

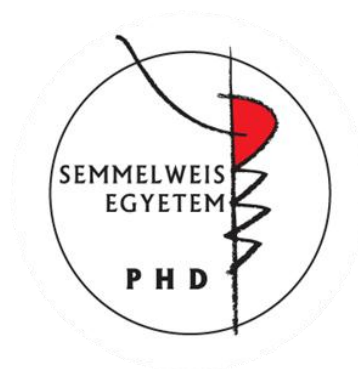
# **Recovery of new diarylheptanoid sources in Betulaceae**

## **Characterisation of the phenolic profile of *Corylus* species by HPLC-ESI-MS methods**

Ph.D. theses

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## Introduction

Natural products have been used to treat human diseases since ancient times. In recent years compounds of plant origin still play an important role in therapeutic drug discovery by providing large chemical diversity and covering an alternative chemical space compared with synthetic derivatives. Consequently, nowadays a significant part of phytochemical researches focuses on screening for potential lead molecules among herbal extracts. Orientation of these researches is mainly based on ethnopharmacology: identification of the major and minor bioactive constituents of traditionally used medicinal plants is a clearly promising way of discovering novel lead compounds. In addition, screening for structural analogues of natural lead molecules in different plants based on taxonomic relatedness also forms an important part of phytochemical studies. However, the cost- and time-consuming procedures of isolation and identification of natural compounds with beneficial biological activity have limited their use in the pharmaceutical industry in the past two decades.

On the other hand, the popularity of herbal remedies is increasing nowadays which comes together with the upsurge of necessity to assure quality, efficacy and safety of these products. Since plant extracts are complex matrices with numerous different constituents, the development of sophisticated analytical methods is a crucial point in the quality control of phytotherapeutics.

Diode-array detection (DAD) and mass spectrometry (MS) together with high-performance liquid chromatography (HPLC) separation offers great selectivity and sensitivity for the qualitative and quantitative analysis of complex plant samples. In addition, coupling bioassays to these analytical procedures (mainly HPLC-MS) allows the rapid and efficient identification of the bioactive constituents of plant extracts.

The aim of our work was the phytochemical evaluation of the *Corylus* (Betulaceae) species native to Hungary: *Corylus avellana* L, *Corylus colurna* L. and *Corylus maxima* Mill. Despite the long-term use of these plants in traditional medicine, their phytochemical exploration is still incomplete. Previous studies on the phenolic constituents of *C. avellana* kernels and leaves focused on the main flavonoid and caffeic acid derivatives, while there is no report concerning the detailed phytochemical composition of *C. colurna* and *C. maxima*. Numerous studies of Betulaceae species revealed that besides other phenolic constituents, diarylheptanoid-type compounds also show a frequent occurrence among these plants; however presence of these constituents is not reported in the *Corylus* species mentioned above. Diarylheptanoids have been proved to possess various pharmacological effects: e.g.

antioxidant, anti-inflammatory, anticancer, anti-adipogenic and antiviral activities suggest their potential utilisation in clinical practice. Therefore, our experiments focused on screening for structural analogues of these compounds in the selected *Corylus* species.

HPLC-ESI-TOF-MS (high-performance liquid chromatography coupled to electrospray ionisation-time-of-flight mass spectrometry) and HPLC-ESI-MS/MS (high-performance liquid chromatography coupled to electrospray ionisation-tandem mass spectrometry) methods were chosen for the simultaneous structural characterisation of the phenolics present in the *Corylus* extracts.

Moreover, in order to broaden information on their biological activity, studies on *in vitro* radical scavenging activity were carried out.

## Objectives

The primary aim of our study was to characterise the phenolic profile of the *Corylus* species native to Hungary: *Corylus avellana* L, *Corylus colurna* L. and *Corylus maxima* Mill. with particular attention to diarylheptanoids, on the one hand in order to screen for structural analogues of diarylheptan derivatives presented in different Betulaceae plants, concerning their various beneficial pharmaceutical effects; on the other hand to reevaluate the phytotherapeutic potential of these plants.

