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ESR STUDIES OF ANTIOXIDATIVE ACTIVITY OF DIFFERENT ELECAMPANE (*Inula helenium* L.) EXTRACTS

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The influence of petroleum ether, ethyl acetate and water extracts of elecampane (Inula helenium L.) on the transformation of the stable 1,1-diphenyl-2-pycrylhydrazil (DPPH) radicals has been investigated by ESR spectroscopy. Using the phytochemist "screening" test a qualitative analysis of extracts has been made.

On the basis of the obtained results it can be concluded that the investigated elecampane extracts have the antioxidative activity due to the hydrogen donor ability of the constituent biomolecules such as tannins, terpenes, polyphenols, etc. The following order of antioxidative activity has been established: ethyl acetate > petroleum ether ≥ water extracts. Also, the investigation showed that the antioxidative activity increased with increasing concentration of all the extracts.

KEY WORDS: ESR, DPPH free radicals, elecampane, successive extraction, antioxidative activity

INTRODUCTION

A free radical is any chemical species capable of independent existence that possesses one or more unpaired electrons. Due to their unstauration with electrons, they are extremely reactive. They can be generated by photolysis, thermolysis, radiolysis, in oxido-reduction processes, enzymatic processes *in vivo*, by the influence of ozone, etc. In addition, free radicals are formed in human cells during normal metabolism and take place in many biochemical reactions. Uncontrolled and abnormal free radical reactions can cause deterioration of physical, biological and health characteristics of food, pharmaceutical and cosmetic products. Excessive quantity of free radicals in human

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body can produce oxidative stress and lead to many pathological states such as mutagenesis and carcinogenesis, aging, rheumatism, arthritis, arteriosclerosis, necrosis, diabetes, etc.

An antioxidant is any substance that when present at low concentrations compared to those of an oxidizable substrate significantly delays or prevents oxidation of that substrate. In order to protect itself from oxidative attack, human organism has its own antioxidative enzymes and compounds (1,2). Nevertheless, in terms of intensified production of free radicals it is necessary to take supplementary antioxidants. The usual way of preserving food and cosmetic products is by using the synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). However, clinical investigations on experimental animals have shown that some of the synthetic antioxidants have carcinogenic effect (3).

Numerous investigations have proved that medicinal herbs contain natural antioxidant compounds such as derivates or isomers of flavones, isoflavones, flavonols, catechins, tocopherols, tannins, carotenoids, terpenoids, and many others. They have the ability not only to prolong the preservation of food products but also to participate as radical scavengers in living organisms (4). Rice Evans et al. (5) have established that poliphenols have the most important role in the antioxidative effect of herbs.

Elecampane (*Inula helenium* L.) is an herb of ancient medical reputation, which used to be candies and sold as sweetmeat. It is used as an important spice, incense and medicine. In the form of tea, elecampane is used to treat respiratory conditions such as asthma, bronchitis and whooping cough, disorders of digestion, intestines and gall bladder and for skin disorders. Elecampane has antibacterial, antifungal and anti-inflammatory activity, it is used as diuretic, carminative and sedative. The pharmacological activity of elecampane is related to the content of mainly sesquiterpene lactones such as alantolactone, isoalantolactone, dihydroalantolactone, etc. However, alantolactone can be irritating to the intestinal tract and oral cavity, and when used in large doses it causes vomiting, diarrhea, convulsions and even paralysis. Elecampane also contains inulin (45%), camphor, chamazulene, waxes, bitter substances, pectin substances, etc.(6).

Elecampane originates from Europe and north Asia. It grows well on sunny, dry ground. The genus *Inula* includes 100-200 species of annual or perennial plants from Europe, Asia and Africa. The base leaves are large, 30-40 cm. Leaves are velvety underneath and rough and hairy from above. Blossoms are 7.5-10 cm, light yellow colored.

In this paper, the most contemporary analytical method for detection of free radicals, ESR spectroscopy, has been employed to investigate antioxidant activity of petroleum ether, ethyl acetate and water extracts (0.1%, 0.25% and 0.5%) of elecampane on the stable DPPH free radicals. Using the phytochemist "screening" test, a qualitative analysis of extracts has been made.

EXPERIMENTAL

Methanol, ethyl acetate, petroleum ether, ethanol, sulfuric acid, formaldehyde and hydrochloric acid were purchased from "Zorka" Šabac. 1,1-diphenyl-2-picrylhydrazyl (DPPH) was from Sigma Chemicals Co., USA.

Plant material, dried root of elecampane, *Inula helenium* L., was collected from the region of Zlatibor.

