QUERCETIN AND RUTOSIDE CONTENT OF SOME *BETULA* SP. BUDS GEMMOTHERAPY EXTRACTS, FROM MOUNTAIN REGIONS

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Abstract

Quercetin is one of the most common polyphenols worldwide, named after the oak forest – quercetum. It is considered one of the most powerful antioxidants due to its five phenolic groups respectively the γ-pyrone cycle. This flavonoid and its glycosylated derivatives as rutoside, hyperoside, quercitrin or iso-quercitrin are present also in the extracts obtained from the buds of different Betula species, extracts that are therapeutic tools for the new branch of phytotherapy named gemmotherapy or meristem-therapy. In the pandemic context, quercetin and its derivatives are successfully used to improve the side or late effects of SarsCov-2 infection. Our study involved the extracts from buds of white birch (*Betula pubescens*) and silver birch (*Betula pendula*) from mountain regions. The quercetin and the rutoside were separated, identified and quantified by HPLC. The total flavonoids were determined by spectral methods. The highest quercetin-containing gemmotherapy extract is obtained from the white birch buds. Due to their rich quercetin content, these extracts can be proposed for the complementary treatment of SarsCov-2 infections and post-COVID symptoms.

Keywords: Quercetin; rutoside; gemmotherapy; HPLC; white birch buds; silver birch buds; SarsCov-2 infection.

INTRODUCTION

Quercetin is an important flavonol, from the class of flavonoids, being a polyphenol known from 1857, when its name was given after the Latin name of oak forest, quercetum. Since that is one of the most studied flavonoids, being widespread in all the vegetal kingdom, from leaves to fruits, seeds, etc. (D'Andrea, 2015; Kim and Park, 2018).

During that time the studies proved this flavonoid had important therapeutic value. As polyphenol possesses a high antioxidant potential that is performed by different mechanisms of action. Quercetin can be oxidized by free radicals, stabilizing the ROS and RNS resulting in reduced radical reactivity (Nijveldt et al., 2001; Kim and Park, 2018).

Due to the antioxidant potential quercetin has beneficial effects on different systems and apparatus of the body. It reduces the oxidative stress induced in diabetes mellitus, in cancer, at the endothelium level, and has antiaging and hepatoprotective effects [1–5]. (Nijveldt et al., 2001; D'Andrea, 2015; Kim and Park, 2018; Batiha et al., 2020; Dengyu et al., 2020).

Quercetin, near its high antioxidant potential, presents also anti-inflammatory, cardio-protective and neuroprotective effects (David et al., 2016).

The same quercetin was also claimed to be responsible also by antiviral and antimicrobial effects. Formulation with quercetin demonstrated to be potent against hepatitis C, influenza-A and other specific viruses that attack the respiratory system (Qiu et al., 2016; Weinjiao et al., 2016). Quercetin is fights also against infections with *E. coli*, *Salmonella enterica* or *Listeria monocytogenes* exhibiting bacteriostatic effect (Maalik et al., 2014). Quercetin promotes immunity by direct regulation of basic functional properties of immune cells (Li et al., 2016).

The studies have demonstrated that rutoside, iso-quercetin or quercitrin have better absorption and bioavailability than quercetin aglyka, exhibiting in the body the biological effects of the aglyka (Kasikci et al., 2016; Li et al., 2016; Almeida et al., 2018; Li et al., 2021).

Quercetin was involved recently as a potential therapeutic tool in the prevention and improvement of different symptoms occurring during and after the SarsCov2 infections. A recent clinical trial performed in Italy showed that the patients treated with a quercetin preparation passed easier and more rapidly through the infection, shorting the time of conversion of tests from positive to negative and at the same time reducing the severity of symptoms (Aucoin et al., 2020; Di Pierro et al., 2021).

All these results regarding quercetin and its derivatives propose the natural sources of these compounds to be very valuable today. This was the aim for which we screened a number of special extracts from birch tree buds to evaluate from the point of view of the content in quercetin and its derivatives.

