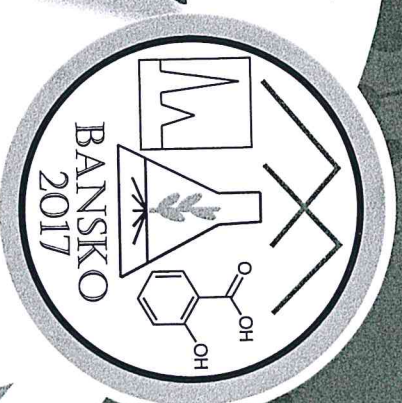


3rd INTERNATIONAL CONFERENCE ON NATURAL PRODUCTS UTILIZATION: FROM PLANTS TO PHARMACY SHELF

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Bansko • Bulgaria



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PP 137

DETAILED LC/PDA/ESI-QTOF-MS/MS ANALYSIS AND BIOLOGICAL PROFILING OF ISOFLAVONES OCCURRING IN AERIAL PARTS OF *TRIFOLIUM MEDIUM* L. (FABACEAE)

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In contrary to popular belief, not only soy or red clover are considered as valuable sources of phytoestrogenic isoflavones. In our studies, aerial parts of *Trifolium medium* L. (zizgag clover), being a fodder plant from the family Fabaceae, were selected as a plant material. The efficient extraction technique, assisted by the operation of ultrasounds (UAE), was developed for obtaining 50% (v/v) ethanolic-aqueous extract, that was further subjected to the vacuum drying procedure. This process led finally to obtaining the lyophilisate (TML), that was purified from ballast compounds using solid-phase extraction on a phenyl J.T.Baker microcolumn. Then, TML was qualitatively analysed using LC-PDA/ESI-MS-QTOF technique in negative and positive ion mode to identify in detail isoflavone constituents.

A 6530B accurate-mass-Q-TOFMS apparatus (Agilent Technologies) with a Zorbax SB-C18 narrow-bore column (dp = 3.5µm, 2.1 x 150 mm) and gradient of acetonitrile (1%) + 10 mM ammonium formate (0.2%) (A) and acetonitrile (95%) + 10 mM ammonium formate (0.2%) (B) as a mobile phase were used. Isoflavone aglycones, namely formononetin, biochanin A, genistein, daidzein and their O-glycosides were identified and quantified together with small amounts of pseudobaptigenin, pratensein and irilone derivatives. Total content of all isoflavone compounds exceeded 12% of dry weight and it was above 2.5 higher than in red clover lyophilisate, used as the reference herbal preparation. The antioxidant potential of TML was evaluated using FCR method and ABTS^{•+} free radical assay. Mean IC₅₀ values obtained in ABTS^{•+} test were 30.24, 2.75 and 0.66 µg/mL for TML, Trolox and gallic acid solutions, respectively.

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ELECTROCHEMICAL INVESTIGATION OF THE BASIC REDOX BEHAVIOR OF RUTIN

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Rutin is one of the most common found flavonoid, containing quercetin as a flavonol aglycone. Its structure consists of a two aromatic (phenolic) rings connected through one heterocycle containing oxygen [1]. The high number of OH groups, as well as, its aromaticity justifies the use of an electrochemical method in its analyses [2]. Cyclic voltammetry has been applied in order to investigate the redox oxidation process of rutin on a glassy carbon electrode, as working electrode. Additionally, the platinum electrode has been used as a reference electrode and a graphite stick has been employed as counter electrode. Its electrochemical properties have been studied in different pH values by use of a few different buffer solutions in pH range from 2.8 to 8.8. The potential applied on the surface of the working electrode in the range of -0.4 to 0.6 V, was used in scanning the redox process of rutin. The reversible oxidation in physiological pH has occurred on $E_{pa} = 0.198$ mV and $E_{pc} = 0.221$ mV, but the results have shown that its oxidation is a pH dependent electron transfer process. Additionally, the adsorption of rutin was noted. The application of these data considering the redox behavior of rutin could be used in prospecting of its mechanism of oxidation and structure-antioxidant activity relationship, as well as developing a method for fast estimation of its antioxidant activity.

References:

- [1] Kang J, et al. (2002) Analytical Letters 35: 677-686.
- [2] Gill ES, Couto RO (2013) Brazilian Journal of Pharmacognosy 23: 542-558.