Extraction. Dried root of *Inula helenium* L. (5 g) was extracted with 70% methanol (250 mL) at 25°C for 72 h. The extract was concentrated under reduced pressure. After removing methanol, the extract was successively treated with petroleum ether (3x20 mL) and ethyl acetate (3x20 mL). The petroleum ether, ethyl acetate and remained water extract were evaporated to dryness under reduced pressure. The yield of extracts was:

- Petroleum ether extract m = 0.186 g
- Ethyl acetate extrac tm = 0.0568 g
- Water extract m = 0.916 g

Phytochemist "screening" test was estimated according to Harborne (7).

Scavenging effect on DPPH radical. A volume of x μL of 1% of the methanolic solution of investigated extracts was added to (400-x) μL methanol and 400 μL 0.4 mM methanolic solution of DPPH. The final concentrations of investigated extracts were: 0.10%, 0.25% and 0.50%. After that the mixture was stirred for 2 min. and transferred to a quartz flat cell ER-160FT. Blank probe was obtained by mixing the 400 μL 0.4 mM methanolic solution of DPPH and 400 μL of methanol.

The scavenging activity (SE) of extracts was defined as:

$$SE = 100\% \cdot (h_0 - h_x)/h_0 \qquad [1]$$

where:

- h_0 - the height of the second peak in the ESR spectrum of DPPH radical of the blank
- h_x - the height of the second peak in the ESR spectrum of DPPH radical in reaction mixture with the addition of the extracts

The ESR spectra were recorded on a Bruker 300E ESR spectrometer (Rheinstetten, Germany) under the following conditions: field modulation 100.000 kHz, modulation amplitude 0.226 G, time constant 40.96 ms conversion time 671.089 ms, center field 3440.00 G, sweep width 100.00 G, x-band frequency 9.64 GHz, power 20 mW, temperature 23°C.

RESULTS AND DISCUSSION

ESR spectrum of stable DPPH free radicals (blank) is shown in Fig 1.

Hyperfine structure of the ESR spectra of DPPH free radicals is the result of interaction of the unpaired electron with two ^{14}N atoms (I=1) and consists of five lines of relative intensities 1:2:3:2:1. Hyperfine splitting constant is a_N=9.03G. The structure of stable DPPH free radicals is shown in Fig. 2.

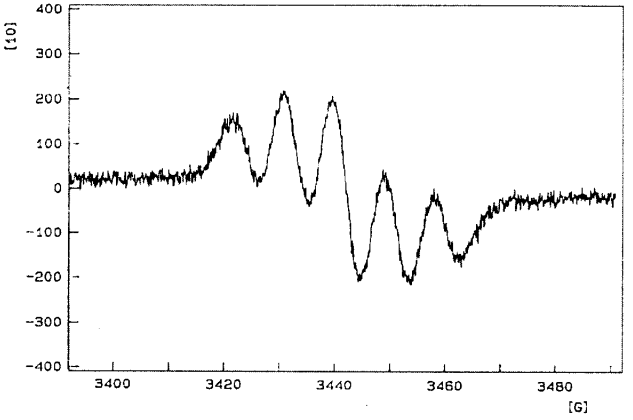

Fig. 1. ESR spectrum of stable DPPH free radicals (blank)

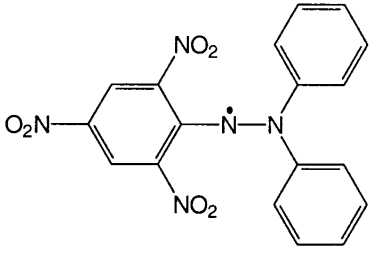

Fig. 2. The structure of stable DPPH free radicals

ESR spectra of DPPH free radicals obtained in the presence of 0.5% petroleum ether, ethyl acetate and water extracts of elecampane are presented in Figs. 3-5.

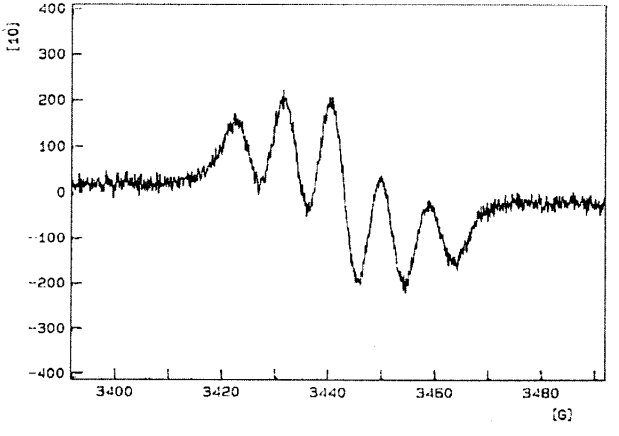

Fig. 3. ESR spectrum of DPPH radicals obtained in the presence of 0.5% petroleum ether extract of elecampane *(Inula helenium* L.)

