. 26 compounds were established and ß-sitosterol was the major constituent (13.03 %), followed by palmitic acid (11.05 %) and terpinen-4-ol (8.96 %). Two essential fatty acids (EFAs), linoleic acid (6.85 %) and -linolenic acid (4.07 %) were also presented.

INTRODUCTION

In living organisms the reactive oxygen species (ROS) and reactive nitrogen species (RNS) are known to cause damage to lipids, proteins, enzymes, and nucleic acids leading to cell or tissue injury implicated in the processes of aging as well as in wide range of degenerative diseases including inflammation, cancer, atherosclerosis, diabetes, liver injury, Alzheimer, Parkinson, and coronary heart pathologies, among others ([1]).

Antioxidants have been used for the prevention and treatment of free radical-related disorders ([2]). Many medicinal plants contain large amounts of antioxidants such as secondary metabolites, which can play an important role in adsorbing and neutralizing free radicals ([3]). Recently, there has been growing scientific interest to find naturally occurring antioxidants because of established carcinogenicity of used synthetic antioxidants.

Several Veronica species are used for the treatment of cancer, influenza, hemoptysis, and against cough, and respiratory diseases in different countries ([456]). Veronica species contain mainly iridoid glucosides, some phenylethanoid and flavonoid glycosides. ([78910]). Different evaluations of Veronica species concerning their possible anti-inflammatory, antioxidant and cytotoxic activities have been reported ([11121314]).

The species V. officinalis is used in traditional Bulgarian medicines for its pharmaceutical properties ([15]). Numerous studies have demonstrated the in vitro antioxidant activity and polyphenol content of many medicinal plants of foreign origin but data about Bulgarian medcinal plants are insufficient. The aim of this study is to establish the antioxidant capacity and the contribution of polyphenols to the antioxidant activity of MeOH and EtOH extracts and fraction from V. officinalis used for treatment of different diseases. In addition, we report our results on the GC-MS analysis of non-phenolic compounds in ethanol extract (the soluble in chloroform part) of this species of Bulgarian origin for the first time.

MATERIALS AND METHODS

PLANT MATERIAL

Aerial parts of V. officinalis were collected in Sevlievo region, Bulgaria. The voucher specimen (SO 105295) has been deposited in the herbarium of faculty of Biology, Sofia University "St. Kliment Ohridski".

PREPARATION OF PLANT EXTRACTS

The air-dried parts of the plant V. officinalis, 150 g were extracted twice with EtOH at 50˚-55˚C for 3 h. The ethanol solutions were evaporated under vacuum to give 16.71 g EtOH extract. In the same conditions was obtained MeOH extract 12.66 g from 100 g plant material. The EtOH extract (8.78 g) was dissolved in petroleum ether -water (3:1) and successively extracted with petroleum ether and chloroform to removing non polar/lipid/compounds. After that the aqueous layer was extracted with ethyl acetate to get ethyl acetate soluble fraction (EtOAc; 11.96 % w/w of EtOH extract).

DPPH RADICAL SCAVENGING ASSAY

The antioxidant activity using the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay was assessed by modifying the method of Blois ([16]).

One ml of 0.1 mM DPPH $^{\bullet}$ methanol solution was added to 3 ml solution of the extracts and fraction or 3 ml pure methanol for the blank sample. The absorbance was read at 517 nm after 30 min incubation. Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid) was used as a reference compound. The Trolox equivalent antioxidant capacity (TEAC) was expressed as mmol Trolox corresponding to one g extract. Unicam UV500 Spectrophotometer (Thermo Spectronic, UK) and 1 cm disposable cuvettes (Brandt, Germany) were used for all the absorption measurements reported.

ABTS RADICAL CATION SCAVENGING ASSAY

The ABTS $^{\bullet+}$ scavenging test was used to determine the antioxidant activity. ABTS$^{\bullet+}$ radical was obtained by reaction between ABTS and potassium persulfate ([1718]). Blank sample was prepared from the daily solution by adding 1 ml ethanol, which gives an absorbance of 0.7 ± 0.01. The radical scavenging activity was assessed by mixing 2 ml of ABTS$^{\bullet+}$ solution with 1 ml ethanol solutions of the investigated plants with different concentrations. The reactive mixture was allowed to stand at room temperature for 10 min and the absorbance was recorded at 734 nm. The Trolox was used as standard. The TEAC was calculated like DPPH assay.

DETERMINATION OF TOTAL PHENOLIC COMPOUNDS

Total phenolic content (TPC) in the investigated extracts and EtOAc fraction was determined by the Folin-Ciocalteu colorimetric method, based on the procedure of Singleton and Rossi ([1920]), using gallic acid as a standard phenolic compound. Briefly, 0.5 ml (three replicates) of the samples was mixed with 3 ml of distilled water and 0.25 ml Folin–Ciocalteu reagent. After 2 min, 0.75 ml of 20% sodium carbonate were added and the volume made up to 5 ml with distilled water. The absorbance of the resulting blue-coloured solution was measured at 765 nm after 2 h with intermittent shaking. Quantitative measurements were performed, based on a standard calibration curve of seven points from 0.01 to 0.2 mg/ml of gallic acid in methanol. The total phenolic content was expressed as gallic acid equivalents (GAE) in mg/g of extract.

