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Marianna Manninen

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## ABSTRACT

The chemical diversity of plants is enormous as plants produce a variety of compounds that play key roles, for instance, in the growth, defence and reproduction of plants. This diversity is not well recognised in chemistry education. To understand the chemical diversity of plants, modern research instrumentation is needed. However, the cognitive and affective outcomes of using modern instrumentation in chemistry education have not been extensively studied. In this thesis, tools for demonstrating the chemical diversity of plants by liquid chromatography and mass spectrometry (LC-MS) and spectrophotometry were created. In addition, first-year university chemistry students' conceptions of plant chemistry were investigated. The changes in students' knowledge of research instruments through hands-on exposure were studied using pre- and post-tests, and the influence of different background factors in learning and experiences were analysed.

The first tool demonstrated the differences in the pigment compositions of autumn leaves. Altogether, 34 upper secondary school and university students analysed the pigment concentrations spectrophotometrically and studied the pigment composition in more detail with LC-MS. With the second tool, 130 upper and lower secondary school and university students studied the defence compounds of leaf buds spectrophotometrically and identified white birch and silver birch samples by their LC-MS fingerprints. This tool provided students a basic understanding of how the instruments function and what they are used for. Their knowledge of the chemistry of plants was concentrated on primary metabolites, but the tool widened their views of specialised metabolites and their functions in plants. To accompany the method for distinguishing the birch species, a chemotaxonomic tool was created for the identification of additional 13 common Finnish deciduous trees and shrubs from the leaf bud extracts. These species were screened for potential markers, which represented a wide range of plant metabolites, thus exemplifying the chemical diversity of leaf buds. The results of the final part of this thesis showed that the use of a deep learning approach resulted in better learning outcomes and positive experiences, whereas the use of a surface approach affected the learning outcomes and experiences negatively. The perceived relevance of research instruments for studies and chemistry research affected positively students' experiences.

KEYWORDS: Chemistry education, modern instrumentation, plant chemistry

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## TIIVISTELMÄ

Kasvit ovat kemiallisesti valtavan monimuotoisia, sillä ne tuottavat laajan joukon yhdisteitä, joilla on merkittäviä tehtäviä liittyen esimerkiksi kasvien kasvuun, puolustautumiseen ja lisääntymiseen. Tämä monimuotoisuus tunnistetaan huonosti kemian opetuksessa. Kasvien kemiallisen monimuotoisuuden ymmärtäminen vaatii moderneja tutkimuslaitteita. Sitä ei kuitenkaan ole laajasti tutkittu, millaisia kognitiivisia ja affektiivisia tuloksia saavutetaan, kun tutkimuslaitteita hyödynnetään kemian opetuksessa. Tässä väitöskirjatyössä kehitettiin työkaluja kasvien kemiallisen monimuotoisuuden havainnollistamiseksi hyödyntäen nestekromatografiaa ja massaspektrometriaa (LC-MS) sekä spektrofotometriaa. Lisäksi kartoitettiin kemian ensimmäisen vuoden yliopisto-opiskelijoiden käsityksiä kasvien kemiasta. Ennakko- ja jälkikyselyiden avulla tutkittiin, miten opiskelijoiden tiedot tutkimuslaitteista muuttuivat käytännön kokemuksen kautta. Lisäksi analysoitiin eri taustamuuttujien vaikutusta opiskelijoiden oppimiseen ja kokemuksiin.

Ensimmäinen työkalu havainnollisti eroja ruskalehtien väriainekoostumuksessa. Yhteensä 34 lukio- ja yliopisto-opiskelijaa analysoi väriainepitoisuuksia spektrofotometrisesti ja tutki väriainekoostumusta tarkemmin LC-MS:lla. Toisen työkalun avulla 130 yläkoulu-, lukio- ja yliopisto-opiskelijaa tutki lehtisilmujen puolustusyhdisteitä spektrofotometrisesti, sekä tunnisti hies- ja rauduskoivunäytteet niiden LC-MS-sormenjälkien avulla. Tämä työkalu antoi opiskelijoille perustiedot siitä, kuinka käytetyt laitteet toimivat, ja mihin niitä voidaan käyttää. He tunsivat ennalta pääasiassa kasvien primäärimetaboliitteja, mutta kyseisen työkalun avulla he oppivat erikoistuneista metaboliiteista ja niiden tehtävistä kasveissa. Koivujen tunnistamiseen käytettävän menetelmän täydentämiseksi luotiin kemotaksonominen työkalu, joka mahdollisti 13 suomalaisen puun tai pensaan tunnistamisen lehtisilmusta tehdyn uutteen avulla. Näille kasvilajeille seulottiin lupaavia markkereita, jotka edustivat laajaa joukkoa kasvien metaboliitteja ilmentäen samalla lehtisilmujen kemiallista monimuotoisuutta. Väitöskirjatyön viimeisen osan tulokset osoittivat, että syväsuuntautunut lähestymistapa oppimiseen vaikutti positiivisesti opiskelijoiden oppimiseen ja kokemuksiin. Pintasuuntautunut lähestymistapa vaikutti puolestaan negatiivisesti. Tutkimuslaitteiden pitäminen merkityksellisinä opintojen tai kemian tutkimuksen kannalta vaikutti positiivisesti opiskelijoiden kokemuksiin.

ASIASANAT: Kasvien kemia, kemian opetus, modernit tutkimuslaitteet

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# Abbreviations

| | |
|---|---|
| AOA | Antioxidant activity |
| DAD | Diode array detector |
| DPPH | 2,2-diphenyl-1-picrylhydrazyl |
| EIC | Extracted ion chromatogram |
| ESI | Electrospray ionisation |
| GC | Gas chromatography |
| H-ESI | Heated electrospray ionisation |
| LC | Liquid chromatography |
| MS | Mass spectrometry |
| PTFE | Polytetrafluoroethylene |
| SIR | Selected ion recording |
| SRM | Selected reaction monitoring |
| TLC | Thin layer chromatography |
| TPC | Total phenolic content |
| UHPLC | Ultrahigh-performance liquid chromatography |
| QqQ | Triple quadrupole mass spectrometer |

# List of Original Publications

This dissertation is based on the following original publications, which are referred to in the text by their Roman numerals:

I Manninen, M., Vesterinen, V.-M., and Salminen, J.-P. Chemistry of autumn colors: quantitative spectrophotometric analysis of anthocyanins and carotenoids and qualitative analysis of anthocyanins by ultra-performance liquid chromatography–tandem mass spectrometry. *Journal of Chemical Education*, 2020; 97: 772–777.

II Manninen, M., Vesterinen, V.-M., Vainio, A.-K., Korhonen, H., Karonen, M. and Salminen, J.-P. Identification of tree species by their defense compounds: a study with leaf buds of white and silver birches. *Journal of Chemical Education*, 2021; 98: 973–981.

III Manninen, M., Karonen, M., and Salminen, J.-P. Chemotaxonomic markers for the leaf buds of common Finnish trees and shrubs: a rapid UHPLC-MS fingerprinting tool for the species identification. Accepted after minor revisions to *Molecules*.

IV Manninen, M., Karonen, M., Lastusaari, M. and Vesterinen, V.-M. Learning approach and the relevance of research instruments are connected to the cognitive and affective outcomes of a laboratory experiment. Submitted to *Chemistry Education Research and Practise*.

# 1 Introduction

Plants are skilled chemists producing a vast range of compounds that are connected to their growth, help in the adaptation to the environment, and regulate organismal processes and metabolism. Especially compounds that aid in the interaction with biotic and abiotic environment, known as specialised metabolites, provide an interesting view to understanding the differences of the plant kingdom. Specialised metabolites are usually categorized according to their biosynthetic pathways under three main molecular families: phenolics, terpenes and alkaloids. The structural diversity among the molecular families is tremendous. Moreover, different plant genera and species are specialised in the production of different compounds. Such species-specific or genus-specific compounds can be used as chemotaxonomic markers.

Compared to primary metabolites that are common to all plants and are essential for cellular functions, specialised metabolites have more specific functions in plants. The functions of specialised metabolites may relate to defence and competition, attraction and stimulation, or abiotic stress (Hartmann, 2007). Specialised metabolites may defend plants against pathogens through antibiotic, antifungal and antiviral properties, or repel unwanted feeders. Some compounds such as pigments may be produced to attract insects and animals to enhance the reproduction of the plant (Andersen & Jordheim, 2010). Other compounds such as phenolics may protect the plant from excess UV radiation (Cheynier et al., 2013; Tattini et al., 2000).

## 1.1 The chemical diversity of common Finnish trees and shrubs

Finnish deciduous trees and shrubs provide interesting examples of the chemical diversity and especially of the structural diversity of plant metabolites in plants. Examples of the compounds are shown in Figure 1 and major compounds in different plant genera are summarised in Table 1. Salicylate-like simple phenolic glucosides (also known as salicinoids, e.g. salicortin; **1**) are characteristic constituents of *Populus* and *Salix* species such as *Populus tremula* and *Salix phylicifolia* (Abreu et al., 2011; Julkunen-Tiitto, 1989). Diarylheptanoids (e.g. oregonin; **2**) are another subgroup of compounds belonging to the molecular family of phenolics. They are

widely distributed in the family of Betulaceae, but they are of special importance as chemotaxonomic markers for *Alnus* species (Ren et al., 2017). Diarylheptanoids can be used in distinguishing between *Alnus glutinosa* and *Alnus incana* (Vidakovic et al., 2017). Another interesting group of compounds due to their limited distribution in the plant kingdom are iridoids (e.g. oleuropein; **3**), which are terpenoids characteristic to the family of Oleaceae (Jensen et al., 2002). Furthermore, the distribution of different iridoid subgroups correlates with the phylogenetic classification (Jensen et al., 2002).

