

IDENTIFICATION OF ACETOVANILLONE (APOCYNIN) FROM WATER-ETHANOL EXTRACT OF THE STEM OF SAPERAVI VINE VARIETY

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Individual substance was extracted by preparation from water-ethanol extract of the stem of Saperavi vine variety. The spectrum examination was done, the melting temperature was determined (114-115°C) and the substance was identified as acetovanillone (apocynin). The characteristics were determined using liquid chromatography (RT – 20,545 min.) and optimal wave length was revealed – 269 nm. The concentration of acetovanillone in the water-ethanol extract of Saperavi vine variety stem was defined as 5.2 mg/l. Antioxidant activity of acetovanillone (EPMR) is 33%. Its content in the stem extract is a positive feature for functional purpose of the target product due to its curative-prophylactic value.

INTRODUCTION

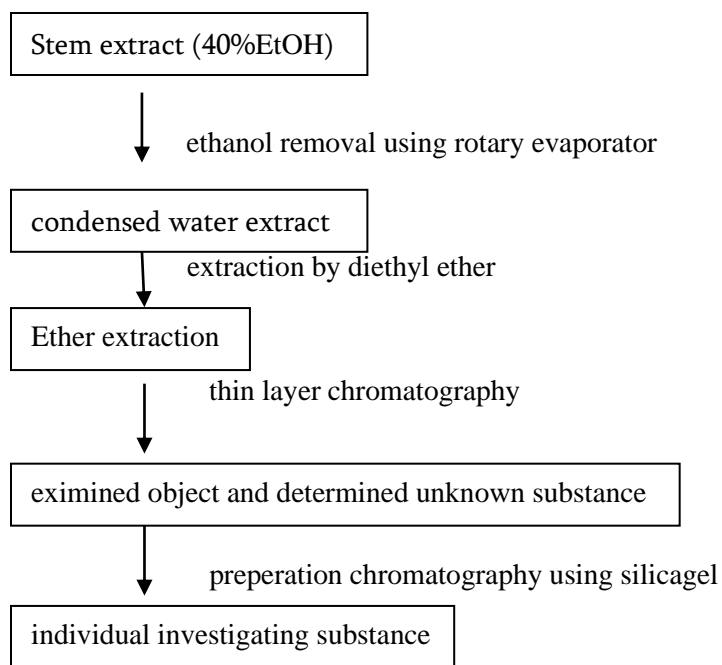
Production of biologically active food additives (biologically active supplement) has become actual since the second part of 20th century. This direction was based on pharmacological, biochemical and scientific achievements of nutrition industry. Biologically active supplement is regarded as a food product. The curative-prophylactic value of these products is due to the biological activity of different kind of chemical components; amongst the phenolic compounds play important part. For this purpose, diverse spectrum of grape phenolic compounds and their high antioxidant activity is very interesting. For this reason, wines gain the functional purpose. Besides technology of producing biologically active food supplements containing grape phenolic compounds are developed. For example, “Mega-Pro” (USA. UK), “Pignojenol” and “Antiox”

(France), “Cholikan” (China), “Enoant” (Ukraine), “Immortel Classic”, “Immortel Red Miracl” [1-3].

New technology of biologically active food supplement is important for this kind of direction and our goal is to study these substances. Because one of the ingredients of biologically active supplement is made from the stem of saperavi vine variety, our aim was to identify its biologically active substances. For this reason, we have conducted the experiment presented in this article, based of which biologically active supplement - acetovanillone (apocynin) was identified from the stem of Saperavi vine variety.

RESEARCH OBJECT AND METHODS

We have used water-ethanol extract of the stem of Saperavi vine variety, which was treated according to the diagram shown below (scheme 1).



Scheme 1. acetovanillone (apocynin) extraction diagram from the stem of Saperavi vine variety water-ethanol extract

We have done qualitative analysis using thin layer chromatography on the silicagel plates (sorbfil, ПТСХ- II-A 10x20), for the system we used organic solvent mixture – chlorophorm:

methanol (80:20) and revealed the chromatographs by diazotisation sulphanic acid. The investigating substance was extracted individually as preparation and have done spectrum analysis: violet – diethylether; “VARIAN”, CARRY 100; infrared – on Ge plate; „THERMO NIKOLET“, AVATAR 370; also we have determined the melting temperature - „MEL TEMP 3“. During the experiment we used individual acetovanillone for comparison.

