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# Extraction and analysis of plant-based products using chromatographic techniques

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## References

MOUČKOVÁ, Kristýna. Extrakce a analýza přírodních látek s využitím chromatografických technik. Pardubice, 2021, 190 pages. Dissertation thesis (Ph.D.). University of Pardubice, Faculty of Chemical Technology, Department of Analytical Chemistry. Supervisor doc. Ing. Petra Bajerová, Ph.D

## Abstract

The subject of the doctoral thesis is the extraction of bioactive compounds from plant material, focusing mainly on extraction of essential oils and flavonoids. The theoretical part is focused on the properties and usage of plant extracts and a description of selected plant matrices. The most extensive part of the theory is describing modern extraction techniques with a focus on green extraction. Furthermore, methods of analysis of extracts, namely gas chromatography with mass spectrometer detector (GC-MS) and flame ionization detector (GC-FID) and high-performance liquid chromatography (HPLC) with UV detector are described. Finally, the theoretical part briefly describes methods for determining the antimicrobial activity of plant extracts in the liquid and vapor phase. All described methodology is shown in pictures and schemes forms.

The experimental part is divided into three main chapters, describing 3 research topics, published in 4 foreign impact journals. The first chapter deals with the chemical composition and antimicrobial activity of hydrolates obtained from selected plants that grow in the Czech Republic (*Lavandula angustifolia*, *Syzygium aromaticum*, *Foeniculum vulgare* and *Laurus nobilis*). The second chapter discusses the chemical composition and antimicrobial activity of essential oils in the liquid, as well as the vapor phase of Asian herbs *(Houttuynia cordata*, *Perciaria odorata, Anthriscus cerefolium* and *Limnophila aromatic*a). The last, third chapter considers use of green microwave extraction with ionic liquid for flavonoid profile determination of leaves originated from Canary fruit trees (*Mangifera sp.* and *Passiflora sp*.).

## Keywords

Green extraction, bioactive compounds, essential oils, distillation, ionic liquids, gas chromatography, liquid chromatography, antimicrobial activity.

**Abstrakt**

Předmětem disertační práce je extrakce bioaktivních látek z rostlinného materiálu, zaměřující se především na izolaci esenciálních olejů a flavonoidů. Teoretická část práce je zaměřena na vlastnosti a využití rostlinných extraktů a popis vybraných rostlinných matric. Nejrozsáhlejší část teorie disertační práce se věnuje moderním extrakčním technikám se zaměřením na zelenou extrakci. Dále jsou v práci popsány metody analýzy extraktů plynová chromatografie s hmotnostní detekcí (GC-MS) a s plamenovým ionizačním detektorem (GC-FID) a vysokoúčinná kapalinová chromatografie (HPLC) s UV detekcí. Nakonec jsou v teoretické části stručně popsány metody stanovení antimikrobiální účinnosti rostlinných extraktů v kapalné a plynné fázi. Veškerá popisovaná metodika je zobrazena v obrázkových a schematických formách.

Experimentální část práce je rozdělena do třech hlavních kapitol, popisující 3 témata výzkumu, publikována ve 4 zahraničních impaktovaných časopisech. První kapitola se věnuje chemickému složení a antimikrobiální aktivitě hydrolátů získaných z vybraných bylin, které se pěstují v České republice (levandule lékařská, hřebíčkovec kořenný, fenykl obecný, vavřín ušlechtilý). V druhé kapitole je diskutováno chemické složení a antimikrobiální aktivita esenciálních olejů v kapalné, a navíc parní fázi z asijských bylin (touleň srdčitá, rdesno vonné, kerblík třebule a bahnatka vonná). Poslední, třetí kapitola se věnuje využití zelené mikrovlnné extrakce iontovou kapalinou pro stanovení profilu flavonoidů z listů Kanárských ovocných stromů (mango a mučenka).

**Klíčová slova**

Zelená extrakce, bioaktivní sloučeniny, esenciální oleje, destilace, iontové kapaliny, plynová chromatografie, kapalinová chromatografie, antimikrobiální aktivita.

