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Research Article

Study on Determination of Synephrine and Artemisinin in *Phellinus vaninii* by High Performance Capillary Electrophoresis

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Abstract: This study established high performance capillary electrophoresis method to determine synephrine and Artemisinin in *Phellinus vaninii*. In 30 mmol/L $Na_2B_4O_7$ buffer solution (pH = 10.00), effective separation of components to be tested was achieved, which provided a new approach of quality control of Chinese medicine *Phellinus vaninii*.

Keywords: Artemisinin, high performance capillary electrophoresis, *Phellinus vaninii*, synephrine

INTRODUCTION

Phellinus vaninii (Japanese "meshimakobu", Chinese "song gen", Korean "sanghwang", English "Meshima", American English "black hoof mushroom") is a medicinal mushroom used in Japan, Korea and China for centuries to prevent ailments as diverse as gastroenteric dysfunction, diarrhea, haemorrhage and cancers. It is shaped like a hoof, has a bitter taste and in the wild grows on mulberry trees. The stem's color ranges from dark brown to black. In Korean traditional medicine, the mushroom is consumed in the form of hot tea. Early research has suggested that *Phellinus vaninii* has anti-breast cancer activity (Gong and Cao, 2009). A paper published by Harvard Medical School reported that *Phellinus vaninii* is a promising anti-cancer agent, but that more research is required to understand the mechanisms behind its anti-cancer activity (Xu and Su, 2013) Nine compounds were isolated from the active ethylacetate fraction of the fruiting body and identified as protocatechuic acid, caffeic acid, protocatechualdehyde, ellagic acid, hypholomine B, hispidin, davallialactone, interfungins A and inoscavin A of which interfungins A is a potent inhibitor of protein glycation (Herrmann *et al*., 2013).

Extracts from fruit-bodies or mycelium of *Phellinus vaninii* stimulate the hormonal and cell-mediated immune function; quench the inflammatory reactions caused by a variety of stimuli and suppress tumor growth and metastasis. Artemisia argyi has effects on eliminating cold and relief pain, warming menstruation and hemostasis, recuperating breath and miscarriage prevention, antibacterial and antiviral. It has been used for the treatment of gastrointestinal tumor, lung cancer, thyroid cancer, abdomen crymodynia, irregular menstruation, uterine deficiency and infertility, hematemesis and so on (Miyazato *et al*., 2013). And Artemisininhas antibacterial, anti-inflammatory, anticancer, spasmolysis and choleretic effects. Synephrine has effects on elevated blood pressure, shock resistance, improving metabolism and so on, which is used for the treatment of bronchial asthma, hypotension in surgery and anesthesia, collapse and shock, etc., in clinical.

Capillary electrophoresis that is a new separation and analysis technology developed in recent years has characteristics of fast, efficiency, trace, high sensitivity, experimental economics, etc., which has a wide range of applications in the chemical, life sciences, pharmaceutical and other fields (Xue *et al*., 2013). This paper established capillary electrophoresis method to determine synephrine and Artemisinin content in *Phellinus vaninii*.

Artemisinin also known as Qinghaosu and its derivatives are a group of drugs that possess the most rapid action of all current drugs against Plasmodium falciparum malaria (Liu *et al*., 2014). Treatments containing an artemisinin derivative (Artemisinin-Combination Therapies, ACTs) are now standard treatment worldwide for *P. falciparum* malaria. Artemisinin is isolated from the plant Artemisia annua, sweet wormwood, a herb employed in Chinese traditional medicine. It can now also be produced using genetically engineered yeast. Chemically, artemisinin is

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a sesquiterpene lactone containing an unusual peroxide bridge. This peroxide is believed to be responsible for the drug's mechanism of action. Few other natural compounds with such a peroxide bridge are known (Herrmann *et al*., 2013). Use of the drug by itself as a monotherapy is explicitly discouraged by the World Health Organization, as there have been signs that malarial parasites are developing resistance to the drug. Therapies that combine artemisinin with some other anti-malarial drug are the preferred treatment for malaria and are both effective and well tolerated in patients. The drug is also increasingly being used in Plasmodium vivax malaria, (Chang and Zhao, 2014) as well as being a topic of research in cancer treatment.

Capillary Electrophoresis (CE) is a family of electro-kinetic separation methods performed in sub-millimeter capillaries and in micro- and nanofluidic channels. Very often, CE refers to Capillary Zone Electrophoresis (CZE), but other electrophoretic techniques including Capillary Gel Electrophoresis (CGE), Capillary Isoelectric Focusing (CIEF), capillary isotachophoresis and Micellar Electrokinetic Chromatography (MEKC) belong also to this class of methods (Stefansson and Novotny, 1994). In CE methods, analytes migrate through electrolyte solutions under the influence of an electric field. Analytes can be separated according to ionic mobility, additionally they may be concentrated by means of gradients in conductivity and pH.