Antioxidant activity of acetovanillone was determined by method of electro paramagnetic resonansce (EPMR) [4].

RESULTS AND DISCUSSIONS

The identified substance find from the water-ethanol extract of Saperavi vine variety stem after thin layer chromatography reveal is shown as redish-orange color spot. It is characterized with high RF-0,93 and does not correspond with other low-molecular phenolic compounds used during experiment, amongst phenolic acids and phenol aldehydes (figure 1).



fig. 1. thin layer chromatography of the diethylether fraction of the water-ethanol extract of the stem (I) individual acetovanillone (II) and acetosyringone (III). System chloroform: methanol (80:20). Revealing substance – diazotisation sulphanilic acid.

According to the data we have decided to use acetovanillone and acetosyringone for comparison. Due to the experiment results relevant to the unknown spot revealed by chromatography, extracted as preparation and purified substance due to the spectrum data and melting temperature is confirmed that it corresponds to acetovanillone (apocynin) (figure 2,3).

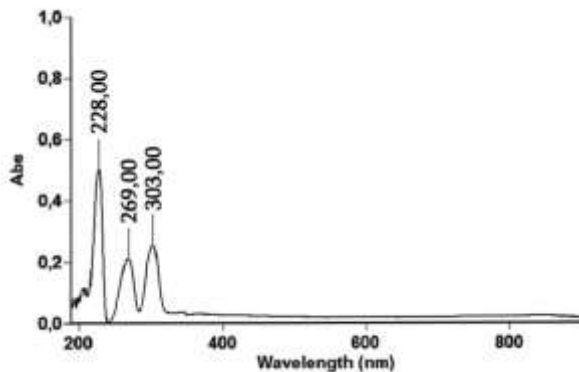
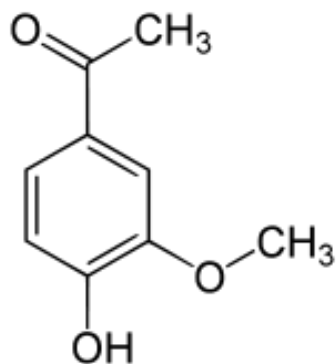


fig.2. ultraviolet spectrum apocynin



acetovanillone (apocynin)

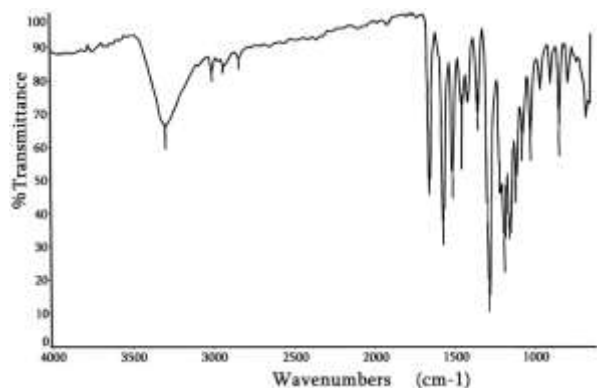


fig. 3. acetovanillone infrared spectrum

Spectrum characteristics are the following: ultraviolet spectrum (diethyl ether) λ max (nm) - 228; 269; 303. Infrared spectrum (on Ge plate) (cm^{-1}) – 3293 (OH-phenolic); 1658 (C =O carbonic group); 1573 (aromatic nucleus C = together with chain); 1511 (aromatic nucleus chain waving); 1450 (C-H chain methoxyl group); 1357 (phenolic OH); 1288 (C-O-C methoxyl); 1187 (methoxyl group- OCH_3).

Investigating mixture is melting at $114-115^{\circ}C$. According to the spectrum data and other data indication investigating substance is totally relevant with compared acetovanillone, based on this it is identified as acetovanillone (apocynin). It could be added that acetosyringone was not found in the extract of stem. Identified substance is soluble in the water, acetone, water-ethanol solvent, poorly soluble in the cold water.

