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HPLC analysis of polyphenols and antioxidant capacity determination of *Scirpus holoschoenus* L. rhizome

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Abstract The aim of the study was to analyse polyphenols compounds from vegetal product *Holoschoeni rhizome* and its antioxidant capacity in order to justify traditional therapeutic using (livery protect effect). The separation, identification and quantification of polyphenols compounds were made through High performance of liquid chromatography (HPLC), standardized method according USP30-NF25 Monograph.
Total antioxidant activity was determined through photochemiluminiscence method as ACL (Antioxidant capacity of lipid soluble substances). In vegetal product *Holoschoeni rhizome* we identified the follows: E and Z resveratrol, vanillin and phenol carboxylic acids (chlorogenic, caffeic, cinnamic and gallic). From all of this we measured E – resveratrol, vanillin, and chlorogenic, caffeic and gallic acids. Antioxidant activity of alcoholic extract from *Scirpus holoschoenus* L rhizome is 23.402 mmols equivalent TROLOX/100 g vegetal product.

Keywords: *Scirpus holoschoenus* L., polyphenols, resveratrol, caffeic acid, antioxidant capacity

1. Introduction

Scirpus holoschoenus L. sin. *Holoschoenus vulgaris* Link. is the studied herb evergreen, with strong rhizome disposed like ray and traditional name is "țipirig", "pipirig" or "dicop" [1, 2].

In Romania, it's spread on wet, sandy ground or even on dried sandy ground, on Danube' s shores and maritime dunes of coastline. We found it in Oltenia (Dunăreni-Bistreț) [3], Dobrogea (Letea, Histria, Vadu, Caraorman, Sf. Gheorghe, Tulcea), Galați (Hanu-Conachi) [4, 5].

M. Abdel-Mogib et al. (2001) studied *Holoschoenus vulgaris* Link sin. *Scirpus holoschoenus* L specie from phytochemicaly point of view and they insulated the follows compounds: 3,5,4'-trimetoxistilben, 2-dimetilpropileten-3,5,4'-trimetoxistilben, 2-dimetilpropileten-3-hidroxi-5,4'-dimetoxistilben, 2-dimetilpropileten-3,4'-dihidroxi-5-metoxistilben şi 3,5,4'-trimetoxistilben and a new acetofenona derivative [6].

In traditional medicine, Russians from Dobrogea use tea of dicop's rhizome for its livery protect effect.

Other species of this genus were phytochemicaly studied and were found benzaldehyde derivative, hidroxy benzoic acids and cinnamic acid in *Scirpus lacustris specie*; caffeic and cumaric acids, quercetol, kaempferol, apigenol and luteol in *Scirpus wichurai*. It was also remarked the presence of some triterpenes, stilbenic derivative, scirpuzina A and B (dimers) and resveratrol in (3,3',4,5'-tetrahidroxistilben) in *Scirpus fluviatilis* [6].

Scirpus americanus and *Scirpus maritimus* extracts proved an important activity on leukaemia lymphoma. *Scirpus lacustris* rhizome extract is bacillicide for *Escherichia coli* [6].

The purpose of the paper is to analyse polyphenol compounds and its antioxidant capacity for *Holoschoeni rhizoma* vegetal product in order to justify traditional therapeutic using (livery protect effect).

2. Experimental

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Holoschoenus vulgaris Link rhizome was reaped from Dobrogea, Vadu village, Corbu township, in October 2008. We washed material in quick water spurt and dried in warm air. We identified the species and a sample of it was kept in Pharmacognosy Laboratory, Faculty of Pharmacy, Ovidius University, Constanta.

The separation, identification and quantification of polyphenols compounds were made through High performance of liquid chromatography (HPLC), standardized method according USP30-NF25 Monograph [7]. Technical data are the follows: HPLC Agilent 1200, with quaternary pomp, DAD, thermostat, degassing system, autosampler; chromatographic column C18, 250 mm × 4.6 mm; 5 µm (Zorbax XDB or equivalent); mobile phase: A solution (phosphoric acid 0.1%), B solution (acetonitrile), eluted in gradient (**Table 1**); temperature: 35^0C; flow rate: 1.5 mL/min; detection: UV 310 nm; injection volume = 20 µL; analysis time = 22 minutes.

Table 1. Work gradient of HPLC analysis

Time, min.	Soluţion A, mL %	Soluţion B, mL %
0-13	90	10
13	7	22
13	78	22
14	60	40
17	60	40
17,5	90	10
22	90	10

Solution for analyse was dispense as follow: 10 g vegetal product with 5.6283 g% moisture is back-flow extracted 6 hours, with 100 mL methanol 70%. We filtered the result solution and filled out in 100 mL volumetric flask, with methanol 70%.