1. In order to gain preliminary information about the phenolic constitution of the *Corylus* crude drugs, we aimed to determine their total phenolic, flavonoid, and tannin contents according to the methods of Ph. Hg. VIII.
2. Plant phenolics are widely reported to act as antioxidants by scavenging free radicals, thus comparison of *in vitro* scavenging activity of the *Corylus* extracts prepared with ethyl acetate and methanol to those of well-known antioxidant phenolics was chosen as the starting point for further analyses.
3. For qualitative analysis of the phenolic composition of the *Corylus* extracts we aimed to optimise high-performance liquid chromatographic separation of the compounds and acquire structural information about the constituents by different detection methods: diode array detection, electrospray ionisation-time-of-flight mass spectrometry and electrospray ionisation-tandem mass spectrometry. Since literature data on the structural

characterisation of diarylheptanoids by mass spectrometry is limited, we also aimed detailed investigation of their mass spectrometric behaviour.

4. Our objective was to develop and validate high-performance liquid chromatographic-tandem mass spectrometric methods for the quantitative determination of the main phenolic compounds in the *Corylus* extracts.
5. We aimed to reveal the contribution of certain compounds of the *Corylus* extracts to the antioxidant activity by developing a high-performance liquid chromatographic-mass spectrometric method coupled with DPPH free radical scavenging assay.

## **Materials and methods**

### **Plant material**

Leaves and bark of *Corylus avellana* L. were collected in Nógrád, Nógrád county, Hungary (June 2010). Leaves and bark of *Corylus colurna* L. were collected in Göd, Pest county, Hungary (August 2012). Leaves and bark of *Corylus maxima* Mill. were collected in Pálfişzeg, Zala county, Hungary (August 2013).

For all the plant material mentioned above, 50-50 g samples were collected from three trees after flowering stage. Plant samples were authenticated in the Department of Pharmacognosy, Semmelweis University, Budapest, where voucher specimen are deposited.

### **Extraction and sample preparation**

Soxhlet extraction was performed using a laboratory-scale apparatus. Dried and milled plant samples (10 g each) were extracted consecutively with 250-250 ml of *n*-hexane at 70 °C, chloroform at 65 °C, ethyl acetate at 80 °C and methanol at 65 °C, for 6 h each. The extracts were evaporated to dryness under reduced pressure in a rotary evaporator at 50 °C. The dried extracts were dissolved in HPLC grade methanol to obtain sample solution concentrations of 25-30 mg/ml. The solutions were filtered through Phenex-RC 15 mm, 0.2 µm syringe filters.

### **Antioxidant activity assays**

Antioxidant activity of the *Corylus* extracts and hirsutenone, quercetin, kaempferol, caffeic acid, ascorbic acid, myricetin-3-*O*-rhamnoside and trolox standards was determined using DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS {2,2'-azino-bis-(3-ethylbenzothiazoline-6-

sulfonic acid)), as free radicals. During the assay, 50  $\mu$ l of the samples of five different concentrations, in three parallels were added to 2.5 ml of the free radical solutions. The decrease of absorbance was recorded against a blank sample (methanol and ethanol for DPPH and ABTS, respectively) at 515 nm and 734 nm, respectively. The concentrations belonging to the half maximal inhibition ( $IC_{50}$  value as  $\mu$ g/ml), were determined by linear regression.

## **Characterisation of phenolics in the *Corylus* extracts by HPLC-DAD-MS**

### **HPLC-DAD-QMS conditions**

For chromatographic separation an Agilent 1100 HPLC system was utilised. The *Corylus* samples were separated on a Kinetex-XB C18 column (150  $\times$  4.6 mm, 2.6  $\mu$ m) maintained at 40°C. Injection volume was 10  $\mu$ l. The following gradient elution program was applied at a flow rate of 0.5 ml/min; where eluent A was 0.2 % (v/v) acetic acid, eluent B was acetonitrile: 0 min: 0% (v/v) B, 5 min: 5% (v/v) B, 15 min: 10% (v/v) B, 30 min: 20% (v/v) B, 60 min 50% (v/v) B, 70 min 100% (v/v) B. Chromatograms were acquired at 254, 280 and 350 nm, as the most selective wavelengths for the detection of flavonoids and diarylheptanoids. UV spectra were recorded between 200 and 400 nm. During the LC-MS analyses ESI was operated in the negative ion mode, which provided better sensitivity due to the phenolate group of the investigated compounds.