Gemmotherapy is the name of a new branch of phytotherapy that uses just those parts of plants that contain mainly undifferentiated, meristematic tissues, with a higher therapeutic potential due to the different phytochemical profile in comparison with adult parts of plants used by classical phytotherapy. The extracts used in gemmotherapy are obtained from fresh buds and young shoots, harvested in a very well-defined time of their development for an optimal biological effect that is at a deep, molecular level, but is also mild and natural. These young parts of plants are rich in primary metabolites, but also in secondary ones and mainly in polyphenols (Tetau, 1998; Ledoux and Gueniot, 2014; Pitera di Clima and Nicoletti, 2018). The studies regarding the phytochemical profile of these extracts are almost missing from specific scientific literature. The scientific proof of the mechanism of action respectively of the biological effects of these extracts are also mainly missing.

The goal of this study was to demonstrate that gemmotherapy extracts from different birch species can be used to improve the symptoms of different respiratory system diseases due to their contain in quercetin and quercetin derivatives, being valuable in the convalescence after or prevention of complications in case of SarsCov2 infection. By this study we wish to contribute also to the better valorization of the implicated species, using also other vegetal materials than those studied until this moment.

MATERIALS AND RESEARCH METHOD

a. Vegetal material and the preparation of gemmotherapy extracts used in the study

In this study were used extracts prepared by PlantExtrakt Ltd., Rădaia, Cluj, Romania (www.plantextrakt.ro; contact@plantextrakt.ro). There were used extracts from white and silver birch buds. The vegetal materials were harvested from the wild flora of the mountains near Cluj, Romania, in February 2020. From all vegetal materials were taken samples for identification, performed in the PlantExtrakt company quality control laboratory. For each species voucher specimens were retained in the company herbarium.

The extracts were prepared according to the French and European Pharmacopoeias in a mixture of 96% vol. ethanol and 100% glycerol (1:1) (Pharmacopee Francaise ed. 11, 2020; European Pharmacopoeia, ed. 11, 2023). The vegetal raw material was processed in a fresh state, first cut then mixed with the solvent using a ratio of 1:20, plant material – solvent. The extraction was performed by cold maceration, by mixing periodically the mixture of plant material with solvent. After 20 days the liquid was decantated and the plant material was pressed at a maximum of 400 atm. The extraction liquids were mixed and these final solutions represent the gemmotherapy extracts.

The solvents used for extraction are of pharmaceutical grade, purchased from SC Coman Prod SRL, Ilfov, Romania and Spiga Nord, Italy. The collection of the plant materials was made according to the Good Agricultural Practices for Collection, taking into consideration the keeping of biodiversity and under Eco certification Ro-008.

b. Determination of total flavonoids by UV-Vis spectrophotometry

The determination of total flavonoids was performed according to Romanian Pharmacopoeia, 10th edition (1993). The determinations were performed on a Cintra 101 spectrophotometer (GBC, Australia). To 1 ml aliquots of each extract were added 3 ml of 2.5% aluminium chloride solution and 5 ml 10% sodium acetate solution. The mixtures were diluted to 25 ml with methanol. The blank solutions were prepared identically using 8 ml of water in place of aluminium chloride and sodium acetate solutions. In the same manner, were prepared also the standard solutions containing 2–25 ug/ml quercetin. These solutions were used to build the calibration curve that has a correlation factor of 0.9997 and a limit of quantification of 1.47 ug/ml. All determinations were performed in triplicate and Excel software from Microsoft Office was used for data statistics. All reagents were of analytical grade and purchased from Merck, Germany. The standard quercetin was obtained from Phytolab, Germany.

c. Determination of quercetin and its derivatives by HPLC

The determination of individual flavonoids was performed by liquid chromatography using a Shimadzu Nexera-I HPLC apparatus. The separation was carried out on a Luna C18, silica gel-C18 150 x 4.6 mm x 3 µm column using gradient elution with a mixture of 0.1% formic acid solution with pH corrected to 2.5 and methanol. The composition of the mobile phase varies from 5% methanol to 25% in the first 3 minutes, then to 37% until minute 9, to 54% until the 18th minute and to 95% until the 26th minute. The composition of the mobile phase was maintained for 4 minutes and then arrived at 5% methanol until the end