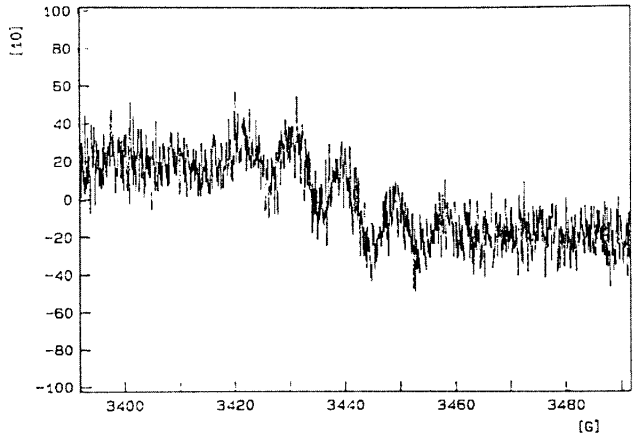

Fig 4. ESR spectrum of DPPH radicals obtained in the presence of 0.5% ethyl acetate extract of elecampane (*Inula helenium* L.)

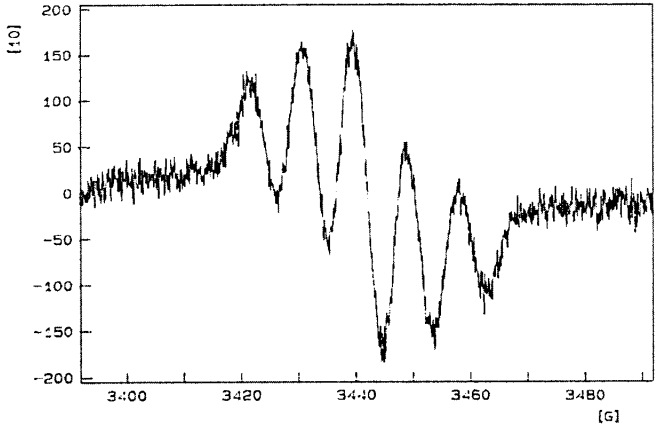

Fig 5. ESR spectrum of DPPH radicals obtained in the presence of 0.5% water extract of elecampane (*Inula helenium* L.)

The scavenging effect of petroleum ether, ethyl acetate and water extracts of elecampane on DPPH radicals is presented in Fig.6.

No change in the shapes of ESR spectra in all examined cases was detected, but the intensity of ESR signals, corresponding to the concentration of DPPH radicals decreased in the presence of each extract. The intensity of ESR signal of DPPH radicals decreased with increasing concentrations of the investigated extracts.

The following order of antioxidative activity can be established: ethyl acetate > petroleum ether ≥ water extracts.

On the basis of the results shown Fig 6 it can be concluded that the strongest antioxidant activity on DPPH free radicals has the ethyl acetate extract (SE=90.49%) used in highest

concentration (0.50%). Besides, the results show that the petroleum ether extract has the similar antioxidative activity as the water extract.

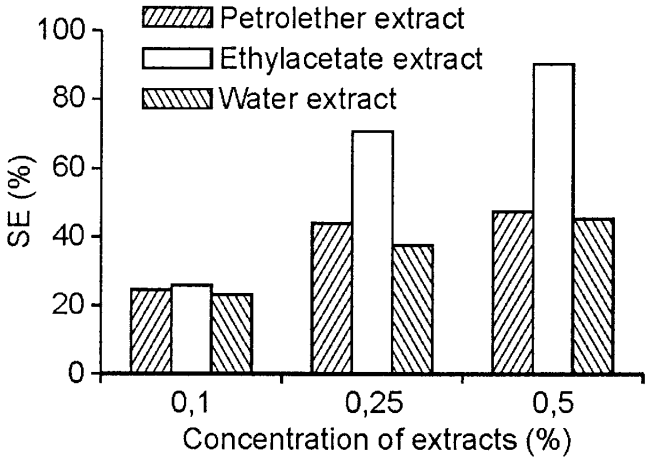

Fig. 6. Scavenging effect of different concentrations of petroleum ether, ethyl acetate and water extracts of elecampane on DPPH radicals

Bohlman et al. (8) established that the elecampane contains sesquiterpenes shown in Fig. 7.

