Journal of Microbiology, Biotechnology and Food Sciences

Baroš et al. 2012 : 1 (February Special issue) 725-732

REGULAR ARTICLE

FREE RADICAL SCAVENGING CAPACITY OF *PAPAVER SOMNIFERUM* L. AND DETERMINATION OF PHARMACOLOGICALLY ACTIVE ALKALOIDS USING CAPILLARY ELECTROPHORESIS

Stanislav Baroš[1], Marianna Karšayová[1], Klaudia Jomová[1], Attila Gáspár[2], Marián Valko[3]*

Address: [1]Constantine the Philosopher University in Nitra, Faculty of Natural Sciences, Department of Chemistry, Tr. A. Hlinku 1, 949 74 Nitra, Slovak Republic

[2]University of Debrecen, Department of Inorganic and Analytical Chemistry, , Hungary

[2]Slovak Technical University, Faculty of Chemical and Food Technology, Bratislava, Slovak Republic

*****Corresponding author: stanislav.baros@ukf.sk

ABSTRACT

The free radical generation is related to the oxidation process in biological systems as well as in foods. It was found that oxidation is affected by antioxidants that can act as radical scavengers. Objective of the present work was to study the free radical scavenging capacity of opium poppy (*Papaver somniferum* L.) extract by using the DPPH test and to verify the suitability of the micellar electrokinetic capillary chromatography (MEKC) technique for analytical assessment and determination of three major poppy alkaloids (thebaine, morphine and papaverine). Because of its generally high separation efficiency, the MEKC is successfully used for analytical evaluation of biologically active substances usually without special claims for sample preparation. The results of DPPH test have shown that poppy contains components capable of terminating free radicals. We have confirmed that nature of the solvent used for the electrophoretic medium in MEKC has a strong influence on the separation efficiency. In our experiments, the most effective solvent was mixture of water to acetonitrile (ratio 4:6).

Keywords: opium poppy, morphine, papaverine, thebaine, MEKC, DPPH

INTRODUCTION

Opium poppy (*Papaver somniferum* L.) is one of the oldest medicinal plants in history. *P. somniferum* is grown for its great medicinal effects, though it is also used as the main narcotic crop of heroin (the diacetyl derivative of morphine), (**Hindson *et al.*, 2007**). The present international research of opium poppy is mostly focused on its medicinal value and botanical characteristic. Opium, the exudates from *P. somniferum* contains more than 30 individual alkaloids and represents the raw material for extraction. *P. somniferum* is a well known source of important morphinane opium alkaloids, mainly morphine, codeine, thebaine, papaverine and narcotine that belong to isoquinoline derivates and show a broad range of pharmacological activities (**Singh *et al.*, 2000**); their major application is in analgesia, sedation and cough depression (**Gotti, 2011**). Some of them have showed in vitro antioxidant properties **(Gülcin *et al.*, 2004; Njoku *et al.*, 2011)**. Antioxidant supplements or food containing antioxidants may be used to help reduce oxidative damage induced by ROS/RNS (Reactive Oxygen/Nitrogen Species), **(Valko *et al.*, 2005)**. Analytical methods are of importance for the determination of these compounds in plant materials because the content of specific morphinane alkaloids could be used to differentiate the origin of opium that is a very important issue to help the law enforcement agencies in checking the illicit production of narcotic drugs. Analytical procedures (high-performance liquid chromatography, capillary electrophoresis) have been reviewed on the analysis of different opium alkaloids. Micellar electrokinetic capillary chromatography (MEKC) has been reported to be a rapid method for determination of alkaloids. In MEKC, the electrophoretic buffer is modified with an ionic surfactant as a micelle modifiers **(Trenerry *et al.*, 1995)**.

This work was focused on the free radical scavenging capacity of commercially available opium poppy extract by means of a free radical-scavenging assay. The quantitative change in the profile of alkaloids after the addition of organic solvent to water in the presence of SDS as a micelle forming agent was observed.

MATERIAL AND METHODS

Plant material and extraction

Papaver somniferum L. seeds (commercially available opium poppy) were grounded in mill, the fine powder was transferred to a flask containing 95% ethanol (poppy to solvent

ratio 1:5) and placed in a water bath at 45°C. The slurry was filtered off and the residue was re-extracted twice. Supernatant was evaporated and dry residue stored at room temperature.