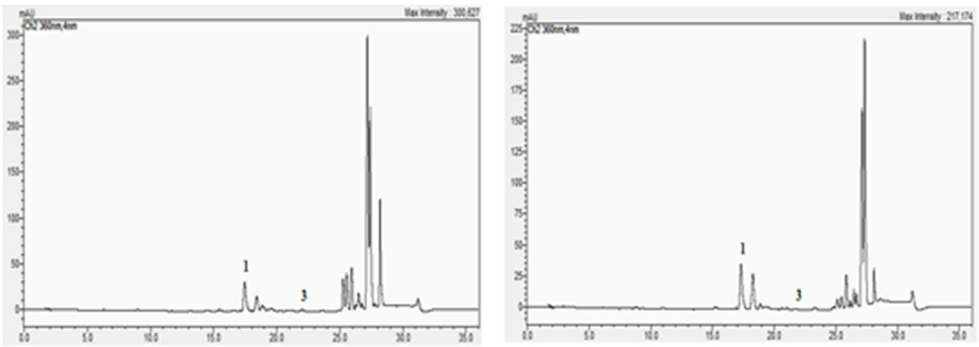
of the analysis (minute 35th). The separation was performed using a flow rate of 0.5 ml/minute. From each extract diluted 1 to 10 with methanol were injected 10 ul. For detection, a DAD spectrophotometer was employed that recorded all data in the range of 190–660 nm. The chromatogram for flavonoid identification and quantification was recorded at 360 nm. As standards were used quercetin, hyperoside and rutoside. The calibration curve data for all these flavonoids are presented in Table 1. All determinations were performed in triplicate and data were analyzed using Excel software from the Microsoft Office package (Criste et al., 2020). All solvents were of HPLC grade, purchased from Merck, Germany and the standards from Phytolab, Germany.

Table 1. Data of calibration curves

Standard	Concentration range, ug/ml	Calibration curve equation	Correlation factor R^2	Detection limit, ug/ml	Quantification limit, ug/ml
Quercetin	90–650	A = 35376*c-95138	0.9995	10.8	16.1
Hyperoside	60–510	A = 35253*c-185515	0.9979	10.5	21.0
Rutoside	60–510	A = 34187*c+67369	0.9985	2	7.9

RESULTS AND DISCUSSIONS

The chromatograms of the extracts recorded at 360 nm are presented in Figure 1. In the used chromatographic condition the flavonoids are separated after minute 10. The quercetin derivatives are separated between 14 and 22 minutes, the first time the glycosides and the last is the quercetin aglyka. It can be observed the presence of quercetin in both extracts, is accompanied by rutoside. The hyperoside could not be identified. The identification was based on the comparison of retention times and UV-Vis spectra shape and maximum absorbances between standards and the compounds separated from extracts. The quantification data are presented in Table 2.



a) **b)**

Figure 1. The HPLC chromatograms of the buds' extracts obtained from (a) white birch; (b) silver birch. 1 = rutosid (17.4 min; 257 and 356 nm); 3 = quercetin (22 min; 255 and 371 nm)

Table 2. Quantification results obtained for studied gemmotherapy extracts by HPLC

Extract from	Quercetin, ug/ml	Rutosid, ug/ml	Total flavonoids expressed in quercetin, mg/ml
White birch buds	37.80 ±0.017	119.90±0.084	1.20±0.095
Silver birch buds	30.95 ±0.011	123.90±0.084	0.94±0.088

Note: Values represent the mean ± standard deviations of three independent measurements

The results show that the highest content of quercetin is in the white birch buds extract. The studied gemmotherapy extracts contain also rutoside in a much higher amount, approximately 3 times more in white birch buds' extract and 4 times more in silver birch buds' extract. The white birch buds extract has the highest content in total flavonoids. If we calculate the percentage of quercetin from the total flavonoids amount we can see that in the white birch buds' extract, this represents 3.15% and in the silver birch buds' extract 3.29%, meaning that both species have similar content in quercetin.

The scientific literature is very poor regarding the phytochemical composition of gemmotherapy extracts that have been introduced in use more frequently in the last 40 years. For this reason, it is very difficult to compare our results with the other researchers. The results, of these special extracts are published for the first time, as far as we can observe in scientific databases. Despite this fact, we try to compare these results with those obtained for leaves or other plant materials from the studied species.

The *Betula* spp. buds' extracts contain the highest total flavonoid content, having 10–13% rutoside, but also the free quercetin is well represented. The leaves of different birch species were extensively studied. A recent study has evaluated the variation in polyphenols content of leaves and buds of different Betula species from Estonia revealing that the buds contain lower quantities of flavonoids in comparison with the leaves. In this study were identified in leaves quercetin and quercetin derivatives like hyperosid, quercitrin, etc. (Raal et al., 2015). Another study could identify *Betula pubescens* buds' fractions containing flavonoids (Isidorov et al., 2021).