### **HPLC-DAD-TOF-MS conditions**

Accurate mass data were acquired on an Agilent 6230 time-of-flight mass spectrometer equipped with a Jet Stream electrospray ion source (ESI). During the LC-MS analyses ESI was operated in the negative ion mode, which provided better sensitivity due to the phenolate group of the investigated compounds. Samples were introduced to Agilent 1260 Infinity HPLC. Mass spectra were processed by Agilent MassHunter B.03.01 software. The *Corylus* samples were separated on a Zorbax SB C18 column (150  $\times$  3.0 mm, 3.5  $\mu$ m) maintained at 25°C. Injection volume was 10  $\mu$ l. The following gradient elution program was applied at a flow rate of 0.4 ml/min; where eluent A was 0.2 % (v/v) acetic acid, eluent B was methanol: 0 min: 30% (v/v) B, 20 min: 100% (v/v) B, 22 min: 100% (v/v) B, 23 min: 30% (v/v) B. Chromatograms were acquired at 254, 280 and 350 nm, as the most selective wavelengths for the detection of flavonoids and diarylheptanoids. UV spectra were recorded between 200 and 400 nm.

### **HPLC-DAD-MS/MS conditions**

For chromatographic separation an Agilent 1100 HPLC system was used. The same column maintained at 25 °C, as well as the same eluents and gradient program at a flow rate of 0.3 ml/min were utilised as for HPLC-TOF-MS analyses of the extracts. Tandem mass spectrometric (MS/MS) analyses were performed on an Agilent 6410 triple quadrupole system equipped with an electrospray ion source (ESI). During the LC-MS analyses ESI was operated in the negative ion mode, which provided better sensitivity due to the phenolate group of the investigated compounds. The Agilent MassHunter B.01.03 software was used for data acquisition, and for qualitative and quantitative analyses. Collision energy was changed between 10–50 eV, according to differences in molecule structures (high purity nitrogen was used as collision gas). For structural characterisation of the compounds retention times, accurate molecular masses and calculated molecular formulas, UV and mass spectral data were compared to literature data and to those of authentic standards, where available.

### **Quantitative analyses by HPLC-MS/MS**

Quantification of myricetin-3-*O*-rhamnoside, quercetin-3-*O*-rhamnoside, hirsutenone and oregonin in the *Corylus* extracts was performed by the external standard method. Quantities were determined by HPLC-ESI-MS/MS in MRM (multiple reaction monitoring) mode. The same HPLC-MS/MS conditions were applied as described above. Before quantification both fragmentor voltage and collision energy were optimized with the use of the sample extracts by parameter ramping, from 70 to 250 V, with steps of 10 V and from 5 eV up to 50 eV, with steps of 5 eV, respectively.

Standard solutions for the calibration were prepared at five different concentrations in triplicates using hirsutenone, oregonin, myricetin-3-*O*-rhamnoside and quercetin-3-*O*-rhamnoside authentic standards in gradient grade methanol.

Retention time repeatability was checked with six successive runs of the *Corylus* extracts. Quality control samples were prepared in three different concentrations for each standard solution. These were used to determine both the intra-day and inter-day precision and accuracy (low, mid and high concentrations of the standards in three parallel runs on the same day and on three successive days, respectively). Blank samples (pure solvents) were analyzed to check the occurrence of any impurity or co-elution with the same  $m/z$  as that of the analytes.

### HPLC-based DPPH scavenging assay

100 µl of the *Corylus* extracts of certain concentrations dissolved in methanol and 100 µl of the DPPH solution (1.2 mg/ml in methanol) were mixed and incubated for 30 minutes at room temperature, protected from light. Then the reaction mixture was directly analysed by HPLC-DAD-QMS. The concentration of the extracts used for the analyses was adjusted by the evaluation of the chromatograms after mixing them with the DPPH solution in different concentrations within the range of 1.0-5.0 mg/ml. The concentration, where the decrease in the peak area of myricetin-3-*O*-rhamnoside, or if that was present in small amounts, that of quercetin-3-*O*-rhamnoside, reached its maximum, was chosen. The control samples were prepared by the addition of 100 µl methanol to 100 µl of the extracts.

