POLYPHENOLS CONTENT OF BERRY LEAVES AND CALLUSES

Tijana D. Kolarević[1]*, Danijel D. Milinčić[1], Aleksandar Ž. Kostić[1], Tatjana Vujović[2], Radosav Cerović[3], Mirjana B. Pešić[1]

[1]*Faculty of Agriculture, University of Belgrade, Serbia*

[2]*Fruit Research Institute, Čačak, Serbia*

[3]*Innovation Center, Faculty of Technology and Metallurgy, University of Belgrade, Serbia*

** Corresponding author: tijana.curic23@gmail.com*

INTRODUCTION

Secondary plant metabolites are sinthetised as stress-responding compounds and provide selective advantages to plants (1). This is a hudge group of compounds with heterogeneous structures. One of the biggest class of these compounds are polyphenols (2). Most of them also act as antioxidants, providing benefits for human health (3). Consequently, food scientists explore possibilities for the isolation and application of these bioactive compounds in food industry. In order to obtain polyphenols, which are present in the plants in low quantities, a conventional plant cultivation is not efficient enough. *In vitro* plant tissue culture techniques, in highly controlled conditions and using appropriate medium, allow, in shorter period of time, production of the whole plant or the plant tissue with ability to synthesize desired compounds, overcoming this problem (4).

AIM

The aim of this study was to determine whether *in vitro* tissues can be a good source of polyphenols and for that purpose *in vitro* shoot and callus cultures were established and their polyphenol content was compared with field-grown plants.

MATERIALS AND METHODS

Callus culture, as well as *in vitro* and *ex situ* leaves from two berry plant genotypes-blackberry (*Rubus* subg. *Rubus* Watson Čačanska Bestrna) and blueberry (*Vaccinium corymbosum* L. Toro) were used. Callus culture and *in vitro* leaves were cultivated on nutritive mediums in clime-chambers, while *ex situ* leaves were obtained from research fields.

Collected samples of leaves and callus cultures were fine grounded and homogenized with liquid nitrogen, then extracted using 80% methanol with 0.1% HCl, evaporated to dryness and suspended in miliQ water (1/10 w/V).

Total phenolic (TPC) and flavonoid content (TFC) were determined using colorimetric assays with Folin-Ciocalteu's reagents and aluminum chloride, respectively.

Results were expressed as mg GAE/g DW for TPC and as mg CE/g DW for TFC.

RESULTS AND DISCUSSION

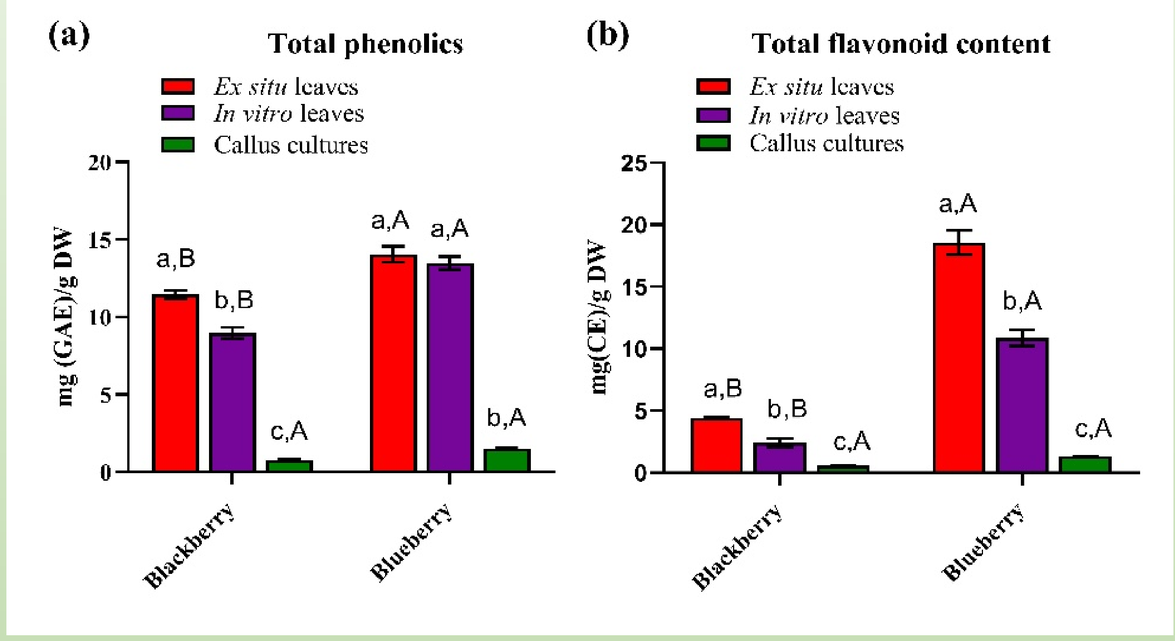

Figure 2. (a) Total phenolic content and **(b)** Total flavonoid content of blueberry and blackberry leaves and callus culture samples. The bars with (±) standard deviation represent mean values

Blueberry Toro has higher both TPC and TFC, comparing all tree types of samples. Field-grown leaves of blueberry have the highest values, 14.06 mg GAE/g DW and 18.56 mg CE/g DW for TPC and TFC, respectively. Similar results are for *in vitro* leaves with 13.47 mg GAE/g DW and 10.88 mg CE/g DW (for TPC and TFC, respectively).

Callus cultures have significantly lower content of both phenolics and flavonoids, again with higher values for blueberry, 1.51 mg GAE/g DW and 1.31 mg CE/g DW (TPC and TFC respectively).

Interestingly, *ex situ* leaves of blueberry appears to have higher TFC than TPC. This can be explained by the fact that different compounds have different interactions with reagents used in these two methods and thus some of the components are "invisible" in spectrophotometric measurement.

CONCLUSION

This study showed that blueberry 'Toro' is better source of phenolic compounds than blackberry 'Čačanska bestrna", both for leaves and callus cultures. Even though callus cultures had significantly lower polyphenol contents, using appropriate plant growth regulators, these can be potentially good for production of specific molecules.

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