Finally, we can conclude that the relatively few references that have been found confirm our results that the studied birch species buds are valuable sources of quercetin and its derivatives.

According to the physicians' observations, birch gemmotherapy extracts can be used in the recovery from respiratory system diseases. The two birch (*Betula pendula* Roth. and *Betula pubescens* Ehrh.) buds extracts have anti-inflammatory and detoxifying effects, being indicated in the prevention of tracheobronchitis relapses. The above-mentioned clinical observations could be related to flavonoids as the presence of quercetin in both studied gemmotherapy extracts, could be among others valuable adjuvants in reducing the risk of infection, and the complications in case of infections or in the improvement of pathologies after the infection with SarsCoV2 virus.

CONCLUSION

The present study demonstrated that the studied birch gemmotherapy extracts contain therapeutically valuable flavonoids. Together with already existing clinical observations these extracts, based also on their content in quercetin and its derivatives, could be considered

to be recommended for prevention of the complications from viral infections and also in convalescence after SarsCoV2 infection.

AUTHORS CONTRIBUTION

Conceptualization, O.N.K., M.E., H.D., T.V.; Data curation, T.V and M.E.; Formal analysis, H.D. and T.V.; Funding acquisition, O.N.K. and T.V.; Investigation, B.V.B., R.F.B., C.E., B.T., M.S., M.J., H.M.; Methodology, B.V.B., R.F.B., C.E., B.T., M.S., M.J., H.M.; Project administration, O.N.K. and M.E.; Resources, B.V.B., R.F.B., C.E., B.T., M.S., M.J., H.M; Software, B.V.B. and R.F.B.; Supervision, O.N.K. and T.V.; Validation, M.E.; Visualization, H.D.; Roles/Writing – original draft, O.N.K., M.E., H.D. T.V.; and Writing – review & editing, B.V.B.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

INSTITUTIONAL REVIEW BOARD STATEMENT

Not applicable.

INFORMED CONSENT STATEMENT

Not applicable.

DATA AVAILABILITY

The data that support the results of this study are available on request from the corresponding author, T.V.

REFERENCES

Almeida A.F., Borge G.I.A, Piskula M., Tudose A., Tudoreanu L., Valentova K., Williamson G., Santos C.N. 2018. Bioavailability of quercetin in humans with a focus on individual variation. *Comprehensive Reviews in Food Science and Food Safety*, 17, 714–731. http://dx.doi.org/10.1111/1541–4337.12342

Aucoin M., Cooley K., Saunders P.R., Cardozo V., Remy D., Cramer H., Abad C.N., Hannan N. 2020. The effect of quercetin on the prevention or treatment of COVID-19 and other respiratory tract infections in humans: a rapid review. *Advances in integrative medicine*, 7 (4), 247–251. http://dx.doi.org/10.1016/j.aimed.2020.07.007

Batiha G.E.S., Beshbishy A.M., Ikram M., Mulla Z.S., Abd El-Hack M.E., Taha A.E., Algammal A.M., Elewa Y.H.A. 2020. The pharmacological activity, biochemical properties and pharmacokinetics of the major natural polyphenolic flavonoid: quercetin. *Foods*, 9, 374. http://dx.doi.org/10.3390/foods9030374

Criste A., Urcan A.C., Bunea A., Pripon Furtuna F.R., Olah N.K., Madden R.H., Corcionivoschi N. 2020. Phytochemical composition and biological activity of berries and leaves from four Romanian sea buckthorn (*Hippophae rhamnoides* L.) varieties. *Molecules*, 25, 1170. http://dx.doi.org/10.3390/molecules25051170

D'Andrea G. 2015. Quercetin: A flavonol with multifaceted therapeutic applications? *Fitoterapia*, 106, 256–271. http://dx.doi.org/10.216/j.fitote.2015.09.018

David A.V.A, Arulmoli R., Parasuraman S. 2016. Overviews of biological importance of quercetin: a bioactive flavonoid. *Pharmacognosy Reviews*, 10(20), 84–89. http://dx.doi.org/10.4103/0973-7847.194044