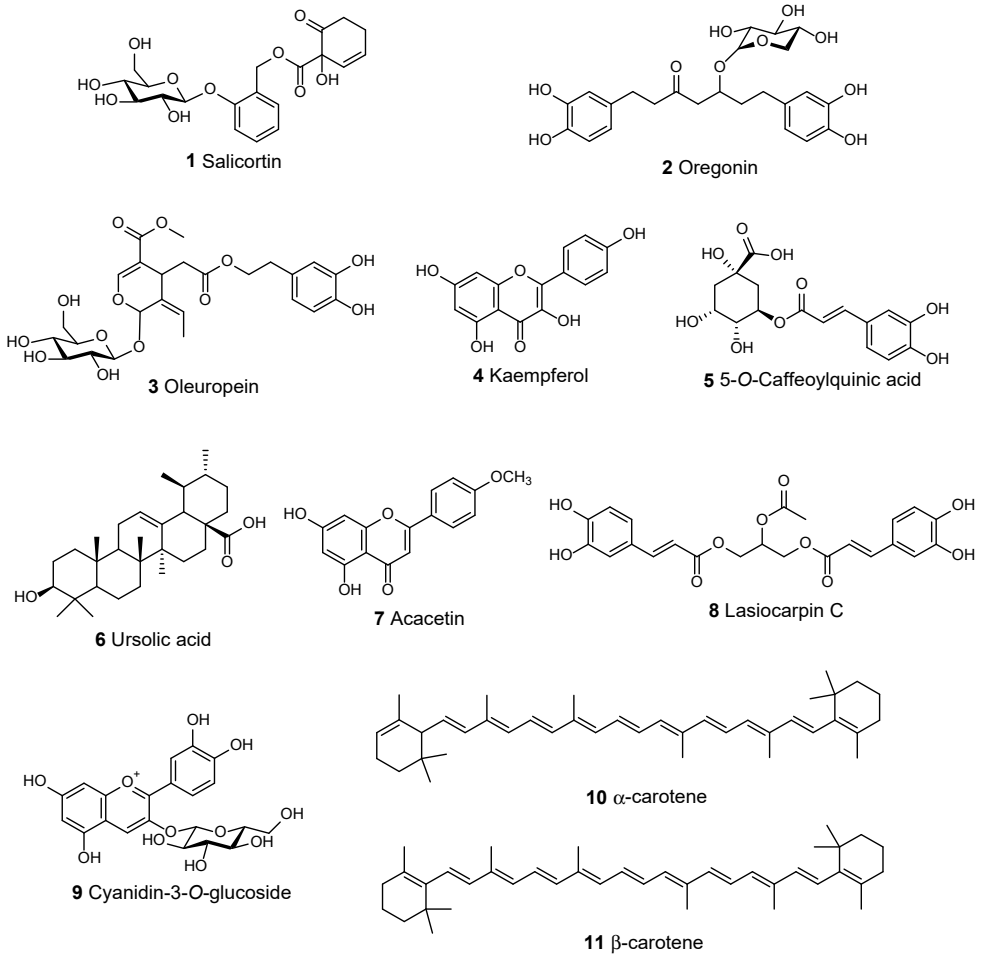


**Figure 1.** Examples of compounds from different molecular families found in Finnish trees and shrubs.

**Table 1.** Overview of the major compounds reported in different plant genera that were included in this thesis.

| Genus (Family) | Common compounds for the genus | References | Plant species studied in this thesis |
|---|---|---|---|
| ***Acer*** **(Sapindaceae)** | Flavonoids, gallotannins, diarylheptanoids, lignans, pentacyclic triterpenoids, hydroxycinnamic acid derivatives | Bi et al., 2016 | *Acer platanoides* |
| ***Alnus*** **(Betulaceae)** | Diarylheptanoids, gallotannins, ellagitannins, flavonoids, tetracyclic and pentacyclic triterpenoids | Ren et al., 2017 | *Alnus glutinosa, Alnus incana* |
| ***Betula*** **(Betulaceae)** | Triterpenoids, diarylheptanoids, proanthocyanidins, ellagitannins, gallotannins, galloylglucoses, flavonoids | Keinänen & Julkunen-Tiitto, 1998; Rastogi et al., 2015; Salminen et al., 2002 | *Betula pendula, Betula pubescens* |
| ***Fraxinus*** **(Oleaceae)** | Coumarins, secoiridoids, phenylethanoids, flavonoids | Kostova & Iossifova, 2007 | *Fraxinus excelsior* |
| ***Populus*** **(Salicaceae)** | Hydroxycinnamic acid derivatives, salicinoids, flavonoids, terpenoids | Guleria et al., 2021 | *Populus tremula* |
| ***Prunus*** **(Rosaceae)** | Terpenes, flavonoids, hydroxycinnamic and hydroxybenzoic acid derivatives, proanthocyanidins, cyanogenic glycosides | Telichowska et al., 2020 | *Prunus padus* |
| ***Quercus*** **(Fagaceae)** | Flavonoids, ellagitannins, proanthocyanidins, hydroxycinnamic and hydroxybenzoic acid derivatives | Burlacu et al., 2020; Şöhretoğlu & Renda, 2020 | *Quercus robur* |
| ***Salix*** **(Salicaceace)** | Flavonoids, hydroxycinnamic and hydroxybenzoic acid derivatives, salicinoids | Tawfeek et al., 2021 | *Salix phylicifolia* |
| ***Sorbus*** **(Rosaceae)** | Flavonoids, hydroxycinnamic and hydroxybenzoic acid derivatives, tetracyclic and pentacyclic triterpenoids | Sołtys et al., 2020 | *Sorbus aucuparia, Sorbus hybrida* |
| ***Syringa*** **(Oleaceae)** | Lignans, coumarins, hydroxycinnamic acid derivatives, iridoids, phenylethanols, flavonoids, pentacyclic triterpenoids | Zhu et al., 2021 | *Syringa vulgaris* |
| ***Tilia*** **(Malvaceae)** | Flavonoids, hydroxycinnamic acid derivatives | Kim et al., 2020; Ziaja et al., 2020 | *Tilia cordata, Tilia* × *europaea* |

Examples of compound groups with a wide distribution in the plant kingdom are flavonoids (e.g. kaempferol derivatives; **4**), phenolic acids such as hydroxycinnamic acid derivatives (**5**) and triterpenoids (**6**) (Table 1). Despite their wide distribution, flavonoids can also be useful chemotaxonomic markers due to chemical stability and the large structural diversity arising from the variety of substituents and the linkage of a carbohydrate moiety. For example, the leaves of *Betula pendula* and *Betula pubescens* contain a variety of flavonoid glycosides, but the leaf surfaces contain flavonoid aglycones such as acacetin (**7**) (Keinänen & Julkunen-Tiitto, 1998). The pattern of external flavonoids has been found to corroborate with their phylogenetic classification (Lahtinen et al., 2006; Valkama et al., 2003).

Besides mature leaves, the chemical composition of leaf buds can have interesting chemical patterns and structural diversity. Asakawa et al. (1977) found phenolic acid glycerols such as lasiocarpin C (**8**) as major components of bud exudates in *Populus lasiocarpa*. These compounds were also identified in *Populus tremula* by Bertrams et al. (2013). Their study showed that phenolic acid glycerols could act as markers for the plant source of propolis, a complex mixture of plant exudates produced by honeybees. Geoffroy et al. (2019) demonstrated the chemical diversity of the bud hot-water extracts of *Acer saccharum* by detecting and identifying 97 compounds. Flavonoids, benzoic acid derivatives and tannins were identified as the main phenolics in the buds.

The diversity of plant pigments in deciduous trees and shrubs is displayed during autumn as the red anthocyanins and yellow carotenoids become visible due to the decay of chlorophylls. Cyanidin-3-*O*-glucoside (**9**) is a widespread anthocyanin in autumn leaves (Ishikura, 1972). When comparing the pigment composition of autumn leaves to spring leaves, it has been shown that the anthocyanin content of leaves can be more diverse in the spring than in the autumn (Ishikura, 1972; Ji et al., 1992). Especially the diversity of aglycone moieties in *Acer* species was greater during spring, as delphinidin derivatives were significantly rarer in autumn leaves (Ji et al., 1992).

In addition, the carotenoid composition varies during the growing season. However, the change is the opposite compared to anthocyanins: the carotenoid composition was the most diverse in the yellow autumn leaves (Czeczuga, 1986). The carotenoids in the autumn were found to be epoxycarotenoids and apocarotenals, which are regarded as oxidation products of α- or β-carotene (**10**, **11**), and degredation of β-carotene or epoxycarotenoids, respectively (Czeczuga, 1986). During the growing season, the maximal concentrations and the timing of the maximum levels of carotenoids differ among different species (Sanger, 1971).

## 1.2 The chemical diversity of plants in chemistry education

The chemistry of plants can be included into chemistry education in multiple ways. There are numerous papers describing courses and laboratory activities with the topic of plant chemistry. For example, Séquin (2005) created a general education course for college non-science students, where general and organic chemistry were introduced through plant themes. Andreoli et al. (2002) built a training course for in-service teachers based on the chemistry of substances found in plants. Both courses connected chemistry to topics that are familiar from everyday life such as plant colours, odours and medicines. Thus, they highlighted the connection between chemistry and the everyday world.

Medicinal plants offer a popular topic for chemistry education. Busta and Russo (2020) created a laboratory module for the determination of natural products in medicinal plants by thin layer chromatography (TLC) and gas chromatography-mass spectrometry (GC-MS). Kirk et al. (2021) described a laboratory course aiming to create a secondary metabolite library of over 200 plant species with medicinal properties. Both courses offered students hands-on experience with analytical methods relevant for their future careers. Moreover, working with the interdisciplinary topic of medicinal plant chemistry improved logical thinking and analysis of text and data, systems thinking, and oral and written communication skills (Busta & Russo, 2020).

The chemical diversity of plants displayed through different courses and laboratory experiments in chemistry education varies. A common approach is to study the presence of a specific compound group in selected species (e.g. Du Toit et al., 2012; Garber et al., 2013; Lavoie et al., 2008). In contrast, Battle et al. (2012) demonstrated more extensively the diversity of plant compounds in the Botanic Garden of Cambridge University through a chemical trail. It introduced the principal components and their applications of 22 familiar plants from all major specialised metabolite classes of terpenes, alkaloids and phenolics. A broad range of compounds was intentionally chosen to avoid the tendency to emphasise the medicinal properties of plants.