This study established high performance capillary electrophoresis method to determine synephrine and Artemisinin in *Phellinus vaninii*. In 30 mmol/L $Na_2B_4O_7$ buffer solution (pH = 10.00), effective separation of components to be tested was achieved, which provided a new approach of quality control of Chinese medicine *Phellinus vaninii*.

MATERIALS AND METHODS

Materials:

Experimental section:

Instruments and reagents: Experimental instruments: CESI 8000-type high performance capillary electrophoresis (U.S.A, AB SciexScientific Instrument Co., Ltd.); HW2000-type chromatography workstation (Nanjing Qianpu Software Ltd.); Capillary (75 μm inner diameter, 60 cm overall length, 51 cm effective length) from Hebei YongnianRuifeng Chromatographic Devices Co., Ltd.); Precision pH meter (Shanghai Leici Instrument Factory).

Synephrine and Artemisinin were purchased from Shanxi Huike Botanical Development Co., Ltd. *Phellinus vaninii* were purchased from the Shandong Weifang Yuandong Pharmaceutical Co., Ltd., (Yaodu Group, lot number 070641). Other reagents used in the experiments were all analytical grade; Double-distilled water.

Experimental methods: Before the start of the experiment, capillary was successively washed with 0.5 mol/L hydrochloric acid solution, double-distilled water, 0.5 mol/L sodium hydroxide solution, double-distilled water, buffer solution, each for 8 min. Between each two run, capillary was only washed with buffer solution for 4 min. After three times run, capillary was cleaned again using the above method.

Measurements were carried out at 20 kV running voltage and 254 nmUV detection wavelength (Although literature reported that two kinds of analytes in general had strong absorption at about 275 nm, it was found the two substances had a strong absorption at 254 nm. So the experiment was measured at 254 nm). Experimental temperature was 24°C. Gravity injection time was 8 sec (7.5 cm height difference).

Sample preparation: First, *Phellinus vaninii* sample were pulverized into powder. *Phellinus vaninii* powder was accurately weighed 0.6352 and 0.8081 g into the conical flask, then respectively added 95 and 50% ethanol each 20 mL, which was extracted at 60°C for 3 h. After filtration, washing and set volume to 25 mL, that were, respectively *Phellinus vaninii* sample solution 1 and 2.

RESULTS AND DISCUSSION

Effects of buffer concentration on migration behavior of synephrine and artemisinin: Preparation different concentrations of borax buffer solution, running synephrine and Artemisinin sample solution, the effects of buffer concentration on the migration behavior of synephrine and Artemisinin were investigated. With the increase of the concentration of borax, synephrinemigration time was little changed and Artemisinin migration time was slightly changed. Meanwhile, electro osmotic flow peaks of synephrine and Artemisinin were exactly at the same position. Considering the size of the bajaur heat and stability of running current, 30 mmol/L borax solution was chose for the further research.

Effects of buffer solution pH on migration behavior of synephrine and artemisinin: The pH value of 30 mmol/L borax buffer solutions was adjusted with 1 mol/L sodium hydroxide. Here, the experiment investigated the effects of buffer solution pH on synephrine and Artemisinin migration time. With the increase of the buffer solution pH value, synephrine and electroosmotic flow peakwere gradually pull opened. At pH of 10.00, synephrine and electro osmotic flow peak were already very obvious, the two substances peak shapes were also better, the peak time was not too long and two substances were also received effective separation from other components in the sample. So, pH 10.00 of buffer solution was chose for quantitative analysis.