## Results

### Antioxidant activity assays

The methanolic extracts of *C. maxima* leaves and bark showed significantly the highest scavenger capacity among the *Corylus* samples in the ABTS test. The bark samples acted noticeably stronger against ABTS free radical than the leaves, while the difference in the DPPH test was not significant. Both the leaves and bark methanolic extracts showed higher antioxidant activity than the ethyl acetate extracts in both *in vitro* tests.

The *C. colurna* bark extracts showed the highest scavenging capacity among the *Corylus* samples in the DPPH test, exceeding also the activity of all the standards except hirsutenone. The scavenger capacity of the bark extracts was found to be higher than that of the leaves in the ABTS test as well. The ethyl acetate extract of the leaves possessed more potent scavenger activity against DPPH free radical, while the methanolic extract acted stronger against ABTS. In case of the bark samples the difference between the activities of the ethyl acetate and methanolic extracts was not explicit.

In case of *C. avellana* significant difference was not observed between the DPPH scavenging capacities of the extracts, while in the ABTS tests the bark samples showed noticeably higher antioxidant activity than the leaves. Although neither in the latter case was significant difference found between the activities shown by the ethyl acetate and methanolic extracts of the bark or leaves.

It has to be noted that neither the DPPH nor the ABTS test can be considered physiological, since none of the two radicals occur in biological systems. Therefore, the results obtained by these methods cannot be extrapolated to *in vivo* data. However, the measured high *in vitro*

scavenging capacity regarding the extracts indicated the presence of potential natural antioxidants, furthermore the fact that the measured antiradical activities did not show any trend, indicated notable differences in the phenolic profile of the extracts. Thus, characterisation of the phenolic fingerprint of the samples was found to be reasonable.

### **Characterisation of phenolics in the *Corylus* extracts by HPLC-MS**

HPLC-ESI-MS methods were developed for the characterisation of phenolics in the *Corylus* extracts that provided uniform platform for the investigation of both flavonoid and diarylheptanoid-type compounds. For the characterisation of the compounds UV spectral data, obtained by LC-DAD, accurate molecular mass and formula, acquired by LC-ESI-TOF and fragmentation pattern, given by LC-ESI-MS/MS analyses were compared to those of authentic standards and to literature data. Altogether twenty diarylheptanoids, nine flavonoid derivatives and eight other compounds, mainly caffeic acid derivatives, were characterised in the *Corylus* extracts. The presence of myricetin- quercetin- and kampferol-3-*O*-rhamnoside was proved in all the extracts of the three *Corylus* species, but the obtained HPLC-fingerprints were different.

The HPLC-DAD-ESI-MS analyses revealed that the main compounds of all the *Corylus* extracts examined were flavonoid derivatives, except for the methanolic extract of *C. avellana* bark, in which a caffeic acid derivative was found to be predominant. According to the HPLC chromatograms, in both the ethyl acetate and methanolic extracts of *C. avellana* leaves myricetin-3-*O*-rhamnoside was the most abundant, while in the ethyl acetate extract of the bark the dominance of quercetin- and kaempferol-3-*O*-rhamnosides was observed. In all the *C. colurna* samples quercetin-3-*O*-rhamnoside, while in the *C. maxima* extracts myricetin-3-*O*-rhamnoside were identified as the main compounds. The presence of myricetin- quercetin- and kampferol-3-*O*-rhamnoside was proved in all the extracts of the three *Corylus* species.

The greatest diversity regarding the structures of the detected diarylheptanoid compounds was observed in the case of the *C. maxima* extracts. Both in the leaves and bark extracts numerous structurally different diarylheptanoids were characterised. In the leaves of *C. avellana* also several diarylheptanoids were detected, however in the bark extracts these compounds were not present. The *C. colurna* samples contained few diarylheptanoid compounds compared with the latter two.