Reference substances (solutions in methanol 70%): E - resveratrol (ChromaDex) = 37 mg/mL, Z – resveratrol = 0,22 mg/mL made by us of trans resveratrol solution exposed to UV ray 254 nm, for 12 hours (**Fig. 1**), caffeic acid = 0.36 mg/mL, chlorogenic acid = 0.37 mg/mL, cinnamic acid = 0.58 mg/mL, vanillin = 0.42 mg/mL, gallic acid = 0.39 mg/mL. Reference substances were 6 times injected. **Table 2** contain retention time of polyphenol compounds. We used a mix of them in order to simplify determinations.

Table 2. Retention time of polyphenol compounds, reference substances

No.	Phenol compound	Retention time
1.	E- resveratrol	14,467 ± 0,017*
2.	Z- resveratrol	15,751 ± 0,058*
3.	caffeic acid	4,598 ± 0,036*
4.	chlorogenic acid	3,501 ± 0,015*
5.	cinnamic acid	15,867 ± 0,007*
6.	vanillin	6,919 ± 0,051*
7.	gallic acid	0,990 ± 0,025*

*standard deviation values of the 6 injections, from statistic calculations (SPSS 10)

In **Fig 2** is presented the mix standard chromatogram used for identification and quantitatively measurements of main compounds. Square of correlation coefficient for calibration curve estimates reproducibility of method. (**Table 3**).

Table 3. Correlation coefficient of calibration curve, square

No.	Phenol compound	r^2
1.	E-resveratrol	0.99965
2.	Z-resveratrol	0.99729
3.	chlorogenic acid	0.99999
4.	caffeic acid	0.99619
5.	cinnamic acid	0.99845
6.	vanillin	0.99691
7.	gallic acid	0.99537

There are many methods (*in vitro* and *in vivo*) to determinate antioxidant activity: TEAC determination, seric malondialdehyde determination and low and total seric glutation determination [8].

We estimated total antioxidant activity of *Holoschoeni rhizome* aqua-alcoholic solution using Chemiluminescence's method in lipid samples (ACL), according to Analytic Jena procedure, Germany, using PHOTOCHEM coupled to PC apparatus [9, 10].

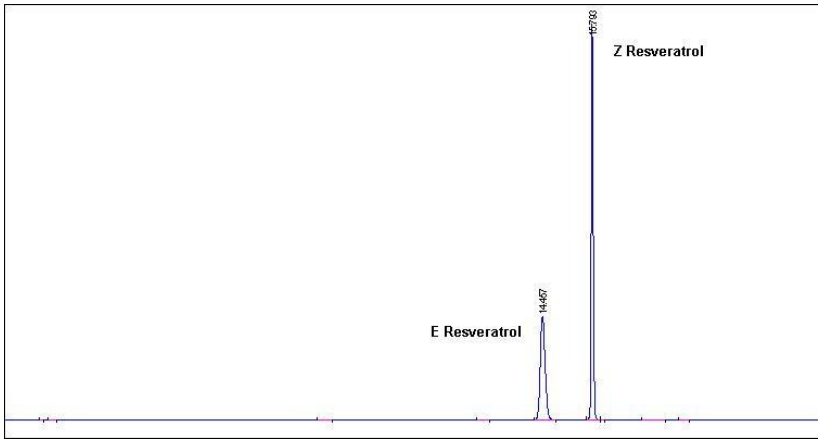

Fig. 1. Resveratrol HPLC chromatogram after UV exposure

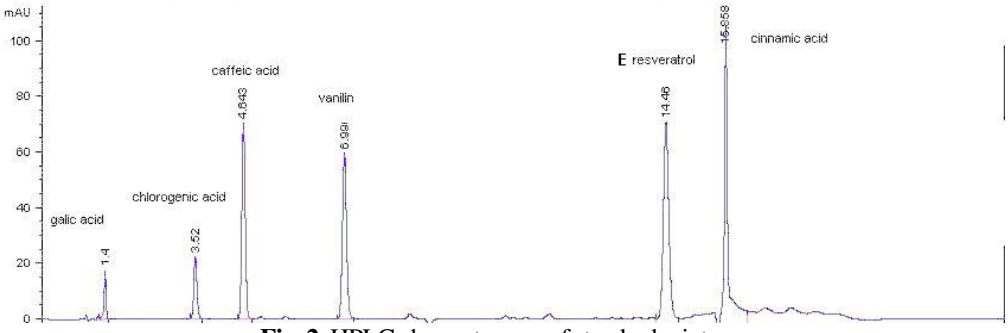

Fig. 2. HPLC chromatogram of standard mixture

Extern source of light is Hg lamp carried with phosphorus with highest energy on $\lambda = 351$ nm and free radicals source is H_2O_2.