Studies on students' conceptions about the chemistry of plants are scarce. The results by Séquin (2005) and Van Bramer and Goodrich (2015) imply that students' knowledge of the concepts and diversity of plant chemistry are insufficient. Séquin (2005) noticed that even though students recognise the importance of plants as sources for sugars, fats, vitamins, and medicines, they do not recognise that these are biosynthetic products of chemical reactions in plants. In the laboratory experiment by Van Bramer and Goodrich (2015), students analysed volatile compounds in plants using solid-phase microextraction and GC-MS. They reported that students were surprised to find same volatile compounds in different plant species. Thus, it seems

that students are not familiar with the biosynthesis or the biological functions of different compounds in plants.

Previous studies have shown that students have difficulties in the understanding of one of the most important concepts regarding plants and chemistry, namely photosynthesis. Stavy et al. (1987) studied the difficulties in understanding the chemical and ecological issues of photosynthesis of 13–15 year old students. They noticed that students had difficulties in describing biological phenomena in chemical and physical terms. Marmaroti and Galanopoulou (2006) showed that a great number of students at that age do not recognise photosynthesis as a chemical reaction. Furthermore, misconceptions about the differences between nutrient and nourishment, energy and matter, and autotrophic and heterotrophic occur still at university level (Södervik et al., 2015).

The studies discussed above demonstrate that plant chemistry themes can provide chemistry education a context which connects the abstract concepts with something that students are familiar with from their everyday life. Moreover, plant chemistry themes can be included into chemistry education in various ways. However, the chemical diversity of the plant kingdom is a topic that seems to be overlooked in education. A recent study indicated that the species recognition skills of Finnish students are poor (Kaasinen, 2019). The previous research implies that students' understanding of the chemistry of plants is poor as well, but more research about that is needed. Seeing the species diversity and the chemistry in plants could help students to understand how different ecosystems and nature work, and therefore help them to understand the consequences of environmental problems such as climate change and the loss of biodiversity.

## 1.3 Modern instrumentation in chemistry education

Modern instrumentation has a vital role, not only in natural compound chemistry, but also in chemistry research and quality control in general. To provide an authentic view of the field of chemistry, students of all educational levels need to be familiarised with the methods and instruments used in modern chemistry research (Nakhleh et al., 2002; Vesterinen et al., 2013). The incorporation of scientific instruments allows students not only to perform experiments that would not be otherwise possible to conduct, but also to learn about gathering, displaying and analysing data (Nakhleh et al., 2002).

The chemistry of plants has provided a context for a broad range of laboratory experiments designed to demonstrate the use of various analytical methods. Cannon et al. (2001) described a general protocol for the isolation, testing for bioactivity and characterisation by spectroscopic methods of specialised metabolites, covering thus multiple techniques relevant to the study of natural compounds. GC-MS has been

used to study volatile compounds such as monoterpenes from plants (Lavoie et al., 2008; Van Bramer & Goodrich, 2015). The basics of chromatography have been introduced through plant pigments in a number of articles (e.g. Curtright et al., 1999; Dias & Ferreira, 2015; Du Toit et al., 2012; McCullagh & Ramos, 2008). Similarly, spectrophotometric methods have been introduced through plant pigments (Edionwe et al., 2011; Galloway et al., 2015; Soares et al., 2002).

As the examples above demonstrate, there are numerous ways to incorporate different research instruments into chemistry education. However, their role in chemical research has not been explicitly discussed in chemistry teaching at upper secondary level (Vesterinen et al., 2013). The inadequate discussion of the relationship between science and technology in the classroom may result in a distorted and over-simplified conception of science (Tala, 2009). In a recent study, first-year university chemistry students were shown to overlook the role of instruments in the way science progresses (An & Holme, 2022). It was noted that a majority of the students viewed instruments as measurement tools, and provided common laboratory accessories such as thermometer, scale and pipet as examples of scientific instruments. Furthermore, the students showed limited understanding of the role of instruments outside of the laboratory. However, a small number of students were able to explicitly connect instruments as important tools for observing phenomena that cannot be detected with human senses.

It has been shown that students generally have positive attitudes toward using instrumentation in the laboratory (Miller et al., 2004). The incorporation of instruments in the laboratory has two benefits: it can help students to connect chemistry with the "real world", as students are aware that instruments are used by professional chemists, and the use of instruments permits students to gain useful skills for the future (An & Holme, 2022; Miller et al., 2004). However, students have exhibited negative feelings regarding learning about instruments and using them in the laboratory (An & Holme, 2022). The negative feelings such as being anxious or scared were derived from a variety of reasons such as a tight schedule or the fear of breaking the instrument.

With regards of the learning outcomes, studies have demonstrated that the level of hands-on exposure has a significant effect on students' knowledge and facility with instruments (Warner et al., 2016). The hands-on exposure leads especially into more technical knowledge, while it does not necessarily lead into better problem solving skills (Warner et al., 2016). Miller et al. (2004) suggested that laboratory experiments should highlight the capabilities of instruments instead of overwhelming students with complex or time-consuming repetitions. Yet, providing students chances to work with instruments in similar contexts may improve their problem solving skills (Warner et al., 2016).

## 1.4 Main aims of the thesis

The chemical diversity of plants has many different dimensions and it offers an alternative way of examining the plant kingdom and relationships between plant species. However, it seems that the topic has not been recognised in chemistry education, even though a multitude of ways for studying plant compounds have been presented. Previous studies have highlighted the benefit of plant chemistry in demonstrating the connection between the science and everyday life. Therefore, it may be able to increase people's interest in chemistry in general.

The incorporation of research instruments into chemistry education has many benefits and it is necessary for providing an authentic view of the whole discipline of chemistry. Despite the frequent use of different instruments in especially laboratory context, relatively little is known of students' interest in using the instruments. Moreover, the learning outcomes of working with the instruments have not been extensively studied.

This thesis had the following four main aims:

1. Create methods suitable for demonstrating the chemical diversity of plants (Articles I-III).
2. Study first-year university students' conceptions about plant chemistry (II).
3. Study how first-year chemistry students' knowledge of research instruments develops through a hands-on exposure (II).
4. Study how students' chemistry learning approach and perceived relevance of instruments affect students' learning and experiences in a hands-on laboratory work including research instruments and plant chemistry (IV).

The experimental methods were developed around two themes: the chemistry of autumn leaves and the chemistry of leaf buds. The methods included the extraction procedure for the plant samples, a simple colorimetric analysis with a spectrophotometer and a more sophisticated analysis by LC-MS. The methods were combined into laboratory exercises that were tested with a number of students from different educational levels. Students at especially lower educational levels have limited opportunities to use modern instrumentation, which is why it was important to provide the chance to use the state-of-the-art instrumentation. Moreover, liquid chromatography and mass spectrometry are commonly used in chemistry research and industry, and therefore relevant techniques for students to learn about.

To analyse university students' understanding of the chemistry of plants and their knowledge of research instruments used in the laboratory experiments, a mind map assignment and pre- and post-tests were developed. In addition, feedback and survey data about students' learning approaches and perceived relevance of research

instruments were collected to study how these background variables affect the cognitive and affective outcomes of the laboratory experiments. The data collection tools were based on the previous work of chemistry learning approaches by Lastusaari et al. (2016) and Lastusaari and Murtonen (2013), and the relevance model by Stuckey et al. (2013). Previous research on learning approaches and the relevance model are discussed in Article IV, and it was therefore omitted from the Introduction of this thesis.

# 2 Materials and Methods

## 2.1 Plant samples and extraction

Majority of the plant samples were collected in the Turku region, South-Western Finland. Collection time for the autumn leaves was September–October 2014 and October 2018. The majority of the samples were collected by a biologist who was also consulted for the species identification of the rest of the samples. After the collection, the autumn leaf samples were either frozen and lyophilised or dried with a household vegetable drier. Majority of the leaf bud samples were collected in April 2020, and the species identification was confirmed by a biologist. The leaf buds were frozen and lyophilised.

The shredded autumn leaves (200 mg) were extracted with acetone-water (80/20, *v/v*) according to the protocol described in Article I. After the evaporation of the extraction solvent, the crude extract was partitioned with liquid-liquid extraction into aqueous phase (5 ml of 4% aqueous formic acid) containing anthocyanins, and hexane phase (8 ml) containing carotenoids. The aqueous phase was filtered with a 0.2 μm polytetrafluoroethylene (PTFE) filter and diluted to one-third concentration prior the LC-MS analyses.

The leaf buds were extracted with ethanol-water (2 ml, 95/5, *v/v*) for either 30 s (Article II) or 10 min (Article III). The structure of the leaf buds was opened by pressing the leaf bud gently with a pipet tip. After that, the leaf bud was dropped into the extraction solvent. Immediately after the extraction, the extract was filtered with a 0.2 μm PTFE filter.

## 2.2 UHPLC-MS analyses

The UHPLC-MS analyses including selected reaction monitoring (SRM) and selected ion recording (SIR) measurements were done with an Acquity UPLC® system (Waters Corp., Milford, MA, USA) coupled with a Xevo TQ triple-quadrupole mass spectrometer (Waters Corp., Milford, MA, USA). The UHPLC system consisted of a sample manager, a binary solvent manager, a column (Acquity UPLC® BEH Phenyl 30 mm × 2.1 mm, 1.7 μm, Waters Corporation, Ireland), and a diode array detector. Acetonitrile (A) and 0.1% aqueous formic acid (B) were used

as solvents, and the elution profile was adjusted for different types of measurements. The high-resolution mass spectra were acquired with a hybrid quadrupole-Orbitrap mass spectrometer (QExactive, Thermo Fisher Scientific GmbH, Bremen, Germany) coupled with a similar Acquity UHPLC system including a similar column.