The free-radical scavenging activity

The free-radical scavenging activity of the prepared extracts was determined according to the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method described previously **(Yen and Chen, 2005)**. Extracts (0.015–1.500 mg) were dissolved in 5 mL of methanol and then added to a methanolic solution containing DPPH (1 mM, 0.4 ml). The mixture was vortexed and then left to equilibrate at ambient temperature for 30 min. The absorbance of the resulting solution was read spectrophotometrically at 517 nm. A methanolic solution of DPPH radical that had decayed and hence no longer exhibited a purple colour was chosen for background correction, instead of pure methanol. The radical scavenging activity (RSA) was calculated as a percentage of DPPH radical discolouration using the equation:

$$RSA = (1 - A_E/A_D).100$$

where A_E is absorbance of the solution containing the extract and A_D is the absorbance of DPPH solution.

Micellar electrokinetic capillary chromatographic procedure

Chemicals used for preparation of standard solution were of analytical grade. The capillary electrophoretic instrument was a HP 3DCE model (Agilent, Waldbronn, Germany). Solvents for sample preparation were: water, water:acetonitrile (4:6, 8:2). Stock standards-solutions of opium alkaloids were: 793 $mg.l^{-1}$ for morphine, 500 $mg.l^{-1}$ for papaverine, 500 $mg.l^{-1}$ for thebaine. Pure reference standards of the three individual alkaloids were dissolved and analyzed in a given solvent at a volume 2 µl of each alkaloid (morphine, thebaine and papaverine). A sample of standard solution had a volume 30 µl. Measurements in different systems were carried out under the following conditions: silica capillary 68 cm x 50 µm, voltage 25 kV, injection pressure 5000 Pa for 2 seconds, pH=7, 50 mM sodium dodecyl sulphate (SDS) in buffer consisting of 1:1 mixture of 0.01 M sodium tetraborate, and 0.01M potassium dihydrogen orthophosphate, UV detection at 200, 240 and 270 nm. The electrophoregrams were recorded and processed by Chemstation software 7.01 version (Agilent). Spiking of pure reference alkaloid standards in the opium extract was used for peak identification.

RESULTS AND DISCUSSION

The molecules possessing radical-scavenging properties present in methanolic poppy extract can quench DPPH free radicals. This documents Figure 1 which shows dose-response dependences for radical scavenging activity of methanolic poppy extracts. Most probably the mechanism proceeds through hydrogen donation or electron donation, conceivably via a free-radical attack on the DPPH molecule. The reaction results in conversion to a colourless product (i.e. 2,2-diphenyl-1-hydrazine, or a substituted analogous hydrazine), resulting in a decrease in absorbance at the 517 nm band. Generally, the more rapidly the absorbance decreases, the more potent is the antioxidant activity of the extract. One should bear in mind that the interpretation of the results is not straightforward. One cannot assume that the decrease in absorbance at 517 nm absorption maximum is entirely attributed to the antioxidant donating a hydrogen atom or an electron to DPPH. The results have shown that poppy contains components capable of terminating free radicals. Thus free radical scavenging properties of poppy should be investigated in the future in greater details. This work is currently underway. Since foods containing antioxidants may help to reduce oxidative damage of molecules the study of antioxidant properties of substances coming from natural sources is indeed justified.

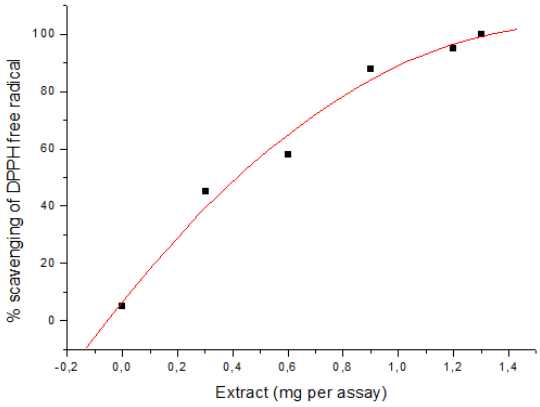

Figure 1 Scavenging effect of methanolic opium poppy extracts on 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH).

The opportunity of application of different separation principles have made capillary electrophoresis very versatile technique suitable for analysis and quality control of herbal drugs and medicinal plants (**Francis *et al.*, 2008; Lurie *et al.*, 2003, 2004; Hindson *et al.*, 2007**). In MEKC, anionic surfactant SDS, has been used a micelle modifiers Electrophoretic mobility of structurally different papaverine is monitored throughout the range is significantly

lower than in other studied alkaloids. The nature of the solvent used for the electrophoretic medium has a strong influence on the separation selectivity **(Li, 1992)**. The solvent effect efficiency is probably partly due to the differences in the dielectric constants of the solvents. The Figure 1 shows electrophoregram of alkaloid separation of poppy extract dissolved in water. The separation has showed the weak separating potential with only papaverine detectable signal at 240 nm.