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# 1 Goals of the doctoral dissertation

The following goals were set for the doctoral dissertation:

1) Summary of traditional and modern green extraction methods for extraction of natural substances

- ✓ The topic summary with reflection of current scientific situation

2) Summary of general principles of separation and identification of non-polar substances contained in essential oils by GC-MS and GC-FID

- ✓ Interpretation of MS spectra of selected natural substances contained in essential oils

3) Chemical composition and antimicrobial activity of extracts obtained by traditional hydrodistillation / steam distillation

- ✓ Isolation and analysis of hydrolates from herbs grown in the Czech Republic and determination of their antimicrobial properties
- ✓ Isolation and analysis of essential oils from Asian herbs and determination of their antimicrobial properties in the vapor and liquid phase of the essential oil

4) Use of green extraction by ionic liquid to determine the profile of flavonoids from the leaves of exotic fruit trees

- ✓ Synthesis of ionic liquids
- ✓ Optimization of ionic liquid extraction using a statistical model
- ✓ Comparison of ionic liquid extraction with a conventional extraction method
- ✓ Influence of the structure of the used ionic liquid on the composition of the extract
- ✓ Determination of selected flavonoids in several leaf cultivars of two exotic fruit trees

# 2 Introduction

Plant oils and extracts have been used for a wide variety of purposes since the time immemorial [1]. These purposes vary from the use of rosewood in perfumery, lime in flavouring drinks or application of lemongrass oil for the preservation of food. However, use of especially medicinal plants and their products are the most interesting part of plant-based products. Medicinal plants and their products such as essential oils are the main source of natural remedies. They have been used for ages as the most affordable means of treating diseases. As it has been proven several times, essential oils and other plant extracts have diverse biological properties. They are bactericidal, virucidal, fungicidal, antiparasitic, antioxidant and insecticidal. In recent years, there has been a renewed interest in plant extracts and their use in the pharmaceutical industry and medicine [2-7]. Among other things, this interest is related to the possible toxicity of synthetic drugs daily used for treating diseases, or many side effects. Another factor that has again increased interest in natural extracts is the increased resistance of microorganisms to commercially available drugs and emerging infectious diseases for which we have no treatment available. Phytomedicine is currently accepted worldwide as a valid alternative system of treatment in the form of drugs, functional foods, etc. and is recognized and supported by the World Health Organization. [8]. To use essential oils and plant extracts for treating various diseases caused by bacteria, it is important to understand the relationship between their chemical composition and the potential antimicrobial activity [9-11].

To obtain high quality extracts from plant it is highly important to choose proper extraction technique. The concept of medicinal plant preparation for experimental purposes includes proper and timely collection of the plant, identification, and verification of the plant by an expert and adequate processing of the plant material (drying, grinding). This is followed by extraction, or further fractionation and isolation of the bioactive compound. From a pharmaceutical point of view, the extraction of plant materials is a separation process in which an extract is obtained from medicinal plant parts and tissues by standard procedures using selective solvents. These extraction techniques separate plant metabolites soluble in a given solvent from an insoluble cellular residue. The purpose of standardized extraction procedures is to achieve therapeutically desired doses and to isolate the desired compounds with a suitable selective solvent [12]. Thus, obtained extract can be used as a medicinal agent in the form of tinctures, oils, liquid extracts, or further processed to be incorporated into any dosage form, such as tablets and capsules. All these products contain a complex mixture of many medicinal plant secondary metabolites, such as alkaloids, glycosides, terpenoids and flavonoids. The

extract can be further processed by various fractionation techniques to isolate a single chemical individual (e.g., codeine) to be used as a modern drug. The basic parameters that determine the quality of plant extracts and oils are used plant part for extraction, used extraction agent and the extraction technology and the concentration of the required drug substance in the extract.