### 2.2.1 UHPLC-ESI-QqQ-MS analyses of anthocyanins

The following elution profile was used: 0−0.1 min 10% A in B (isocratic); 0.1−2.0 min 10−50% A in B (linear gradient); 2.0−2.1 min 50−90% A in B (linear gradient); 2.1−3.2 min column wash and stabilization. The flow rate was 0.65 ml/min, and the injection volume was 5 µl. Mass analyses were performed using an electrospray ionisation (ESI) source and positive ionisation. The ESI conditions were: capillary voltage, 3.4 kV; source temperature, 150 °C; desolvation temperature, 650 °C; desolvation and cone gas ($N_2$), 1000 and 100 l/h, respectively; and collision gas, argon. The SRM parameters are described in Table 1 in Article I. The data was processed with the TargetLynx software (V4.2 SCN982 © 2017 Waters Inc.)

### 2.2.2 UHPLC-HESI-Q-Orbitrap-MS analyses of leaf bud markers

The elution profile was as follows: 0−0.3 min, 10% A in B; 0.3−3.1 min, 10−75% A in B (linear gradient); 3.1−3.5 min, 75 % A in B; 3.5–3.6 min, 75–95% A in B; 3.6–5.0 min column wash and stabilization. The flow rate was 0.65 ml/min, and the injection volume was 5 µl. The full scan MS data (*m/z* 150–2250, resolution of 35 000, automatic gain control $3\times10^6$) and MS/MS data (dd-$MS^2$, TopN of 7, resolution of 17 500, automatic gain control of $1\times10^5$, stepped normalized collision energies (NCE) of 30, 50 and 80) were measured for negative ions. The parameters for the heated ESI source (H-ESI II, Thermo Fisher Scientific GmbH, Bremen, Germany) were set as follows: spray voltage, −3.0 kV; sheath gas ($N_2$) flow rate, 60 (arbitrary units); aux gas ($N_2$) flow rate, 20 (arbitrary units); sweep gas flow rate, 0 (arbitrary units); capillary temperature, +380 °C. In-source collision-induced dissociation energy was 30 eV. The data were processed with Thermo Xcalibur Qual Browser software (Version 4.1.31.9, Thermo Fisher Scientific Inc., Waltham, MA, USA). For further processing of the data, the raw MS files were converted to .mzXML format by the MS Convert Software, included in the ProteoWizard package (Chambers et al., 2012), and the mzXML files were processed with MZmine 2.53 (Pluskal et al., 2010).

### 2.2.3 UHPLC-ESI-QqQ-MS fingerprinting of leaf buds

In the analysis of birch samples, following elution profile was used: 0−0.3 min 40% A in B (isocratic); 0.3−1.2 min 40−75% A in B (linear gradient); 1.2−1.5 min 75% A in B (isocratic); 1.5–1.6 min 75–95 % A in B (linear gradient); 1.6−3.5 min column wash and stabilization. The flow rate was 0.65 ml/min, and the volume of partial loop injection was 1.5 µl. Mass analyses were performed using an ESI source and negative ionization. The ESI conditions were similar to the ones used in the anthocyanin analysis, except for the lower capillary voltage of 1.8 kV. The SIR measurement was performed for the ions at *m/z* 365.0 and 439.6.

In the fingerprinting analysis of other species, the gradient was similar to the one used in the Orbitrap analysis. The ESI conditions were similar to the ones used in the birch analyses. The SIR parameters are presented in Article III.

## 2.3 Colorimetric analyses

### 2.3.1 Total anthocyanin and carotenoid content of autumn leaves

The quantitative measurements of anthocyanins and carotenoids were done with a portable spectrophotometer (DR1900, Hach, Loveland, CO, USA) using micro cuvettes from Hellma Analytics (volume, 700 µL; path length, 10 mm; optical glass, black walls). The aqueous phase was analysed at 520 nm for presence of anthocyanins, and the hexane phase was analysed at 450 nm for carotenoids. Anthocyanin and carotenoid concentrations were calculated using predefined calibration curves of cyanidin (5, 10, 20, and 40 µg/ml) and β-carotene (0.25, 0.5, 1.0, and 2.0 mg/ml).

### 2.3.2 Total phenolic content of leaf bud extracts

The total phenolic content of leaf bud extracts was estimated with a modified Folin−Ciocalteu assay (Salminen & Karonen, 2011). First, 150 µl of the plant extract was mixed with 1 ml of the Folin−Ciocalteu reagent. Then, 2 ml of 20% $Na_2CO_3$ (*m/v*) solution was added to the tube, and the colour of the solution was recorded after 20 min by a spectrophotometer at 730 nm and/or comparing the colour to a colour chart. The TPC was calculated using a calibration curve of gallic acid (25, 50, 100 and 300 µg/ml).

### 2.3.3 Antioxidant activity of leaf bud extracts

A modified 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Tuominen, 2013) was used to determine the antioxidant activity of the leaf bud extracts. A volume of 450 µl of the bud extract was mixed with 3 ml of 0.25 mM DPPH solution, and the colour was recorded by a spectrophotometer at 517 nm and/or compared to a colour chart after 15 min. The AOA was calculated using gallic acid (2 mg/ml) as a positive control, and ethanol-water (95/5, *v/v*) as a negative control.

## 2.4 Questionnaires and tests

### 2.4.1 Relevance of Research Instruments (RoRI)

The RoRI questionnaire was developed to evaluate university students' views of research instruments in chemistry. The questionnaire included statements about the relevance of research instruments for chemistry studies, research and employment. The items were designed based on the relevance model by Stuckey et al. (2013). The answers in the 25 statements were collected on a five-point Likert scale. Mean variables were formed according to the statistical analyses and used in the correlational analyses.

### 2.4.2 ChemApproach

The ChemApproach questionnaire by Lastusaari et al. (2016) was used to analyse university students' chemistry learning approaches. The questionnaire is designed to analyse specifically university chemistry students' learning approaches, and for that reason, it was used in this thesis work. The statements of the questionnaire are related to four learning approach categories: submissive surface (SubSurf), technical surface (TechSurf), active deep (ActDeep) and practical deep (PraDeep). Mean values of each category were calculated for all students, and these mean variables were used in the statistical analyses.

### 2.4.3 Pre- and post-tests of students' knowledge of research instruments

The pre- and post-tests measured university students' knowledge of spectrophotometry, liquid chromatography and mass spectrometry. The pre-test was taken one or two weeks before the laboratory experiment, and the post-test was done one week after the laboratory experiment. In both cases, students were given 15 min time to answer the following questions:

- Describe how a spectrophotometer works.
- Describe how a liquid chromatograph works.
- Explain what an ion source is.
- Describe how a mass spectrometer works.

Different concepts regarding the instruments were classified and their frequencies were counted in the students' answers. The answers were also scored according to the correct concepts, and total scores were counted from the pre- and post-tests for the statistical analyses.

### 2.4.4 Mind maps of the chemistry of plants

The mind map task was used to measure university students' knowledge of plant chemistry during the laboratory work. Mind maps have been demonstrated to offer an informative assessment and research tool for science education, and they can give insight into students understanding of scientific phenomena (Burrows & Mooring, 2015; Van Zele et al., 2004). At the beginning of the laboratory work, students were given 15 min to create a mind map with the title "Compounds in plants and their functions". At the end of the laboratory work, students could supplement their mind maps with a different coloured pencil. The occurrence of different compound groups and biological functions connected to them were analysed from the mind maps, as well as the total numbers of compounds and functions.

### 2.4.5 Feedback

Feedback was collected from all student groups. In the feedback collected from the secondary school students, students were asked to choose the most and least interesting part of the experiment. The feedback from the university students from the leaf bud experiment was collected as a survey, which included statements about students' interest in the topic, instruments and scientific aspects of the experiment as well as understanding of the different methods. The answers were collected on a five-point Likert scale.

## 2.5 Participants

Summary of the students who participated in this thesis and the survey data collected from them is presented in Table 2. The university students doing the laboratory work about the chemistry of autumn leaves were the participants in a chemistry education course for BSc students in University of Turku. Whereas, the university students doing the laboratory work about the chemistry of leaf buds were the students of a first-year laboratory course on experiments in general chemistry in University of

Turku. The participation of the secondary school students was arranged as a visit to the Department of Chemistry at the University of Turku.

**Table 2.** Summary of the participants' educational level, laboratory work, and questionnaires and tests done by the participants.

| Educational level | Number of students | Laboratory work | ChemApproach | RoRI | Pre-test about instrumentation | Post-test about instrumentation | Mind maps | Feedback |
|---|---|---|---|---|---|---|---|---|
| **Upper secondary** | 18 | Chemistry of autumn leaves | no | no | no | limited | no | yes |
| **University** | 16 | Chemistry of autumn leaves | no | no | no | limited | no | yes |
| **Lower secondary** | 13 | Chemistry of leaf buds birches | no | no | no | no | no | yes |
| **Upper secondary** | 59 | Chemistry of leaf buds of birches | no | no | no | no | no | yes |
| **University** | 53 | Chemistry of leaf buds of birches | yes | no | yes | yes | yes | yes |
| **University** | 103 | Chemistry of leaf buds | yes | yes | yes | yes | yes | yes |

## 2.6 Statistical analyses

Statistical analyses were performed using IBM SPSS Statistics 27.0. Reliability estimates for internal consistency (Cronbach's α) were calculated for each mean variable formed from RoRI and feedback items. Correlations between ChemApproach, RoRI and feedback mean variables were computed to study interactions between them. Statistical comparisons between pre- and post-test means were done using a *t*-test.

# 3 Results and Discussion

## 3.1 Tools for studying the chemistry of leaves and buds

The first tool was the laboratory experiment designed to demonstrate the differences in the anthocyanin and carotenoid compositions in autumn leaves. The complete description of the protocol is described in Article I. In short, the experiment consisted of the extraction of the pigments from the leaves and separation of anthocyanins and carotenoids by liquid-liquid extraction. The anthocyanins and carotenoids were analysed quantitatively by a spectrophotometer, and the anthocyanin composition was studied qualitatively with UHPLC-ESI-QqQ. The extraction and separation procedure was optimised for the facilities available in a regular school laboratory. The analysis of anthocyanins was performed by group-specific SRM methods for the six most common anthocyanidins and their derivatives. Therefore, the presence of different anthocyanidin groups could be concluded from the SRM chromatograms.

