Macedonian pharmaceutical bulletin, 68 (Suppl 2) 217 - 218 (2022)
Online ISSN 1857 - 8969
DOI: 10.33320/maced.pharm.bull.2022.68.04.100
Short communication

Flavonoids productivity of wild growing and *in vitro* cultivated *Hypricum* species

Antoaneta Trendafilova[1], Viktoriya Ivanova[1], Ina Aneva[2], Kalina Danova[*1]

[1]*Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria*
[2]*Institute of Biodiversity and Ecosystem Research, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria*

Introduction

Over 1300 medicinal plants are being used in Europe, 90 % of them sourcing from Nature (Chen et al. 2016). Although widely studied throughout the years medicinal plant *Hypericum perforatum* still remains a source of scientific research due to the richness of its pharmacological activities, determined by the complexity of the phytochemical composition of *Hyperici herba*. Research has led to elucidation of the most important biologically active substances in the plant – polyphenolic compounds, flavonoids, naphthodianthrones and phloroglucinols, terpenes, possessing antidepressive, antitumor, antiviral and antibiotic activity (Danova 2015). The Balkan flora holds a priceless natural resource with a vast and still unexplored potential in the search of new sources of biologically active compounds.

The aim of the present work was to compare the flavonoids productivity of three *Hypericum* species (*H. perforatum, H. tetrapterum* and *H. richeri*) collected from the wild with plant material derived from their biotechnological development.

Materials and methods

Plant material

Hypericum species were collected from their natural habitats in Bulgaria as follows: *H. perforatum* and *H. tetrapterum* - Western Balkan region, *H. richeri* - from two different accessions - at the Vitosha and Rila mountains.

Tissue culture initiation

Shoot segments of the wild growing species (the samples of Vitosha Mountain for *H. richeri*) were surface sterilized by 30 sec. immersion in 70 % ethanol, followed by 5 min sterilization in 0.1 % $HgCl_2$ and triplicate washing in sterile distilled water. For axillary shoot formation the following medium formulation was used: the basic Murashige and Skoog (MS) medium (1962), supplemented with Gamborg vitamins (Gamborg et al. 1968), 6.5 g/l agar, 20 g/l sucrose and 0.5 mg/l N^6-benzyladenine (BA), at 25 ± 0.2 °C and 16/8 hours photoperiod. The obtained axillary shoots were transferred to the basic MS culture medium, supplemented with 30 g/l sucrose (with addition of 0.2 mg/l BA and 0.1 mg/l indole-3-butyric acid (IBA) for *H. richeri*).

Shoot culture experiment

The tissue culture experiment included the following plant growth regulator (PGR) treatments: Hyp_M0 - PGR-free control and Hyp_M2 - 0.2 mg/l BA + 0.1 mg/l IBA.

Extraction of the plant material

Dry plant material of the wild collected and shoot cultures derived *Hypericum* representatives (100 mg) was defatted by maceration in chloroform (3x10 ml) and further on extracted by methanol (3 x 10 ml). The extraction steps were 1 for 24 hr at room temperature, and 2 for 15 min in an ultrasonic bath at 25 °C.

Total flavonoids determination

Total flavonoids were assayed spectrophotometrically by the method of Zhishen et al. (1999) and the results were expressed as mg catechin equivalents per 1g DM.

*k_danova@abv.bg

HPTLC characterization of individual components

HPTLC analysis was done with pre-coated HPTLC glass plates (20 × 10 cm, Si G60 F254, Merck) using a Camag HPTLC system (Switzerland). Ethyl acetate:CH_eCl_2:acetic acid: formic acid: water (100:25:10:10:11 v/v/v/v/v) was used as a mobile phase. The chromatographic spots were visualized by UV light at 366 nm before and after spraying with NP reagent. Rutin, hyperoside and chlorogenic acid were used as standards.

Results and discussion

The three species showed a good response to the PGR supplementation applied, as multiplication rate increased up to 50 axillary shoots formed per explant within the culture period of 12 weeks in Hyp_M2 culture medium as compared to up to 4-5 shoots per explant formed in the Hyp_M0 supplementation. Noteworthy, long-term cultivation in the PGR-free medium was not possible for *H. richeri*, due to its slow growth and subsequent necrosis and decay in the Hyp_M0 medium.

The highest levels of total flavonoid compounds were established for *H. richeri* collected from the Rila Mountain, followed by the ones of *H. perforatum*. *H. tetrapterum* showed intermediate levels and the samples of *H. richeri* obtained from the Vitosha Mountain had the lowest flavonoid levels.

The highest *in vitro* flavonoid production was established for *H. tetrapterum* in the PGR-free medium supplementation. Noteworthy, flavonoid levels in *H. tetrapterum* dropped more than two times with the addition of PGRs. For *H. perforatum*, the PGR treatment slightly increased the flavonoid content up to levels almost reaching the ones of the PGR-free *H. tetrapterum. H. richeri* showed intermediate flavonoid production in the Hyp_M2 medium, being higher than *H. tetrapterum* lower than *H. perforatum*, grown in the same medium formulation.

The HPTLC comparison of the methanol extracts of the samples in the presence of the referent chlorogenic acid, rutin, hyperoside and hypericin showed both similarity and differences between samples. Thus, the wild collected samples were shown to be richer in individual flavonoid components. Noteworthy, *H. tetrapterum* samples did not contain rutin, with the most prevailing components in them being mono-glycosides such as hyperoside and isoquercetin, while *H. perforatum* and *H. richeri* produced both rutin and hyperoside. Previous research on *H. perforatum* has evidenced the presence of rutin, hyperoside, isoquercitrin, avicularin, quercitrin, and quercetin as major constituents of a flavonoid-rich extract of the species. Research showed the potent free radical scavenging activity and antioxidant activity of the preparation (Zou et al. 2004).

Conclusion

Although not so widely studied as *H. perforatum*, other representatives of the genus, characteristic for the Balkan flora are prospective candidates for phytochemical investigation and biotechnological development.

Acknowledgements

The authors are thankful to the financial support of grant КП-06-Н39/6, National Scientific Fund, Bulgaria

References

Chen, S.L., Hua, Y., Luo H.M., Wu, Q., Li, C.F., Steinmetz, A., 2016. Conservation and sustainable use of medicinal plants: problems, progress, and prospects. Chin. Med. 11, 37. https://doi.org/10.1186/s13020-016-0108-7.

Danova, K., 2015. Potential of the Balkan Flora as a Source of Prospective *Hypericum* Genotypes for the Conventional and Biotechnological Delivery of Phytopharmaceuticals. In: Davis, H.R., (Ed), *Hypericum*: Botanical Sources, Medical Properties and Health Effects, Nova Science Publishers, USA, pp. 19-52, ISBN: 978-1-63482-701-0, 19-52.

Gamborg, O.L., Miller, R.A., Ojima, K., 1968. Nutreint requerments of suspension culture of soybean root cells. Exp. Cell. Res.. 50, 151-158. https://doi.org/10.1016/0014-4827(68)90403-5.

Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plantarum. 15, 473-497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x.

Zhishen, J., Mengcheng, T., Jianming, W., 1999. The determination of flavonoid content in mulberry and their scavenging effects on superoxide radicals. Food Chem. 64, 555-559. https://doi.org/10.1016/S0308-8146(98)00102-2.

Zou, Y., Lu, Y., Wei, D., 2004. Antioxidant activity of a flavonoid-rich extract of *Hypericum perforatum* L. *in vitro*. J. Agric. Food Chem. 52, 5032-5039. https://doi.org/10.1021/jf049571r.