Traditional techniques for extracting medicinal and aromatic plants include distillation, especially steam distillation (SD) and hydrodistillation (HD), as well as various solid-liquid extraction (SLE) applications. Examples of SLE are maceration, infusion, digestion or Soxhlet extraction. These conventional solvent extractions have been used in the home environment since time immemorial. However, there are disadvantages associated with these traditional extraction techniques. They are uneconomical due to excessive consumption of time and energy and often not environmental due to the use of toxic solvents. Therefore, nowadays, much attention is paid to the incorporation of the Green Chemistry principles in analytical extraction methods. One of the main strategies is the use of new solvents to replace conventional organic solvents, which are characterized by their high volatility, flammability and toxicity [13, 14]. For these reasons, green extractions, such as microwave-assisted extraction [15], ultrasound assisted extraction [16] or supercritical fluid extraction [17] have begun to appear and be used. Moreover, modern miniaturized techniques such as microdistillation [18], thermomicrorodistillation [19], molecular distillation [19], single-droplet microextraction [20], hollow fibre liquid phase microextraction [21] are also included, ionic liquid extraction (ILE) [22-24] or eutectic solvent extraction [2, 25, 26]. A special group of extractions are solid phase extractions, such as stir bar sportive extraction [27, 28] or solid phase microextraction [29-31]. These two extraction techniques are often used for the analysis of essential oils in plant materials. However, it only serves for qualitative or semi-quantitative analysis. The disadvantage of these methods is the impossibility of industrial use, as it is not possible to obtain an extract for further use, eg. for the production of cosmetic products [32, 33].

# *3* Chemical Composition and Antibacterial Activity of Natural Hydrolates Isolated from *Lavandula angustofolia, Laurus nobilis, Syzygium aromaticum and Foeniculum vulgare*

The aim of this study was to broaden the current knowledge of the chemical composition and antimicrobial activity of hydrolates obtained from lavender (*Lavandula angustifolia*), clove (*Syzygium aromaticum*), fennel (*Foeniculum vulgare*), and bay leaves (*Laurus nobilis*). All these natural matrices are widely available, but to date studies have not looked at testing hydrolates obtained from these matrices. Individual hydrolates obtained by distillation were subjected to chemical analysis by gas chromatography with flame ionization detector (GC-FID) and gas chromatography coupled to mass spectrometry (GC-MS). The antimicrobial activity of the obtained hydrolates was determined by the disc diffusion method, and minimal inhibitory/bactericidal concentration (MIC/MBC) was evaluated by microdilution method. In this chapter results and discussion of this research are described as published in the article ŠILHA, D. *et al*., Chemical Composition of Natural Hydrolates and Their Antimicrobial Activity on Arcobacter-Like Cells in Comparison with Other Microorganisms, Molecules, 2020, vol. 25, no. 23, s. 5654. ISSN: 1420-3049.

## *3.1 Results and Discussion*

### 3.1.1 Chemical Composition of Hydrolates

Analysed hydrolates, especially from lavender and laurel, were rich in several compounds. When comparing the results with respect to the hydrolate obtained by hydrodistillation (HHD) and steam distillation (HSD), more compounds were detected in the extracts from steam distillation (SD) hydrolates. This was probably caused by the more favourable conditions during steam distillation. During hydrodistillation, the plant material is in contact with boiling water throughout the distillation, and some of the volatile compounds may be converted (via oxidative reactions, polymerization and hydrolysis) into different substances [34]. The content of individual compounds is expressed as a percentage of the total peak area in the chromatograms obtained by the GC-FID analysis of HSD_SPE extracts and HHD_SPE extracts, so these are approximate values.