The second tool described in Article II highlighted the differences in the chemistry of leaf buds of white birch (*Betula pubescens*) and silver birch (*Betula pendula*). It included the rapid extraction of the compounds from the surface of a single leaf bud, analysis of the total phenolic content (TPC) and antioxidant activity (AOA) of the leaf bud extract, and the identification of the birch species from the UHPLC-MS fingerprint. The TPC and AOA could be estimated visually based on a colour scheme, or measured spectrophotometrically. The UHPLC-MS fingerprint was obtained based on a SIR method measuring two markers: one specific to white birch, the other specific to silver birch. This approach resulted in species-specific LC-MS profiles upon which the identification was possible.

The third tool was a result of the method development process described in Article III. It supplemented the birch identification tool by enabling the identification of 13 additional common Finnish deciduous trees and shrubs from the leaf bud extracts. Compared to the method designed for the birch species, the third method had two modifications in addition to the SIR methods of the species-specific markers of the additional species: the extraction time of the leaf buds was prolonged into 10 min from 30 s, and the chromatographic gradient profile was different. The modifications allowed the detection of a wider range of compounds.

### 3.1.1 The pigment diversity of autumn leaves

The pigments of autumn leaves of altogether nine species in several replicates were analysed by upper secondary school and university students (Article I). The spectrophotometric results showed substantial differences in the anthocyanin and carotenoid concentrations between samples (Figure 2). The leaf colour was in agreement with the ratio of anthocyanins to carotenoids, i.e. yellow leaves (Figure 2A) contained higher amounts of carotenoids and lower amount of anthocyanins than red leaves (Figure 2B). Significant differences between species could not be concluded nor would it be reasonable due to the concentration changes during leaf senescence (Lee et al., 2003; Sanger, 1971). Instead, studying the development of anthocyanin and carotenoid concentrations during the growing season until the leaf senescence could be an interesting project for a science course.

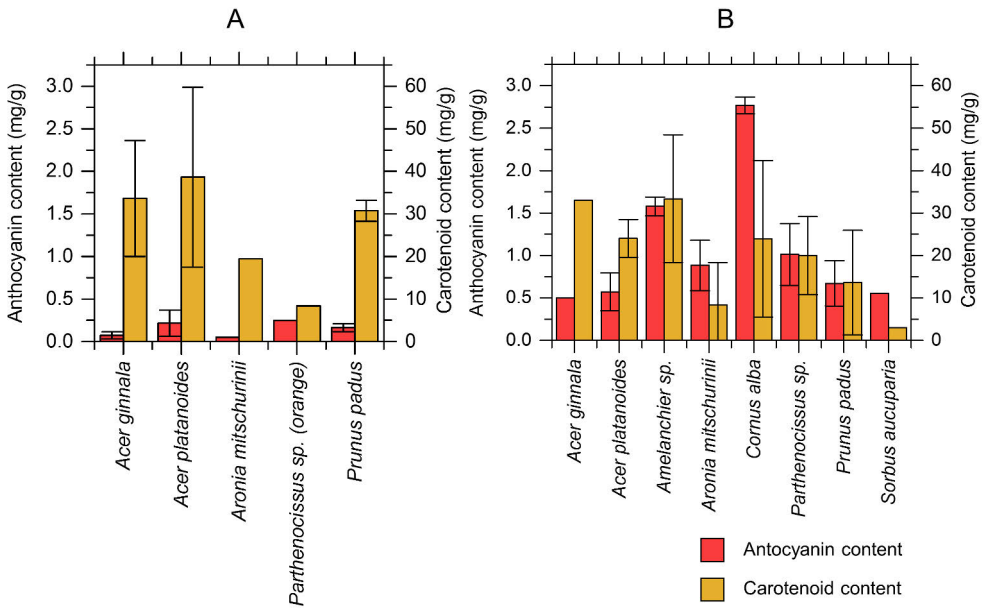


**Figure 2.** Anthocyanin and carotenoid concentrations of yellow (A) and red (B) leaves measured spectrophotometrically by the students.

The UHPLC-QqQ-MS analysis revealed both differences and similarities in the anthocyanin compositions between different species. All species contained anthocyanins of at least three different anthocyanidin groups (Table 3). Cyanidin and its derivatives were detected in all of the species that contained anthocyanins. The ubiquity of the anthocyanidin cyanidin was not surprising based on previous studies (Ishikura, 1972; Ji et al., 1992). On the other hand, malvidin derivatives were not

detected in any of the species. *Prunus padus* was the most diverse species with regards of anthocyanin content, as the samples contained derivatives of all groups of anthocyanidins except for malvidin derivatives.

**Table 3.** The distribution of anthocyanidin derivatives among the red leaves of the studied species studied by LC-MS.

| Anthocyanidin group | *Acer ginnala* | *Acer platanoides* | *Amelanchier* sp. | *Aronia mitschurinii* | *Cornus alba* | *Parthenocissus* sp. | *Prunus padus* |
|---|---|---|---|---|---|---|---|
| **Delphinidin** | + | +/++ | - | + | - | - | +/++ |
| **Cyanidin** | ++ | ++ | ++ | ++ | ++ | ++ | ++ |
| **Petunidin** | - | + | - | tr/- | - | + | + |
| **Pelargonidin** | - | - | + | + | + | + | + |
| **Peonidin** | + | + | + | - | ++ | + | tr/++ |
| **Malvidin** | - | - | - | - | - | - | - |

++, high intensity of the signal ($10^5$ or $10^6$); +, low intensity of the signal ($10^4$); tr, traces; -, not detected

### 3.1.2 Defence compounds in leaf buds

The experimental results obtained by the university students in Article II showed that the levels of TPC and AOA per leaf bud can vary significantly, and distinguishing between *B. pubescens* and *B. pendula* was not possible based on the TPC and AOA values alone. However, the university students' results of a wider selection of species showed that significant differences in the TPC and AOA between different species could be observed (Figure 3). The species richest in phenolic compounds were *P. tremula* and the *Betula* species. The AOA of these species was also at a high level. Interestingly, the AOA of all *Alnus incana* samples was at a high level, even though the TPC values were not that high compared to other species. The species with lowest content of phenolic compounds were the *Tilia* species, *Quercus robur* and *Syringa vulgaris*. In most cases, the low TPC reflected also in the AOA values.

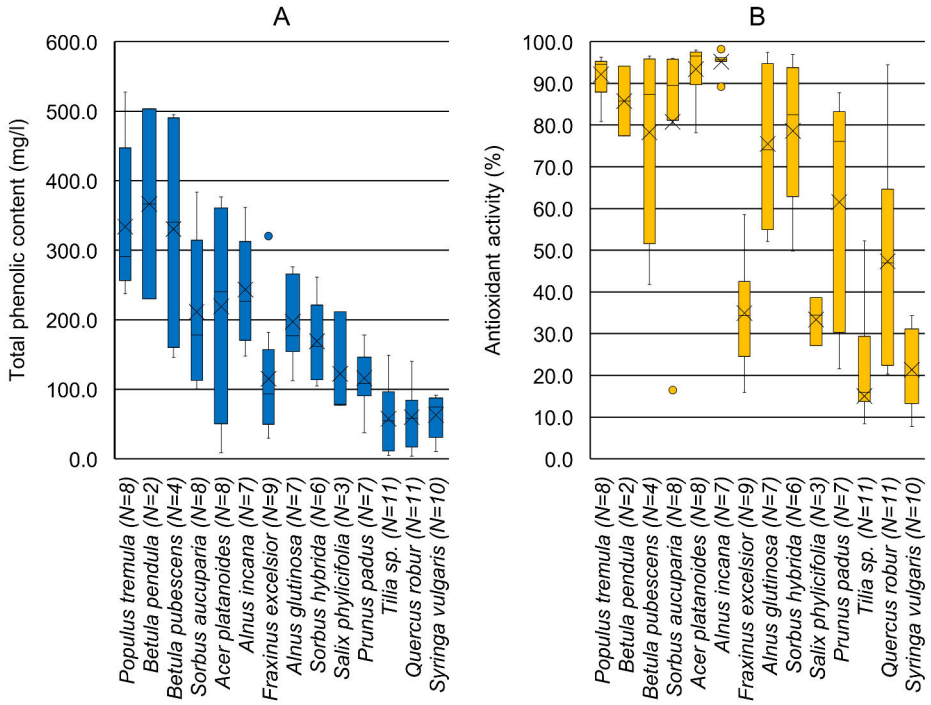


**Figure 3.** University students' results of the total phenolic contents (A) and antioxidant activities (B) of the studied species.

### 3.1.3 The species-specific markers and LC-MS fingerprints

In search of species-specific markers for the leaf buds of 13 common Finnish trees and shrubs, MZmine 2, a software for mass spectrometric data processing, was demonstrated as a useful tool for discovering potential marker candidates. One replicate per species was analysed with UHPLC-ESI-Q-Orbitrap MS, and the data was transferred to MZmine 2 in order to compare the presence of detected ions in different leaf bud extracts. More specifically, extracted ion chromatograms (EICs) were created for all ions with an intensity above $1\times10^5$, and all such features, i.e. detected variables with a retention time and an *m/z* ratio, were aligned into a combined list (i.e. feature list).

From the feature list, 3–7 marker candidates with the highest peak area were chosen for further testing. The species-specific SIR methods of these markers provided good specificity with regards of intensity (Figure 4A) and profile (Figure 4B). Furthermore, the MZmine 2 data revealed differences in the chemical diversity of the leaf bud extracts. *A. glutinosa* and *A. incana* were the most chemically diverse species, while *T. x europaea* and *T. cordata* were the least chemically diverse species.