### Quantitative analyses by HPLC-MS/MS

The results of the quantitative analyses by HPLC-MS/MS clearly indicated that myricetin-3-*O*-rhamnoside and quercetin-3-*O*-rhamnoside were present in the extracts in much higher amounts than the investigated two diarylheptanoids, namely hirsutenone and oregonin.

In our studies both myricetin-3-*O*-rhamnoside and quercetin-3-*O*-rhamnoside were present in all the extracts in amounts above the LOQ of the applied HPLC-MS/MS method. *C. colurna* leaves ethyl acetate extract was found to be the richest in quercetin-3-*O*-rhamnoside, while *C. avellana* leaves ethyl acetate extract in myricetin-3-*O*-rhamnoside. The ethyl acetate extracts were richer in both the flavonol-glycosides, except for the *C. maxima* extracts where this trend was unequivocal, moreover, in most cases the opposite was observed.

Regarding oregonin and hirsutenone the results showed that the quantity of the two compounds was below the LOQ of the applied HPLC-MS/MS method both in *C. avellana* bark and in *C. colurna* leaves extracts. Similarly, oregonin was not detected in the leaves of *C. avellana*, while hirsutenone in the bark of *C. colurna*. In the *C. maxima* extracts it was possible to quantify both oregonin and hirsutenone. The highest quantities of the two diarylheptanoids were measured in *C. maxima* leaves ethyl acetate extract; this was found to be richer in hirsutenone, while the other three contained higher amounts of oregonin.

In general it could have been observed that in case of all the samples, the ethyl acetate extract contained the two investigated diarylheptanoids in higher amounts compared with the corresponding methanolic extract. These results confirm that diarylheptanoids show better solubility in ethyl acetate, thus it can be the appropriate solvent of choice for the enrichment of the *Corylus* extracts in these compounds.

### HPLC-based DPPH scavenging assay

Our qualitative and quantitative results supported the assumption that the phenolic compounds played an important role in the high antioxidant activity of the *Corylus* extracts. The dominance of flavonol-3-*O*-glycosides was observed in the case of all the examined samples, while diarylheptanoids were present in the extracts as minor constituents. Therefore, only the correlation between the content of the two main flavonoid derivatives, namely myricetin-3-*O*-rhamnoside and quercetin-3-*O*-rhamnoside in the extracts, and the antiradical power presented in the DPPH and ABTS *in vitro* tests was investigated by plotting the 1/IC<sub>50</sub> data as the function of the corresponding compound concentrations. No correlation has been found neither with the myricetin-3-*O*-rhamnoside nor with the quercetin-3-*O*-rhamnoside content of

the samples, nor with the sum of the two. These observations let us conclude that the scavenger capacity of the major constituents was influenced by the minor compounds, e.g. diarylheptanoids and caffeic acid derivatives. Therefore, examination of the contribution of certain compounds to the total antioxidant activity of the extracts was found to be reasonable. Coupling the DPPH assay to chromatographic separation was found to be most appropriate method of choice for this purpose. Such approaches have been developed in order to identify the radical scavenger constituents in plant extracts by monitoring the decrease in the chromatographic peak areas of certain compounds after reaction with DPPH free radical. It has been demonstrated that the peak areas of constituents presenting antioxidant activity significantly decreased, whereas no change was observed regarding the peaks of compounds without scavenging activity.

Based on our results the contribution of certain compounds to the total antioxidant activity could have also been estimated by using the  $\Delta$ Area ratio value. Evidently, these calculations can be used only if the stoichiometry of the DPPH scavenging of the main compounds is similar.

In *C. avellana* leaves ethyl acetate extract relatively high amounts of myricetin-3-*O*-rhamnoside and quercetin-3-*O*-rhamnoside were determined. According to the HPLC-based results the contribution of the two compounds to the DPPH scavenger activity was nearly 80% that resulted in moderate to high DPPH activity of the extract. Similar results were obtained regarding the methanolic extract of the leaves; moderate to high content of the two previously mentioned flavonoids with nearly 90% contribution to the scavenger capacity determined moderate to high antioxidant activity for the whole extract. In the bark extracts quercetin-3-*O*-rhamnoside was proved to be dominant regarding the DPPH scavenging effect, moderate amount of this compound led to moderate antioxidant activity.