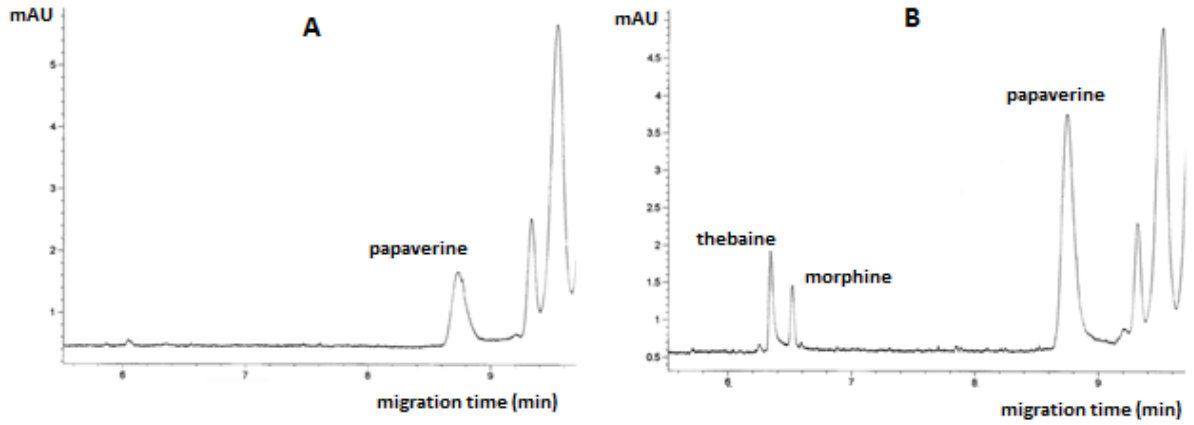

Figure 2 Electrophoregrams of poppy extract dissolved in water: (A) pure sample, (B) sample with standards (B). Measured at wavelength 240 nm.

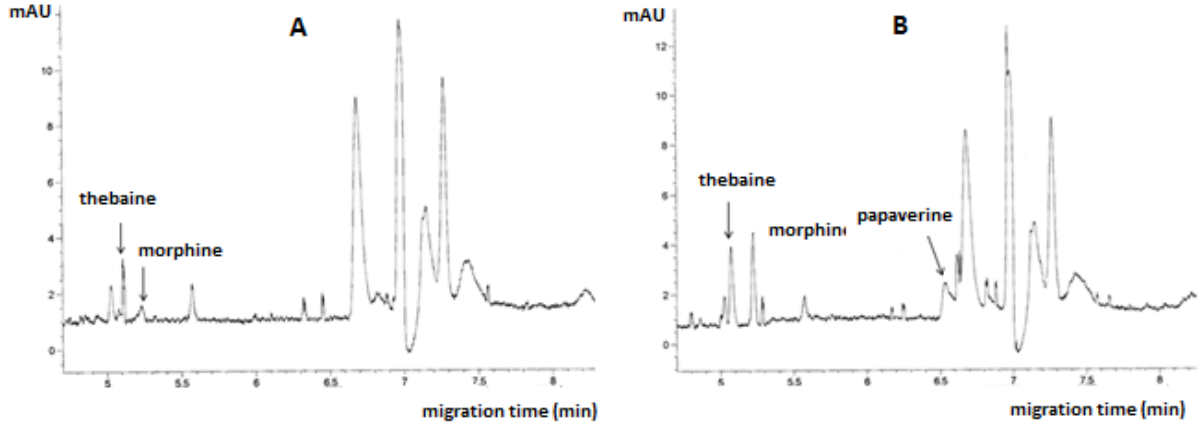

Figure 3 Electrophoregrams of poppy extract dissolved in a mixture of water : acetonitrile (4:6): (A) pure sample, (B) sample with standards. Measured at wavelength 200 nm.

The efficiency of the separation has been found to be most intensive in mixture of water and acetonitrile (4:6) at 200 nm. However, only thebaine and morphine peaks were detected (Fig. 3). Papaverine signal was not detected in the electrophoregrams at any wavelength with the same electrophoretic conditions. A mixture with higher proportion of

water to acetonitrile (8:2) was unsuitable for MEKC separation indicating that the higher proportion of acetonitrile improves the separation process.