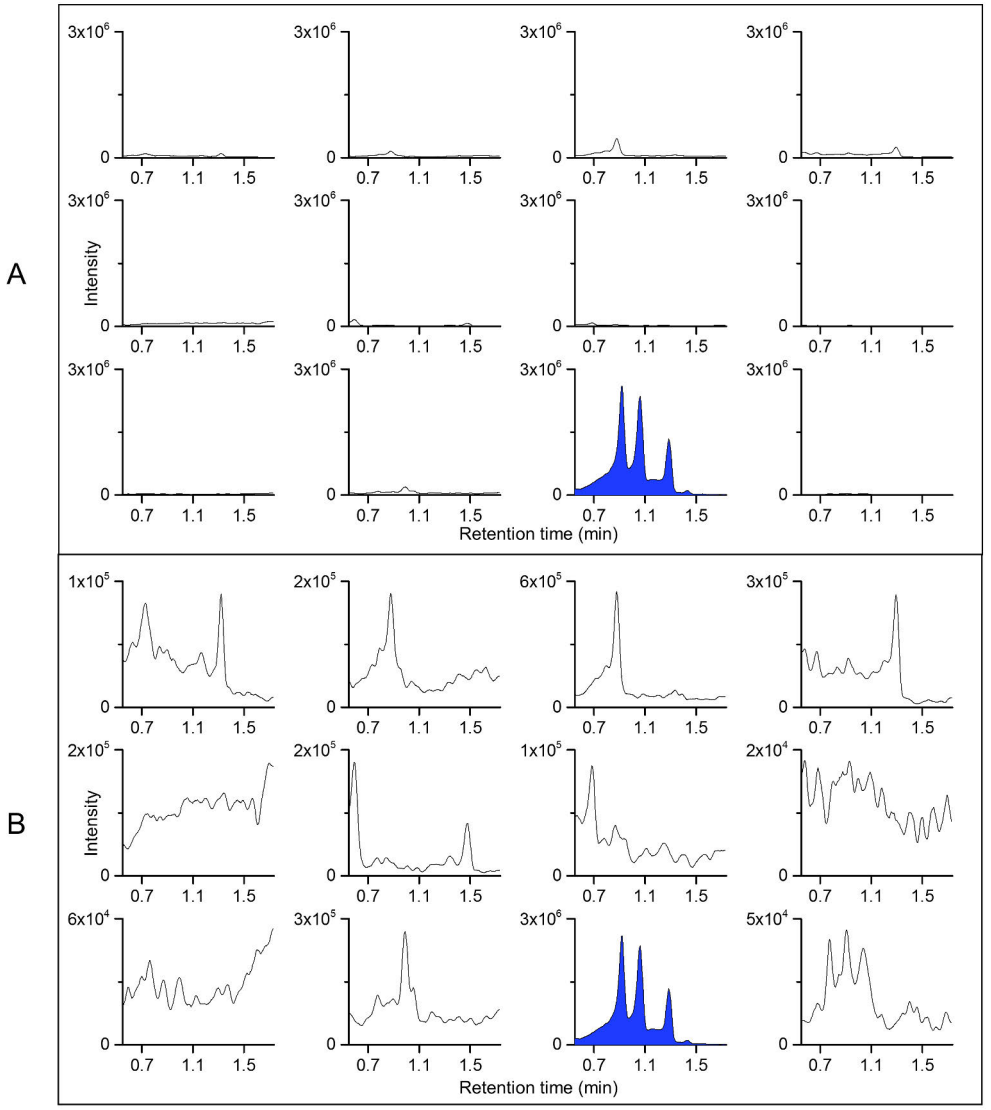


**Figure 4**. The LC-MS fingerprints of 12 species with the species-specific method for *Syringa vulgaris* (highlighted with blue colour). (A) The y-axes were scaled according to the most intensive peak of all samples, which is was produced by *S.vulgaris*. (B) The y-axes were scaled to the most intensive peak of each sample, which demonstrates the significant difference of the chromatographic profile of *S. vulgaris* compared to the others. Figure modified from Article III.

Alternative SIR methods were created using the main ions from the QqQ full scan spectra as markers. All species were screened in 4–10 replicates with UHPLC-ESI-QqQ MS, and 3–6 main ions from the full scan spectra were chosen to be used in the SIR methods. This approach produced repeatable fingerprints for all species.

Figure 5 shows an example of three species: *Sorbus hybrida* (A), *Alnus glutinosa* (B) and *Salix phylicifolia* (C). The repeatability was evaluated qualitatively based on the fingerprint profile: all replicates exhibited the same main peaks and no additional peaks were detected.

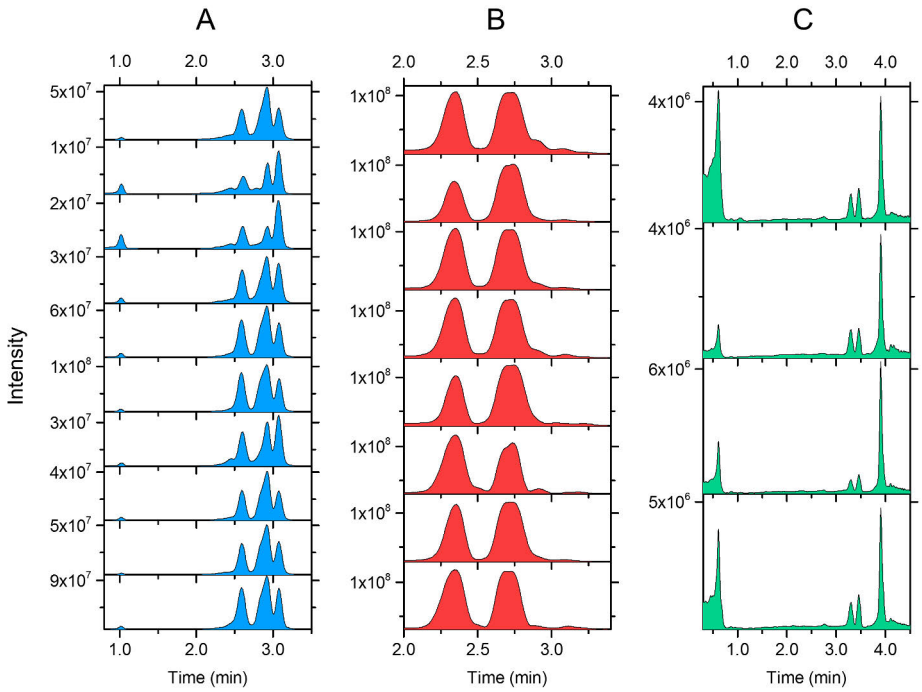


**Figure 5**. Within-species repeatability of the LC-MS fingerprints of (A) *Sorbus hybrida*, (B) *Alnus glutinosa* and (C) *Salix phylicifolia* using the main ions as markers. Figure from Article III.

The final LC-MS fingerprinting method consisted of markers mainly obtained with MZmine, but for *A. glutinosa*, *S. aucuparia* and *S. hybrida*, the best repeatability and specificity were obtained with the SIR method using the main ions as markers. In combination with the markers for birches, a fingerprinting method was developed, which could be used for the identification of 15 species from a single leaf bud in less than 6 min analysis time. Figure 6 demonstrates the reference fingerprints of each species.

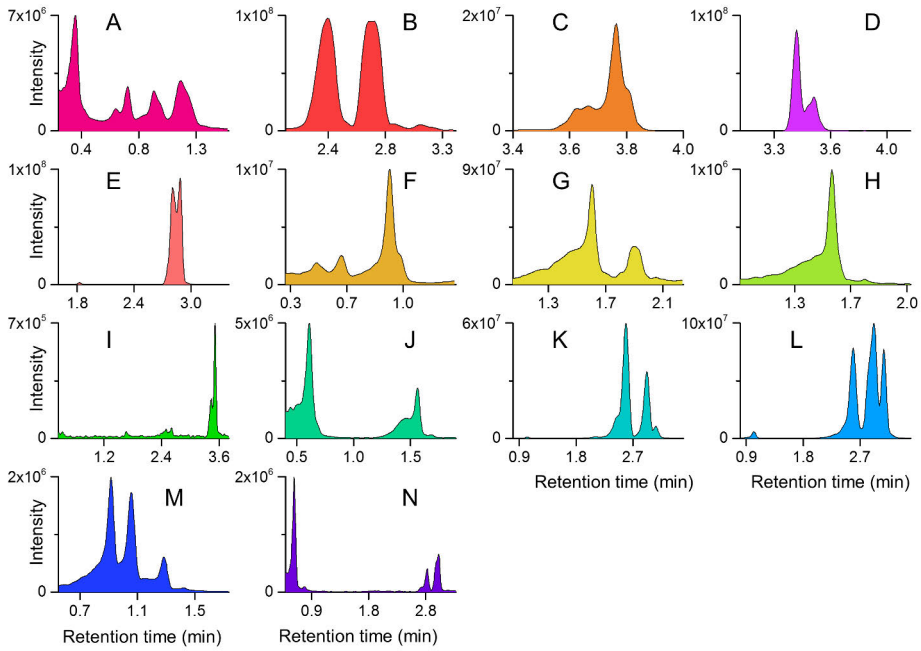


**Figure 6**. The LC-MS fingerprints of the studied species with the species-specific methods. (A) *Acer platanoides*, (B) *Alnus glutinosa*, (C) *Alnus incana*, (D) *Betula pendula*, (E) *Betula pubescens*, (F) *Fraxinus excelsior*, (G) *Populus tremula*, (H) *Prunus padus*, (I) *Quercus robur*, (J) *Salix phylicifolia*, (K) *Sorbus aucuparia*, (L) *Sorbus hybrida*, (M) *Syringa vulgaris*, and (N) *Tilia cordata*. *Tilia* × *europaea* produced a similar fingerprint with *T. cordata*.