After identification of acetovanillone we have decided to make the quantitative analysis in the stem of Saperavi vine variety. For this purpose we have done liquid chromatography of individual acetovanillone on “Varian ProStar” chromatography. The conditions of chromatography were the following: Column-Supercosil LC-18-DB. 25 smx4,6mm, 5µm, eluent A-0,5% water solution of H₃PO₄; eluent B-50% acetonitrile; 0,5% H₃PO₄; 49,5% - H₂O. The flow rate 1ml/min. Wave length – 280 µm. Detector-violet. Chromatography was done at 228, 229, 303 nm wave length. Amongst the maximum peak was revealed at 269 nm. According to the data chromatography analysis of investigating extract was done at 269 nm. Due to the liquid chromatography of acetovanillone is characterized by delay time RT – 20,545 min. (figure 4a,b).

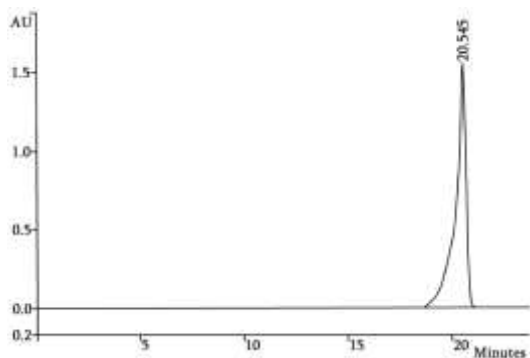


fig. 4a. liquid chromatography of acetovanillone

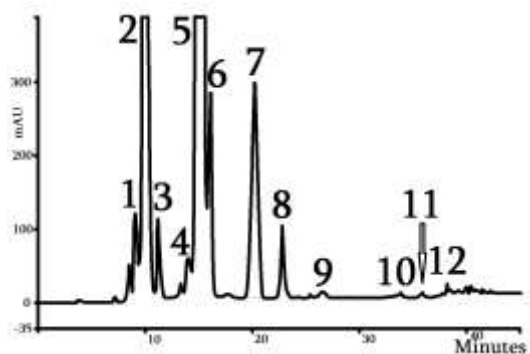


fig. 4b. liquid chromatography of the extract of Saperavi vine variety stem. 7- acetovanilone

The concentration of acetovanillone in water-ethanol extract is 5,2 mg/l. Due to the biological activity its existence in the stem extract and correspondingly in biologically active supplement,

has the positive feature due to the curative-prophylactic value. It could be mentioned also that antioxidant activity of individual acetovanillone water-ethanol solvent (C – 10 mg/l) is 33%.

According to the medical researches biological activity of apocynin was determined. Besides its pharmacological features was first depicted in 1883 year by German pharmacologist Oswald Schmiedeberg. Apocynin was extracted from “Apocynum Cannabinum” roots. Apocynin for a long time was used for treatment of heart diseases; also, in 1971-year apocynin was extracted from the plant “picrohiza kurroa” spread in Himalaya, its roots was used for treating heart, liver diseases; asthma and hepatitis A [5-9].

Biological activity of apocynin is revealed by anti- flammable and anti-rheumatism effect. Also, the main source of super oxidative radical - NADPH – oxidase is determined by inhibiting features and other medical researches, that NADPH – oxidase develops super oxidative radical which has negative effect and activates of the diseases like, ischemic insult, disorder of blood circulation in the brain, Alzheimer’s and Parkinson’s disease. The scientists mention that apocynin has curative value towards the before mentioned diseases [10].

CONCLUSION

As a result of research, for the first time biologically active phenolic low molecular compound acetovanillone (apocynin) was identified from the water-ethanol extract of the stem of Saperavi vine variety and defined by liquid chromatography. As one of the ingredient of our бад – biologically active supplement is the extract of the stem of Saperavi vine variety, the existence of apocynin has positive feature as curative-prophylactic value.

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Идентификация ацетованилона (поницина) из водно-спиртового экстракта гребней винограда сорта саперави

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В результате проведенного эксперимента в водно-спиртовом экстракте гребней винограда сорта саперави, впервые идентифицирован и хроматографически определен низкомолекулярное, биологически активное фенольное соединение- ацетованилон (поницин). Ввиду того, что экстракт гребней винограда является ингредиентом экспериментального БАД-а, наличие в нем ацетованилона (поницина) характеризуется, как положительный показатель с точки зрения его лечебно-профилактической ценности.