Solution for analyse was dispense as follow: 10 g vegetal product is back-flow extracted 6 hours using methanol 50%. We filled out the result solution in 100 mL volumetric flask. 1mL of this solution was filled out in 50 mL with ethanol 50%. We took samples of 5 µL, 10 µL, 20 µL and 30 µL of this solution. We calculated antioxidant capacity in comparison with TROLOX standard (2 carboxilic–6-OH-2, 5, 7 acid – tetrametilcroman). This standard helps us to plot calibration curve for 4 concentrations (0.5, 1, 2 and 3 nmols TROLOX).

This reaction takes place in the presence of a light quant registered by detector. (photomultiplicator). The result is expressed in units of TROLOX (mmols).

3. Results and Discussions

According to HPLC analysis, we identified E- and Z-resveratrol, vanillin and chlorogenic, caffeic, cinnamic, and gallic acids (**Fig. 3**) from all peaks of sample. E–resveratrol (24.11 mg %), vanillin (5.18 mg %), chlorogenic acid (6.86 mg %), caffeic acid (6.28 mg %) and gallic acid (291.00 mg %) were quantitatively measured. These substances have antiviral and low level lipids effects [11]. Antioxidant capacity of alcoholic extract from *Scirpus holoschoenus* L. rhizome is 23.402 mmols equivalent TROLOX/100 g vegetal product.

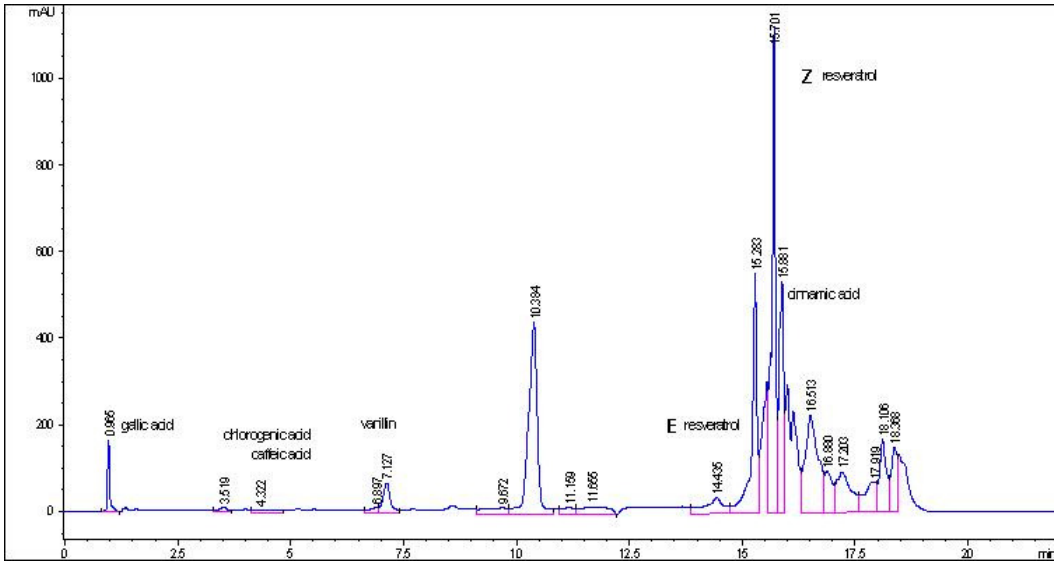

Fig. 3. HPLC Chromatogram of *Holoschoeni rhizoma* polyphenol compounds (methanol extractive solution 70%)

We consider that analysed vegetal product might favour cure of liver diseases with inflammation and steatosis because of its antiviral, antioxidant and low level lipids effects. Pharmacological properties of identified compounds prove this statement [12].

4. Conclusions

Holoschoeni rhizome vegetal product contain E and Z resveratrol, vanillin and phenol carboxylic acids (clorogenic, cafeic, cinamic and galic). The concentrations of studied polyphenol compounds are: E – resveratrol (24.11 mg %), vanillin (5.18 mg %), and chlorogenic acid (6.86 mg %), caffeic acid (6.28 mg %) and gallic acid (291.00 mg %).

According to our study regarding identifying and dosing some active principles, we consider that using *Holoschoeni rhizome* vegetal product in traditional medicine is justified because of its antioxidant, antiviral and lower levels of lipids properties.

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