The UHPLC-DAD-MS/MS QOrbitrap data was used for the identification of the markers used in the final method. The characterisation was made based on the comparison of UV spectra, exact masses and main fragment ions of the markers with published data. The markers were structurally diverse and belonged into many different subclasses of phenolics and terpenoids (Table 4). Many of the identified compounds were in accordance with previous literature concerning the species in question, meaning that the compound had been previously identified in the species and had been proposed to have chemotaxonomic relevance. For example, all markers of *S. vulgaris* were secoiridoids, which are members of the terpenoid family. These compounds have been reported in different parts of *S. vulgaris* (Tóth et al., 2016; Wozniak et al., 2018).

**Table 4.** The number of individual compounds used as chemotaxonomic markers in the LC-MS fingerprinting methods for the studied species and the compound classes of the markers.

| Species | Number of compounds | Compound classes of the markers |
|---|---|---|
| ***Acer platanoides*** | 6 | Benzoic acid glycosides |
| ***Alnus glutinosa*** | 6 | Triterpenoid saponins |
| ***Alnus incana*** | 3 | Unknown |
| ***Betula pendula*** | 1 | Triterpenoid |
| ***Betula pubescens*** | 1 | Coumarin |
| ***Fraxinus excelsior*** | 4 | Phenylethanoids, coumarin, unknown |
| ***Populus tremula*** | 4 | Phenolic acid glycerols |
| ***Prunus padus*** | 2 | Flavanone, hydroxycinnamic acid derivative |
| ***Quercus robur*** | 1 | Unknown |
| ***Salix phylicifolia*** | 2 | Flavanol, salicinoid |
| ***Sorbus aucuparia & Sorbus hybrida*** | 7 | Triterpenoids |
| ***Syringa vulgaris*** | 4 | Secoiridoids |
| ***Tilia cordata & Tilia × europaea*** | 3 | Flavanol, unknown |

Some of the markers used in the fingerprinting method contribute to the TPC and AOA results obtained for the leaf bud extracts. In general, it can be assumed that a high TPC causes a high AOA, as phenolic compounds can act as antioxidants (Leopoldini et al., 2011). For example, all markers to *P. tremula* were phenolic acid glycerols, which have been reported as main compounds in *P. tremula* leaf bud extracts in previous studies (Bertrams et al., 2013; Okińczyc et al., 2018, 2021). The phenolic acid glycerols may therefore explain the high TPC and therefore the AOA of *P. tremula* leaf buds (Figure 3). The leaves of *Acer platanoides* and the leaf buds of *Acer saccharum* are rich with benzoic acid glycosides, indicating that benzoic acid glycosides are quantitatively significant in the leaf buds of *A. platanoides* as well (Geoffroy et al., 2019; Kim et al., 2020). Therefore, the benzoic acid glycosides could be responsible for the high TPC and AOA observed in the students' leaf bud samples. In contrast, the high TPC and AOA results of both *Betula* species are more likely caused by the epicuticular flavonoids that are present in high quantities in young leaves (Valkama et al., 2003).

The markers demonstrate the chemical diversity of the studied species in multiple different ways. However, the markers to differentiate *T. cordata* and *T.* × *europaea* could not be discovered, which is an indication of the chemical similarity

of the species. On the other hand, *S. aucupari*a and *S. hybrida* had joint markers, but the fingerprints were significantly different. Thus, they were qualitatively similar, but quantitatively differences were found in the main compounds. Qualitatively the chemical diversity of the studied species can be seen through the structural diversity of the markers. The markers represent different branches of the biosynthetic pathways, and corroborate well with previous literature of the special characteristics of different plant genera.

## 3.2 University students' conceptions about plant chemistry

One of the main goals for the laboratory experiments developed in this thesis was to demonstrate the chemical differences between plants, and to encourage students to combine chemical and biological knowledge. A mind map task was used to evaluate students' overall knowledge of the chemistry of plants and the learning outcomes of the laboratory experiment. By requesting students to use a different coloured pencil when making modifications after the laboratory experiment, changes and new concepts could be observed in the data analysis.

University students' mind maps of the compounds in plants and their functions varied notably in terms of quality and quantity. The minimum number of compounds was one, the maximum was 24 (mean 7.2). Compounds related to photosynthesis were common (on average 2.5 per mind map), whereas other primary metabolites were rarer (1.3 per mind map). Commonly mentioned compounds were sugar, water, cellulose and chlorophyll (Figure 7).


**Figure 7**. Different compound groups present in the university students' ($N = 57$) initial mind maps. The size of the slice is proportional to the frequency of the compound in the mind maps. Figure from Article II.

Common compounds from the category of specialised metabolites were phenolic compounds, pigments and toxins. On average, students' mind maps contained 2.3 specialised metabolites. Students were encouraged to make additions to their mind maps after the laboratory work with the leaf buds. Majority of the additions were linked to phenolic compounds and antioxidants. With the help of the laboratory work, students were able to describe more accurately that phenolic compounds protect plants from herbivores and UV radiation. Antioxidants, on the other hand, were related to protection from oxidation reactions before and after the laboratory experiment.

Besides studying what students know about plant chemistry, one of the aims of the thesis was to discover how students' chemistry learning approach affects their understanding of the topic. Learning approaches can be divided into surface and deep approaches, and the division derives from the work by Marton and Säljö (1976). In general, a person using a surface learning approach tries to memorise the topic

without understanding of the bigger picture, which may hinder learning. On the other hand, the use of a deep approach is more likely to result in better academic achievement (e.g. Cano, 2005; Case & Gunstone, 2003; Sæle et al., 2017). The students' learning approaches were studied using the ChemApproach survey by Lastusaari & Murtonen (2016), which divides surface learning approaches into submissive surface (SubSurf) and technical surface (TechSurf) approaches. A student using the TechSurf approach uses more active techniques than pure memorisation for surface learning.

The statistical analyses in Article IV suggested that the TechSurf learning approach might have a negative effect on the understanding of plant chemistry. A small, but statistically significant negative correlation was observed between the TechSurf score and the total number of compounds in mind maps (-0.188, $p = 0.018$). The TechSurf approach also correlated negatively with the number of compounds related to photosynthesis (-0.185, $p = 0.021$) and the number of compounds added at the end of the laboratory work (-0.203, $p = 0.011$). Interestingly, the SubSurf learning approach did not affect the quality of the mind maps. The SubSurf approach differs from the TechSurf approach in that student has very little interest in deep learning of chemistry and is not keen to make effort in enhancing the learning (Lastusaari & Murtonen, 2013).

In accordance with the present results, previous studies on first-year students' understanding of photosynthesis have also demonstrated the negative correlation between surface learning approach and the quality of students' mind maps (Hazel & Prosser, 1994). However, the results in Article IV suggested that a student using specifically the TechSurf approach has difficulties in combining knowledge over discipline boundaries, which is essential for understanding the chemistry of plants or other complex multidisciplinary concepts.

## 3.3 University students' knowledge and perceived relevance of research instruments

Pre- and post-tests were used to analyse students' knowledge of spectrophotometry, liquid chromatography and mass spectrometry qualitatively and quantitatively (Article II). Different concepts in the answers regarding the instruments were classified and quantified, and the answers were scored based on the number of correct concepts included in the answers. To support students' learning about the research instruments, they were provided with a short explanation of the basic functioning of liquid chromatography, a triple quadrupole mass spectrometer and the fingerprinting method in the instructions for the laboratory experiment (Supporting Information of Article II). These topics were also discussed during the laboratory experiment.

The pre-test showed that students had very little prior knowledge of the instruments and their functioning. The qualitative analysis revealed several misconceptions regarding spectrophotometry and liquid chromatography. A common misconception about spectrophotometry was to connect it with the refraction of light and the refraction index of a substance. Liquid chromatography was often confused with TLC. The question about mass spectrometry showed that some students were capable of describing the functioning of a sector instrument, but it may hinder students' learning of other types of mass analysers as the functioning of other mass analysers is based on very different physical phenomena.

The hands-on exposure improved students' answers about the instruments in terms of what the instrument is used for, and how the measurement is performed. In other words, the practical experience was reflected into technical knowledge, as observed earlier by Warner et al. (2016). In addition, the number of most obvious misconceptions decreased and correct concepts were connected to the instruments more often in the post-test (see Tables 1 and 2 in Article II). However, many students still confused spectrophotometer with refractometer and thin layer chromatography with liquid chromatography. These misconceptions were not discussed during the laboratory work, which could have been beneficial for students' learning outcomes. It has also been shown that the level of exposure to instrumentation affects students' knowledge and facility with instrumentation (Warner et al., 2016). Therefore, to develop a deeper understanding of the basic principles regarding the functioning of the instruments, longer exposure could have been required.

The quantitative results of the pre- and post-tests were also compared with background variables such as chemistry learning approach (Article IV). As expected, the SubSurf score correlated negatively with the total score both in the pre- and post-test about the instruments, meaning that students using the SubSurf approach scored lower in the pre- and post-tests. If a student uses pure memorisation without deeper understanding, it may be difficult to understand the functioning of the instruments, which requires understanding of multiple concepts relating to the physical and chemical properties of compounds (Karonen et al., 2021). Interestingly, the use of the deep learning approach did not affect the learning, which could have been expected based on previous research (e.g. Cano, 2005; Case & Gunstone, 2003; Sæle et al., 2017). Other studies have shown that the deep learning approach is related to the quality of learning, but not to the quantitative results such as course grades (Minbashian et al., 2004; Trigwell & Prosser, 1991; Trigwell et al., 2012). According to Minbashian et al. (2004), one explanation could be that students who apply deep approach opt for depth rather than the number of factual details in their answers.

The knowledge of research instruments did not correlate with students' perceived relevance of research instruments either in the pre-test or in the post-test. On average, the perceived relevance of research instruments to chemistry studies,

industry and research was at a high level (Figure 8). Previous studies have not either been able to enhance students' performance in individual tasks through a relevance intervention (Hulleman et al., 2010), but instead, the performance over a longer period of time in terms of final course grades has been improved (Hulleman & Harackiewicz, 2009; Hulleman et al., 2010). Furthermore, academic performance is a sum of different factors, but perceived utility of a course can ultimately lead into enhanced performance (Simons et al., 2004). Therefore, it is possible that the perceived relevance and even the learning approach could require more time to impact the learning outcomes of research instruments, and a longitudinal experiment could reveal effects on the performance.