GAS CHROMATOGRAPHY AND GC-MS ANALYSIS

The part of ethanol extract was mixed with chloroform at room temperature, and after filtration the chloroform soluble part containing non-phenolic compounds was obtained. It was analyzed by GC and GC-MS. GC analysis was carried out on a PERKIN-ELMER Auto System GC, equipped with FID and split/splitless injector and a glass capillary column –RSL 200 (30 m x 0.25 mm x 0.25 μm film), carrier gas He with linear velocity 42 cm.min^{-1}, temperature programmed – from 60°C to 310°C, with 10°C/min. GC/MS analysis was performed on Hewlett-Packard GCD System G 1800A. The optimum conditions of analysis were employed: ionization type: EI; ionization energy: 70 eV; temperature of ion sours: 200˚C. The column and temperature program were the same as in GC analyses. The GC–MS peaks were identified by comparison with data from the literature and the profiles from the Wiley 275 and NIST 05 libraries.

RESULTS AND DISCUSSION

DPPH RADICAL SCAVENGING ASSAY

The antioxidant activity of the MeOH, EtOH extracts and EtOAc fraction of EtOH extract from aerial parts of V. officinalis was reported for the first time. The DPPH scavenging activities expressed as trolox equivalent antioxidant capacity (TEAC) were presented in Table 1.

The effect of the investigated samples on DPPH radicals has been checked at various concentrations from 1.2 to 14.4 μg/ml and from 0.6 to 9.6 μg/ml for the extracts and fraction, respectively. Since TEAC is a quantification of the effective antioxidant activity of the samples, a higher TEAC would imply greater protective action. The EtOAc fraction of V. officinalis exhibited the best DPPH scavenging capacity,

followed by EtOH and MeOH extracts.

ABTS RADICAL CATION SCAVENGING ASSAY

The results, calculated as trolox equivalent antioxidant capacity (TEAC), are shown in Table 1. The investigated extracts and fraction possessed the free radical-scavenging properties in different degrees. Their suppressive effect on ABTS cation radicals was assayed at various concentration regions: from 5 to 25 μg/ml and from 2 to 10 μg/ml for the extracts and fraction, respectively. At this assay the EtOAc fraction was the most active like DPPH method. The lowest TEAC value was established for MeOH extract.

{image:1}

TOTAL PHENOLIC CONTENT

It is well known that plant polyphenols are widely distributed in the plant kingdom and that they are sometimes present in surprisingly high concentrations ([21]). The phenol content in the investigated plant material analyzed is presented in Table 2. The results showed that the total phenolic content in EtOAc fraction of V. officinalis was the highest as compared to the extracts. This may be due to the presence of high bioactive compounds in EtOAc fraction. A positive correlation ($R^2 = 0.99$) between antioxidant activity in both $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ scavenging assays and total phenolic content was found (Fig. 1). This correlation suggests that phenolic compounds are likely to contribute to the antioxidant potential of the investigated extracts and fraction.

{image:2}

{image:3}

GC AND GC-MS ANALYSIS

The composition of EtOH extract from aerial parts of V. officinalis was determined by GC–MS analysis for the first time (Table 3). According to the results obtained EtOH extract of this plant contains a number of non phenolic compounds: terpenes, saturated and unsaturated fatty acids and esters, steroids (sterols and sterenes), p-hydroxyphenylethyl alcohol, maltol and loliolide. ß-sitosterol was the most abundant component (13.03 %) in the extract.

{image:4}

The widely distributed phytosterols (ß-sitosterol, campesterol and stigmasterol) may offer protection from the most common cancers such as colon, breast and prostate cancer ([22]).

Palmitic acid was predominant in relation to other fatty acids. In the investigated extract were found also α-linolenic and linoleic acid, which belong to the group of so called essential fatty acids (EFAs). These two EFAs are precursors in the biosynthesis of biologically important lipids and have also been reported in the reduction of coronary heart decease ([23]). It was established that omega 3 series of fatty acids might act as indirect anti- rather than pro-oxidant ([24]).

Terpinen-4-ol, neophytadiene, hexahydrofarnesyl acetone, vitamin E, phytol and squalene were identified for the first time in the species from the genus Veronica. Several investigations revealed in vitro antioxidant activity of monoterpenes(γ-terpinene) and diterpenes (vitamin E, phytol) or essential oils([25]).

CONCLUSIONS

V. officinalis was used as a medicinal plant in Bulgaria. In this study, we pointed out that this plant not only has those well-known bioactivities but also that the polar extracts and EtOAc fraction from V. officinalis exhibited an excellent antioxidant activity based on various in vitro assays. The total phenolic content correlates well with the radical scavenging activity. V. officinalis can be used as an easily accessible source of natural antioxidants.

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