In the *C. colurna* leaves ethyl acetate extract the highest quercetin-3-*O*-rhamnoside content was measured among the investigated samples, although the contribution of the compound to the antioxidant activity was relatively low. Furthermore, only moderate scavenger capacity was measured regarding the whole extract. These results might indicate antagonistic interaction between the antioxidant components. Similar conclusion could have been drawn regarding the methanolic extract. The highest DPPH scavenging activity was obtained in the case of the *C. colurna* bark extracts. Quercetin-3-*O*-rhamnoside was found to be predominant in the antioxidant effect, which was present in high amounts in the ethyl acetate extract. It was also observed that the methanolic extract possessed lower scavenging activity compared with the ethyl acetate extract in accordance with the significant difference in their quercetin-3-*O*-

rhamnoside content. Although, it has also to be mentioned that in the methanolic extract kaempferol-3-*O*-glucuronide, the main compound, was found to contribute mostly to the scavenger capacity.

In the *C. maxima* leaves extracts moderate amounts of myricetin-3-*O*-rhamnoside and quercetin-3-*O*-rhamnoside were determined, although the extracts showed low DPPH scavenging activity. This can be contributed to the large diversity of these extracts regarding antioxidant compounds that makes several interactions possible. In the bark extracts also moderate amounts of the two flavonoids were measured, neither their contribution to the DPPH scavenger capacity was explicit, which led to moderate or low antioxidant activity regarding the whole extract.

It could have been generally concluded that the correlation between the amount of the main antioxidant compounds and the scavenger capacity of the extracts is not always equivocal and let us assume the presence of different interactions among the constituents.

## Conclusions

1. Total phenolic, flavonoid and tannin contents of the *Corylus* crude drugs were determined according to the spectroscopic methods described in Ph. Hg. VIII. Based on our results it could be concluded that all the crude drugs contained notable amounts of polyphenol compounds, with *C. maxima* bark being the richest in these constituents.
2. Since plant phenolics are widely reported to act as antioxidants, and the *Corylus* crude drugs were proved to be rich in these constituents, determination of the *in vitro* radical scavenging activity of the ethyl acetate and methanolic extracts was found to be reasonable. Two *in vitro* tests using DPPH and ABTS as free radicals were utilised. Our results indicated that all the extracts possessed notable activity in both *in vitro* tests compared to well-know antioxidant standards. Comparison of the results of the different extracts to each other revealed that no trend occurred regarding the scavenger capacity. These observations let us conclude that there were differences in the phenolic profile of the extracts studied.
3. HPLC-ESI-MS methods were developed for the characterisation of phenolics in the *Corylus* extracts that provided uniform platform for the investigation of both flavonoid and diarylheptanoid-type compounds. For the characterisation of the compounds UV

spectral data, obtained by LC-DAD, accurate molecular mass and formula, acquired by LC-ESI-TOF and fragmentation pattern, given by LC-ESI-MS/MS analyses were compared to those of authentic standards and to literature data. Altogether twenty diarylheptanoids, nine flavonoid derivatives and eight other compounds, mainly caffeic acid derivatives, were characterised in the *Corylus* extracts. The presence of myricetin- quercetin- and kampferol-3-*O*-rhamnoside was proved in all the extracts of the three *Corylus* species, but the obtained HPLC-fingerprints were different. Therefore, it could be concluded that the developed HPLC-DAD-ESI-MS methods can be successfully utilised for the identification and differentiation of the *Corylus* extracts obtained from the three species. One of the novel findings of our qualitative studies was the characterisation of diarylheptanoids, since no previous paper had reported the presence of these compounds in *Corylus* species. Further studies, including isolation and identification of these constituents by NMR spectroscopy, would be worthy to perform. In addition to the the former results, the phytochemical investigation of *C. maxima* and *C. colurna* and the characterisation of their phenolic compounds have been reported in this study for the first time. Results of the detailed investigation of the phenolic profile of the *Corylus* species might explain their previously reported antioxidant, anti-inflammatory, anti-microbial and hepatoprotective effects. Besides, comparison of the phenolic profile of each *Corylus* species to other members of the Betulaceae family would be interesting from the chemotaxonomic point of view as well.