Dengyu Y., Tiancheng W., Miao L., Peng L. 2020. Quercetin: its main pharmacological activity and potential application in clinical medicine. *Oxidative Medicine and Cellular Longevity*, 1–13. http://dx.doi.org/10.1155/2020/8825387

Di Pierro F., Iqtadar S., Khan A., Mumtaz S.U., Chaudhry M.M., Bertuccioli A., Derosa G., Maffioli P., Togni S., Riva A., Allegrini P., Khan S. 2021. Potential clinical benefits of quercetin in the early stage of COVID-19: results of a second, pilot, randomized, controlled and open-label clinical trial. *International Journal of General Medicine*, 14, 2807–2816. http://dx.doi.org/10.2147/IJGM.S318949

European Pharmacopoeia 2023. 11th Edition, EDQM,.

Farmacopeea Romana 1993. Ediția a 10-a, Editura Medicala, București, România.

Isidorov V.A., Nazaruk J., Stocki M., Bakier S. 2021. Secondary metabolites of downy birch buds (Betula pubescens Erch.). *A Journal of Biosciences: Zeitschrift für Naturforschung C*. http://dx.doi.org/10.1515/znc-2021–0036

Kasikci M.B., Bagdatlioglu N. 2016. Bioavailability of quercetin. *Current Research in Nutrition and Food Science Journal*, 4, 146–151. http://dx.doi.org/10.12944/CRNFSJ.4.Special-Issue-October.20

Kim J.K., Park S.U. 2018. Quercetin and its role in biological functions: an updated review. *EXCLI Journal*, 17, 856–863. http://dx.doi.org/10.17179/excli2018-1538

Ledoux F., Gueniot G. 2014. La phytoembryotherapie, l'embryon de la gemmotherapie; Editions Amyris SPRL, Bruxelles, Belgium.

Li H., Li M., Fu J., Ao H., Wang W., Wang X. 2021. Enhancement of oral bioavailability of quercetin by metabolic inhibitory nanosuspensions compared to conventional nanosuspensions. *Drug Delivery*, 28 (1), 1226–1236. http://dx.doi.org/10.1080/10717544.2021.1927244

Li Y., Yao J., Han C., Yang X., Tabassum Chaudhry M., Wang S., Liu H., Yin Y. Quercetin, inflammation and immunity. Nutrients 2016, 8, 167. http://dx.doi.org/10.3390/nu8030167

Maalik A., Khan F.A., Mumtaz A., Mehmood A., Azhar S., Atif M., Karim S., Altaf Y., Tariq I. 2014. Pharmacological applications of quercetin and its derivatives: a short review. *Tropical Journal of Pharmaceutical Research*, 13 (9), 1561–1566. http://dx.doi.org/10.4314/tjpr.v13i9.26

Nijveldt R.J., Van Nood E., Van Hoorn D.E.C., Boelens P.G., Van Norren K., Van Leeuwen P.A.M. 2001. Flavonoids: a review of probable mechanism of action and potential applications. *The American Journal of Clinical Nutrition*, 74, 418–425. http://dx.doi.org/10.1093/ajcn/74.4.418

Pharmacopee Francaise 2020. 11th Edition, https://ansm.sante.fr/pharmacopee/preparations-homeopathiques-anglais

Pitera di Clima F., Nicoletti M. 2018. Gemmoterapia. Fondamenti scientifici della moderna meristemoterapia. 2nd ed.; Nuova Ipsa Editore srl, Palermo, Italy.

Qiu X., Kroeker A., He S., Kozak R., Audet J., Mbikay M., Chretien M. 2016. Prophylactic efficacy of quercetin 3-b-O-D-glucoside against Ebola virus infection, *Antimicrobial Agents and Chemotherapy*, 60 (9), 5182–5188. http://dx.doi.org/10.1128/AAC.00307-16

Raal A, Boikova T, Pussa T. 2015. Content and dynamics of polyphenols in Betula spp. leaves naturally growing in Estonia. *Records of Natural Products*, 9 (1), 41–48.

Tetau M. 1998. Gemmotherapy, a clinical guide. 2nd ed.; Editions du Detail Inc., Paris, France,.

Weinjiao W., Richan L., Xianglian L., Jian H., Shibo J., Shuwen L., Jie Y. 2016. Quercetin as an antiviral agent inhibits influenza A virus (IAV) entry. *Viruses*, 8, 6. http://dx.doi.org/10.3390/v8010006