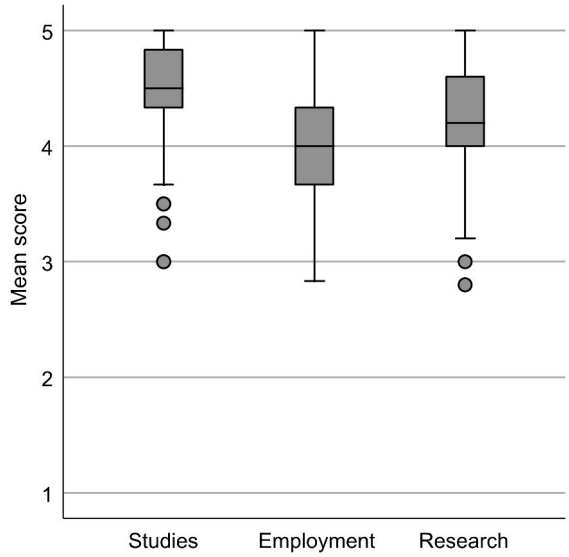


**Figure 8.** University students' mean scores (*N* = 95) in the RoRI survey for the relevance of research instruments in terms of studies, employment and research.

## 3.4 University students' experiences

Generally, both laboratory experiments, i.e. the chemistry of autumn leaves and the chemistry of leaf buds, received positive feedback from all students. Especially the younger students enjoyed the colourful parts of the experiments, liquid-liquid extraction in the case of autumn leaves, and DPPH and Folin–Ciocalteu assays in the case of leaf buds. The feedback survey designed for the university students was dedicated on measuring students' interest in the different aspects of the leaf bud experiment and how well they understood the protocols. The university students found the nature-related topic, instruments and chemistry research interesting. The

students also felt they understood well what they were doing in the experiment and why. Not surprisingly, students found it the most difficult to understand how the fingerprinting method works.

Both the learning approach and the perceived relevance of research instruments were connected with students' experiences about the laboratory experiment. Three variables from the feedback survey were studied, namely interest for the topic, interest in the instruments and understanding of the methodology. The ActDeep learning approach correlated positively with all three variables, whereas the SubSurf approach correlated negatively with understanding. In other words, students using the ActDeep approach expressed interest for the topic of the experiment, were interested in the instruments, and understood the methodology. Students using the SubSurf approach felt they did not understand the methodology of the experiment. Previous research has also found a connection between deep approach and positive emotions, while lower positive emotions and the use of surface approaches were connected (Trigwell et al., 2012). The negative correlation between understanding and the SubSurf approach was an expected result as the SubSurf score correlated negatively with the learning outcomes as well. However, the TechSurf score correlated positively with interest for the topic, despite the poorer performance in the task about plant chemistry.

The relevance of research instruments for studies correlated positively with all three variables of the feedback survey. Thus, students who held instruments relevant for their studies, were interested in the topic and using the research instruments in practise, and they understood the methods used. In addition, the perceived relevance of instruments to chemistry research was connected to understanding and interest in using the instruments in the experiment. These results reflect those of Hulleman and Harackiewicz (2009) and Hulleman et al. (2010), who also found that their relevance intervention was able to promote students' interest.

Figure 9 summarises the effects of learning approach and perceived relevance of instruments in the outcomes of the laboratory experiment of leaf buds. The cognitive outcomes were only affected by the learning approach, whereas both, the learning approach and instrument relevance had an effect on the affective outcomes. Generally, the use of a deep approach and finding research instruments relevant were positively connected with the outcomes, whereas a surface approach had a negative effect.

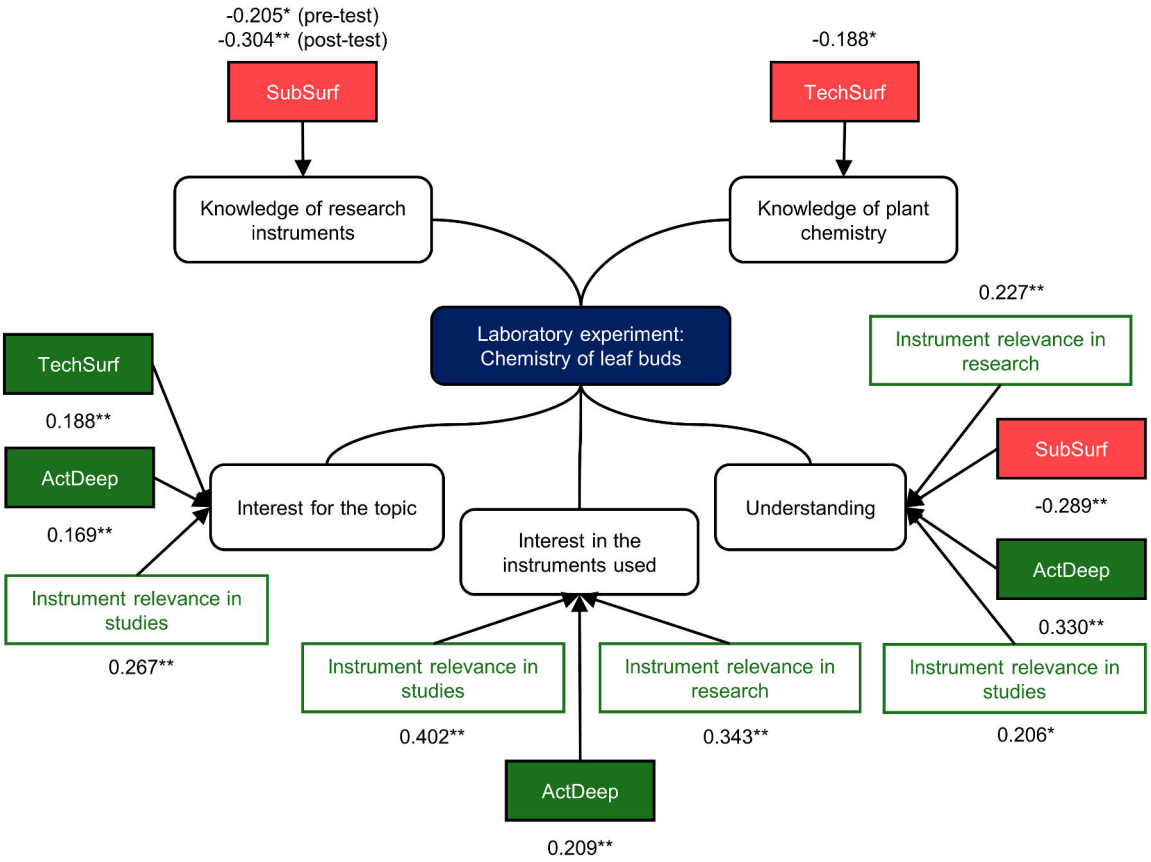


**Figure 9.** The correlations between learning approach or instrument relevance and the cognitive and affective outcomes of the laboratory experiment of leaf buds. *, correlation is significant at the 0.05 level (2-tailed) **, correlation is significant at the 0.01 level (2-tailed). Figure from Article IV.

# 4 Conclusions

The chemical tools developed in this thesis were used to study the chemical diversity of leaves and leaf buds. The analytical methods included simple colorimetric tests providing quantitative information of the samples, and more sophisticated LC-MS based analyses providing more detailed information of the samples on a molecular level. The tools were successfully tested with students from different educational levels. The students' experimental results demonstrated the variation in anthocyanin and carotenoid concentrations in autumn leaves, and differences in the anthocyanin compositions. The defence compounds of leaf buds were analysed through the measurement of total phenolic content and antioxidant activity of the leaf bud extracts. The results revealed differences between species, but also significant variation between plant individuals, which is expected due to biological and biochemical reasons. The qualitative differences in the chemistry of different species were utilised in an LC-MS fingerprinting method, which enabled the identification of 15 Finnish trees and shrubs in less than 6 min from a single leaf bud.

The mind maps of the university students showed that, on average, students' prior knowledge of the chemistry of plants was rather limited and most frequently associated with photosynthesis. The mind maps did not reflect the chemical diversity of the plant kingdom. Thus, ways of incorporating plant chemistry into education are needed. The results indicate that the tools developed in this study were able to widen students' understanding of the compounds that plants produce, and what functions they may have. However, the chemistry of plants is a multidisciplinary topic, which requires knowledge over discipline boundaries, and students using a surface chemistry learning approach may have difficulties in that. Nevertheless, including plant chemistry into chemistry education helps to connect chemistry with the everyday life and therefore may increase people's interest in chemistry.

Besides demonstrating the chemical diversity of plants, students learned about what kind of instrumentation is used and how it is used in modern chemistry research, which is necessary for providing an authentic view of chemistry as a discipline. The university students' previous knowledge of spectrophotometry, liquid chromatography and mass spectrometry was rather limited, and they would have needed more time to develop a deeper understanding of the basic principles

related to the functioning of the instruments. The survey results showed that first-year chemistry students hold research instruments relevant for their studies, employment and chemistry research. The perceived relevance was positively connected to their experiences of a laboratory experiment utilising modern instrumentation. Therefore, it could be useful for chemistry educators to emphasise the importance of research instruments to chemistry. Furthermore, students' experiences and learning outcomes were affected by their chemistry learning approaches.

In conclusion, this thesis work provides tools for increasing people's understanding of the chemistry of plants and the role of modern instrumentation in it. The results gave insight on university chemistry students' understanding of plant chemistry and instrumentation. As the experimental tools are suitable for younger students, an interesting topic for future research could be to study their understanding of plant chemistry, instrumentation and the factors affecting those. The university students seem to understand the value of research instruments to chemistry research and industry, but younger students' ideas of the role and relevance of instruments have not been studied. Additionally, younger students' understanding of plant chemistry seems to be an understudied area which should be addressed in future studies. Understanding the chemistry of plants may help to increase understanding of the value of plants to life.

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