4. For the determination of the two main flavonoid compounds, namely myricetin-3-*O*-rhamnoside and quercetin-3-*O*-rhamnoside, and the two most abundant diarylheptanoids, hirsutenone and oregonin, a HPLC-ESI-MS/MS method using MRM (multiple reaction monitoring) mode was developed and validated. The method was successfully utilised for the quantitative analyses of the *Corylus* samples, and might be useful in future studies aiming the quality control of different *Corylus* extracts used in medication. In addition, this quantitative method would be applicable for the investigation of the influence of cultivar, geographical origin, seasonal variations and ripening stage on the yield of the compounds with relevant biological activity.
5. Our qualitative and quantitative results supported the presumption that the phenolic compounds played significant role in the high antioxidant activity of the *Corylus* extracts. The dominance of flavonol-3-*O*-glycosides was observed in the case of all the examined samples, while diarylheptanoids were present as minor constituents. Literature data

reporting high antioxidant activity regarding not only flavonoids but also diarylheptanoids, and the fact that no correlation has been found between the antiradical power and the quantity of the main flavonoids in the extracts let us conclude that the scavenger capacity of the major constituents was influenced by the minor compounds, e.g. diarylheptanoids. Therefore, a high-performance liquid chromatographic-mass spectrometric method coupled with DPPH free radical scavenging assay was developed in order to gain information about the contribution of certain constituents to the total antioxidant activity. Based on our results we could conclude that in some cases the quantity of the main flavonol-3-*O*-glycosides in the extracts strongly affected the scavenger capacity, but in other cases, where several compounds played role in the antioxidant effect, the contribution of the main compounds was not equivocal. Thus, further studies aiming the clarification of the interactions between the antioxidant compounds (synergism, antagonism or additive effect) are needed, in order to investigate the possibility of the preparation of fractions with evaluated antioxidant effect from the extracts.

## Publications

### Publications related to the thesis

**Riethmüller E**, Könczöl Á, Szakál D, Végh K, Balogh GyT, Kéry Á. (2016) HPLC-DPPH Screening Method for Evaluation of Antioxidant Compounds in *Corylus* Species. Nat Prod Comm, 11: 641-644.

Alberti Á, **Riethmüller E**, Béni Sz, Kéry Á. (2016) Evaluation of Radical Scavenging Activity of *Sempervivum Tectorum* and *Corylus Avellana* Extracts with Different Phenolic Composition. Nat Prod Comm, 11:469-474.

**Riethmüller E**, Tóth G, Alberti Á, Végh K, Burlini I, Könczöl Á, Balogh GyT, Kéry Á. (2015) First characterisation of flavonoid- and diarylheptanoid-type antioxidant phenolics in *Corylus maxima* by HPLC-DAD-ESI-MS. J Pharm Biomed Anal, 107: 159-167.

**Riethmüller E**, Tóth G, Alberti Á, Végh K, Béni Sz, Balogh GyT, Kéry Á. (2015) Diarylheptanoidok előfordulása a mogyoró nemzetség Kárpát-medencében honos fajaiban [Occurrence of diarylheptanoids in *Corylus* species native to Hungary]. Acta Pharm Hung, 85: 29-40.

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**Riethmüller E**, Alberti Á, Tóth G, Béni Sz, Ortolano F, Kéry Á. (2013) Characterisation of diarylheptanoid- and flavonoid-type phenolics in *Corylus avellana* L. leaves and bark by HPLC/DAD-ESI/MS, Phytochem Anal, 24: 493-505.

### Further scientific publications

Földi T, Kupai J, Túrós GyI, Németh T, Rojik E, **Riethmüller E**, Balogh GyT, Huszthy P. (2016) Application of flow chemistry to macrocyclization of crown ethers. J Flow Chem article in press

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Tóth A, **Riethmüller E**, Alberti Á, Végh K, Kéry Á. (2012) Comparative phytochemical screening of phenoloids in *Lysimachia* species. Eur Chem Bull, 1: 27-30.