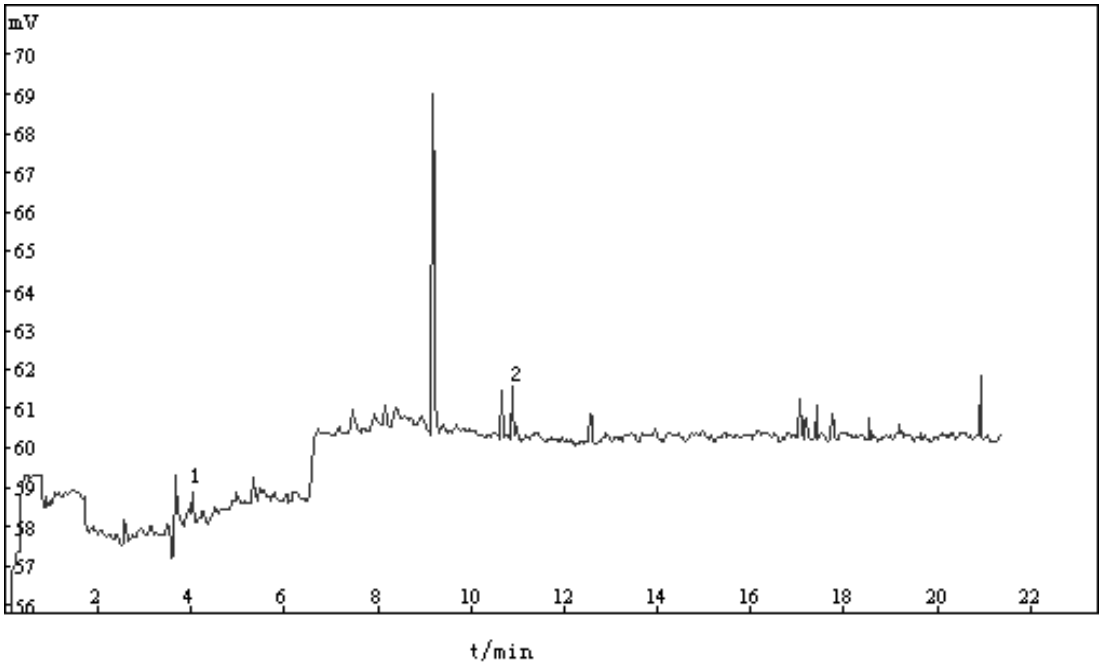

Fig. 1: The electrophorogram of *Phellinus vaninii* sample solution 1 (1: Synephrine; 2: Artemisinin)

Standard curve: First, preparation different concentration of synephrine and Artemisinin standard solution, each standard solution was run for three times under the above-identified analysis conditions. Taking concentration as the abscissa and peak area as the ordinate, the standard curve was drew. Linear regression equation (peak area: y μV•s, density:x mg/mL) and the linear range were as follows:

Synephrine:

y = 18.74 + 476746.71x (r = 0.998), 1~0.0625 mg/mL

Artemisinin:

y = -18260.98 + 1377750x (r = 0.999), 1.75~0.0273 mg/mL

Recovery: Sample solutions run for four times, measured average recoveries of synephrine and Artemisininin sample solution 1 were, respectively 84.3% (RSD = 8.1%) and 81% (RSD = 6.1%). Measured average recoveries of synephrine and Artemisinin in sample solution 2 were, respectively 78.2% (RSD = 7.3%) and 81.6% (RSD = 9.1%).

CONCLUSION

In 30 mmol/L borax buffer solution (pH = 10.00) conditions, *Phellinus vaninii* sample solution 1 and 2 were run each for five times and the results were averaged. Taking experimentally measured peak area into the linear equation and calculating the concentration, it was converted into the contents of synephrine and Artemisinin. The electrophorogram of the sample solution 1was shown in Fig. 1. The contents of two substances in *Phellinus vaninii* sample solution 1 were as follow: Synephrine 0.0358 mg/g, RSD = 8.7% (n = 5); Artemisinin: 0.591 mg/g, RSD = 6.7% (n = 5). The contents of two substances in *Phellinus vaninii* sample solution 2 were as follow: Synephrine 0.0188 mg/g, RSD = 7.1% (n = 4); Artemisinin: 0.420 mg/g, RSD = 6.3% (n = 4). Thus, the results showed that 95% ethanol extraction rate was more than 50% ethanol extraction rate forsynephrine, 95% ethanol extraction rate was less than 50% ethanol extraction rate for Artemisinin.

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REFERENCES

Chang, L. and Y. Zhao, 2014. Studies on preparation and properties of Vc nano liposomes. J. Appl. Sci. Eng. Innovat., 1(4): 237-240.

Gong, X.Y. and X.J. Cao, 2009. Measurement and correlation of solubility of artemisinin in supercritical carbon dioxide. Fluid Phase Equilibr., 284(1): 26-30.

Herrmann, S., K.K. Jessing, N.O.G. Jrgensen, K.E. Cedergreen and B.W. Strobel, 2013. Distribution and ecological impact of artemisinin derived from *Artemisia annua* L. in an agricultural ecosystem. Soil Biol. Biochem., 57: 164-172.

Liu, J., L. Guo, L. Yang, Z. Liu and C. He, 2014. Study on the rheological property of cassava starch adhesives. Adv. J. Food Sci. Technol., 6(3): 374-377.

Miyazato, H., M. Nakamura, S. Hashimoto and S. Hayashi, 2013. Odor-active (*E*)-4-methyl-3-hexenoic acid in roasted coffee generated in the maillard reactions of L-Isoleucine with sugars. Adv. J. Food Sci. Technol., 5(10): 1367-1374.

Stefansson, M. and M. Novotny, 1994. Resolution of the branched forms of oligosaccharides by high-performance capillary electrophoresis. Carbohyd. Res., 258: 1-9.

Xu, P. and Y. Su, 2013. Study on fresh keeping effect of chitosan antistaling agent for several kinds of fruits. Adv. J. Food Sci. Technol., 5(12): 1577-1579.

Xue, Y., N. Chen, L. Chunying, X. Wang and S. Chengjun, 2013. Simultaneous determination of seven preservatives in cosmetics by dispersive liquid-liquid microextraction coupled with high performance capillary electrophoresis. Anal. Method., 5(9): 2391-2397.