CONCLUSION

The first results of oppium poppy methanolic extract indicated presence of compounds which are able to scavenge free radicals. The addition of organic solvent to the buffer affected the separation of complex mixture of opium poppy extract significantly. The effect of wavelength was also observed. From the alternatives tested, the water to acetonitrile solvent (ratio 4:6) at 200 nm occurred to be the most suitable for this purpose.

Acknowledgment:
This work was supported by the Institutional project of Constantine the Philosopher University in Nitra under No. VII/2/2011 and CEEPUS project CII-HU-0010-02-0708.

REFERENCES

FACCHINI, P. J. - BIRD, D. A. - BOURGAULT, R. - HAGEL, J. - LISCOMBE, D. K. - MACLEOD, B. P. - ZULAK, K. G. 2005. Opium poppy: a model system to investigate alkaloid biosynthesis in plants. In *Canadian Journal of Botany*, vol. 83, 2005, no. 10, p. 1189-1206

FRANCIS, P. S. - ADCOCK, J. L. - COSTIN, J. W. – PURCELL, S. D. - PFEFFERA, F. M. – BARNETT, N. W. 2008. Chemiluminescence detection of opium poppy (*Papaver somniferum*) alkaloids. In *Journal of Pharmaceutical and Biomedical Analysis*, vol. 48, 2008, p. 508–518,doi:10.1016/j.jpba.2008.06.022

GOTTI, R. 2011. Capillaryelectrophoresisofphytochemicalsubstances in herbaldrugs and medicinalplants.In *Journal of Pharmaceutical and Biomedical Analysis*, vol. 55, 2011, iss.4-5, p. 775-801.

GÜLCIN, I. – BEYDEMIR, S. – ALICI, H.A. – ELMASTAS, M. – BÜYÜKOKUROGLU, M.E. 2004. In vitro antioxidant properties of morphine. In *Pharmacological Research*, vol. 49, 2004, p. 59-66

HINDSON, B. J. - FRANCIS, P. S. – PURCELL, S. D. – BARNETT, N. W. 2007. Determination of opiate alkaloids in process liquors using capillary electrophoresis. In *Journal of Pharmaceutical and Biomedical Analysis*, vol. 43,2007, p. 1164–1168, doi:10.1016/j.jpba.2006.09.040

LI, S.F.Y. 1992. Capillary Electrophoresis, Principles, Practice and Applications.In *J. of Chromatogr.Library Ser.,* Vol.52, 1992, Elsevier, Amstredam, Netherland.

LURIE, I.S. - HAYS, P.A. - GARCIA, A.E. - PANICKER, S. 2004. Use of dynamically coated capillaries for the determination of heroin, basic impurities and adulterants with capillary electrophoresis. In *Journal of Chromatography A*, vol. 1034, 2004, p. 227–235, doi:10.1016/j.chroma.2004.01.062

LURIE, I.S. - PANICKER, S. - HAYS, P.A. - GARCIA, A.D. - GEER,B.L. 2003. Use of dynamically coated capillaries with added cyclodextrins for the analysis of opium using capillary electrophoresis. In *Journal of Chromatography A*, vol. 984, 2003, p.109–120, doi:10.1016/S0021-9673(02)01774-0

NJOKU, U.O. - NWODO, O.F.C. - AGU, C.V. 2011. In vitro Effect of Papaverine on Lipid Peroxidation in Sickle Cell Anaemia. In *International Journal of Basic & Applied Sciences*, vol.11, 2011, no.05, p. 67-73.

SINGH, D. V. - PRAJAPATI, S. - BAJPAI, S. - VERMA, R. K. – GUPTA, M. M. – KUMAR, S. 2000. Simultaneous determination of important talkaloids in *Papaver somniferum* using reversed phase high performance liquid chromatography. In *Journal of Liquid Chromatography and Related Technologies,* vol. 23, 2000, no. 11, p. 1757-1764

TRENERRY, V.C. – WELLS, R.J. – ROBERTSON, J. 1995. Determination of morphine and related alkaloids, poppy straw and opium preparations by micellar electrokinetic capillary chromatography. In *Journal of Chromatography A*, vol. 718, 1995, p.217-225.

VALKO, M. - MORRIS, H. – CRONIN, M.T.D. 2005.Metals, Toxicity and Oxidative Stress, In *Current Medicinal Chemistry,* vol. 12, 2005, p. 1161-1208.

YEN, G.-C. – CHEN,H.-Y. 1995.Antioxidant activity of various tea extracts in relation to theiranti mutagenicity. In *Journal of Agricultural and Food Chemistry*,vol. 43, 1995, p. 27–37, DOI: 10.1021/jf00049a007