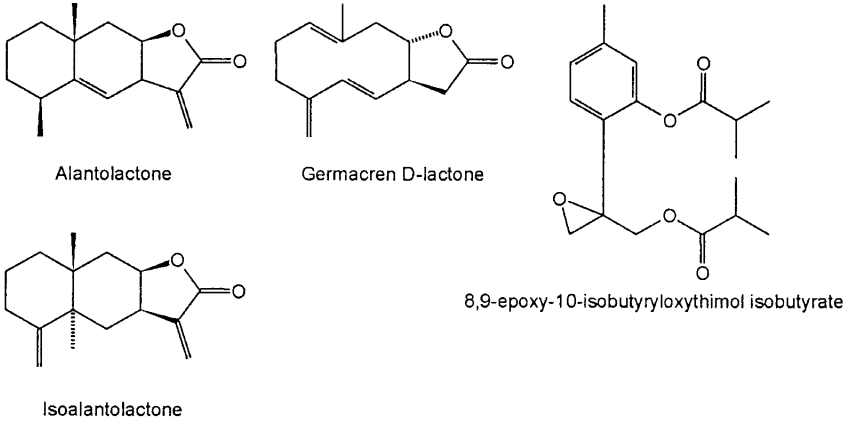

Fig. 7. Sesquiterpenes isolated from elecampane

Harborne (9) showed that the root of elecampane contains polyphenols such as quercetin-7-triglycoside.

Using the phyitochemist "screening" test the presence of tannins and saponins has been proven. Generally, it can be concluded that active organic compounds cause the reduction of the concentration of DPPH free radicals. The activity of the extracts is therefore attributed to their hydrogen-donating ability.

CONCLUSION

- Employing the ESR spectroscopy, the antioxidative activity of elecampane extracts on stable DPPH free radicals was established;
- Antioxidative effect depends on the type and the concentration of the extracts;
- The following order of antioxidative activity has been established: ethyl acetate > petroleum ether ≥ water extracts;
- Antioxidative activity increased with increasing concentration of all the extracts;
- The most significant antioxidative effect (90.49%) is shown with highest concentration (0.50%) of ethyl acetate extract;
- The mechanism of antioxidative activity is probably based on the ability of the biomolecules such as tannins, terpenes, polyphenols, etc. present in elecampane to act as donors of hydrogen atom.

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REFERENCES

1. Auroma, O.I.: Nutrition and health aspects of free radicals and antioxidants. Food Chem. Toxicol. **32** (1994) 671-683.
2. Ramarathnam, N., Osawa, T.,Ochi, H. and S. Kawakishi: The contribution of plant food antioxidants to human health. Trends in Food Science & Technology **6** (1995) 75-82.
3. Chen, Q., Shi, H., and C.T. Ho: Effects of Rosemary extracts and Major Constituents on lipid oxidation and Soybean Lipoxygenase Activity. JAOCS **69** (1992) 999-1002.
4. Milić, B.LJ., Đilas, S.M. and J.M. Čanadanović-Brunet: Interakcije fenolnih jedinjenja i slobodnih radikala sa nesparenim elektronima na O- i N-atomu. Jubilarni naučni skup "Sto godina SHD 1897-1997", Beograd, 25-26. September 1997., p. 36.
5. Rice Evans, C.A., Miller, N.J. and G. Paganga: Stucture-Antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biol. Med. **20** (1996) 933-937.
6. Lawless, J.: The Illustrated Encyclopedia of Essential Oils, Barnes & Noble books, New York, (1999) p. 153.
7. Harborne, J.B.: Phytochemical methods, Chapman and Hall Ltd., London (1976) p.14
8. Bohlman, F., Mahaute, P.K., Jakupović, J., Rastogi, R.C. and A. Natu: New sesquiterpenes lactones from Inula Helenium. Phytochemistry **17** (1978) 1165-1170.
9. Harborne, J.B. and C.A. Williams: Flavone and Flavonol Glycosides, in The flavonoids, advances in research. Eds. J.B. Harborne and T.J. Mabry, London (1982) pp. 286-287.

ЕСР ИСПИТИВАЊЕ АНТИОКСИДАТИВНЕ АКТИВНОСТИ РАЗЛИЧИТИХ ЕКСТРАКАТА ОМАНА (*Inula helenium* L.)

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Извршена је сукцесивна екстракција корена омана 70% метанолом, петролетром и етилацетатом. Утицај добијених екстраката омана на трансформацију стабилних 1,1-дифенил-2-пикрилхидразил (ДППХ) радикала испитан је електрон спин резонантном (ЕСР) спектроскопијом. Квалитативна анализа екстраката извршена је фитохемијским скрининг тестом.

На основу добијених резултата може се закључити да сви испитивани екстракти омана поседују антиоксидативну активност и то према следећем редоследу: етил-ацетатни > петролетарски > водени екстракт. Такође је утврђено да се антиоксида-тивна активност повећава са повећањем концентрације екстракта. Антиоксида-тивна активност испитиваних екстраката условљена је присуством биомолекула, као што су танини, терпени, полифеноли итд., који имају способност да предају водоников атом.

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