

AN IMPROVED MICROSCALE METHOD FOR EXTRACTION OF ISOFLAVONES FROM RED CLOVER (Trifolium pratense)

Marija Kovačević Babić, Marija Viljevac Vuletić, Daniela Horvat Agricultural Institute Osijek, Južno predgrađe 17, 31000 Osijek, Croatia

INTRODUCTION

Red clover (Trifolium pratense L.) is a widely used fodder, but in the past decade has attracted interest as an alternative valuable source of isoflavones with variety of health protective effects. Legumes Isoflavones are present in different chemical forms, but their health beneficial effects are most pronaunced in their free forms – aglycones. The main aim of this study was to obtain the highest content of isoflavones from red clover leaves and whole plant through the various extraction and hydrolysis methods.

Red clover sample were collected at the Agricultural Institute. Briefly, 0.05 g of the powder sample was dissolved in 10 mL of 100% methanol – water mixture (50:50) using 2 M HCl (MTH1), 2M HCl with evaporation to dryness (MTH2), 6M HCI (MTH3), as well as by 80% MeOH without (MTH4) and with evaporation of extract to dryness (MTH5). The extracts were analysed by HPLC (High-Performance Liquid Chromatography) using an external calibration curve for isoflavones quantification. Statistical differences among isoflavones extracted by five different methods were analyzed using analysis of variance followed by post hoc Fisher's Least Significant Difference (LSD) test.



Fig. 1. Average values of isoflavones in whole plant (A) and leaves (B) obtained by five different extraction methods (MTH). Different letters indicate significant difference among isoflavones extracted by five methods at *p* < 0.05 according to the LSD test.

CONCLUSSIONS

The highest concentration of total isoflavones was obtained from the leaf extract that was evaporated to dryness after 2M HCl hydrolysis as follows: formononetin 4358.75 $\mu g/g_{DM}$, biochanin A 2950.23 $\mu g/g_{DM}$, daidzein 186.98 $\mu g/g_{DM}$ and genistein 194.09 $\mu g/g_{DM}$, while in whole plant a lower values of isoflavones were obtained, except for daidzein, as follows: formononetin 2746.17 $\mu g/g_{DM}$, biochanin A 731.41 $\mu g/g_{DM}$, daidzein 428.93 $\mu g/g_{DM}$ and genistein 81.16 $\mu g/g_{DM}$.

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