#### *3.1.1.1 Lavender Hydrolates*

In total, 48 compounds were identified by mass spectrometry and by comparing the calculated retention indexes with the literature data. The identified compounds amounted to 93.2% in the HHD_SPE extracts and 90.0 % in the HSD_SPE extracts. The major group of identified compounds was oxidized monoterpenes (34 identified compounds that accounted for 86.5% in the HHD_SPE extract and 82.3 % in the HSD_SPE extract). The biggest difference in the chemical compositions of both extracts from lavender hydrolates was in the content of linalool (23.2 % in the HHD_SPE, 7.9 % in the HSD_SPE). Linalool is one of the major compounds of lavender oils. Another major compound in lavender oils is linalyl acetate. The decomposition of linalyl acetate to linalool due to hydrolysis has been reported [35], and could be the main cause of the higher content of linalool in the hydrolate obtained by hydrodistillation (HD) due to the more unfavourable conditions in HD than in SD. A relatively high difference was observed in the content of coumarins (namely coumarin and 7-methoxycoumarin; HSD_SPE 3.8 % in total, HHD_SPE 0.9 % in total), and in the proportion of linalool derivatives (mainly furanoxides and pyranoxides; HSD_SPE 23.5 % in total, HHD_SPE 15.7 % in total). The second most abundant compound in the extracts was 1,8-cineol, the content of which was similar (20.6 % in the HSD_SPE extract, 19.5 % in the HHD_SPE extract).

#### *3.1.1.2 Bay Leaves Hydrolates*

In total, 33 compounds were identified in both types of hydrolates. The identified compounds amounted to 78.0 % in the HHD_SPE extracts and 79.4 % in the HSD_SPE extracts. The composition of both hydrolates was similar with respect to the identified compounds. Most of the identified compounds were oxidized monoterpenes (22 compounds accounted for 72.1 % in the HSD_SPE extracts and 73.2 % in the HHD_SPE extracts). The dominant compound in both types of extracts was 1,8-cineol (56.4 % in the HSD_SPE extract, 54.1 % in the HHD_SPE extract). Additionally, the EO of this matrix contains especially high levels of 1,8-cineol, linalool and R-terpinylacetate, as well as benzene compounds (eugenol, methyleugenol and elemicin) [30].

#### *3.1.1.3 Fennel Hydrolates*

In total, 13 compounds were identified in both types of hydrolates, and these compounds amounted to 84.1 % in the HSD_SPE extracts and 85.1 % in the HHD_SPE extracts. The main compounds in hydrolates were estragole and fenchone; both compounds formed almost 50 % of the extracts. Estragole and fenchone are the major components of fennel Eos [36-38]. Furthermore, estragole was characterized as one of the key odorants in fennel EOs [39]. Another

major constituent (often the most abundant compound) of fennel EOs, (E)-anethole, was not identified.

#### *3.1.1.4 Clove Hydrolates*

In total, 9 identified compounds amounted to 99.3 % in both extracts. The major group of identified compounds were phenolic derivatives, namely eugenol (E, 92.7 % in HHD_SPE extract, 89.1 % in HSD_SPE extract) and eugenyl acetate (EA, 9.4 % in HSD_SPE extract, 5.6 % in HHD_SPE extract). The difference in the E/EA ratio is given by the more unfavourable conditions during HD than during SD. Eugenyl acetate is hydrolysed to form eugenol. It is similar to linalyl acetate, which is hydrolysed during the hydrodistillation of lavender oil.

### 3.1.2 Antimicrobial Activity of Hydrolates

The antimicrobial activities of eight samples of non-concentrated and eight samples of concentrated hydrolates from *Lavandula angustifolia Mill*., *Syzygium aromaticum L*., *Foeniculum vulgare Mill*., and *Laurus nobilis L*. against eight strains of Arcobacter-like bacteria, and further against *Staphylococcus aureus* CCM 4223, *Enterococcus faecalis* CCM 4224, *Pseudomonas aeruginosa* CCM 3955, *Escherichia coli* CCM 3954 and the yeast *Candida albicans* CCM 8186 was obtained.

For most of the tested non-concentrated hydrolates HHD and HSD, no antimicrobial activity against the observed microorganisms was recorded. Most of the studied arcobacters were not suppressed by non-concentrated hydrolates at all. However, a very weak antimicrobial activity of clove hydrolate was reported just against *A. thereius* LMG 24488 (HHD, inhibition zone 6.5 ± 0.3 mm; HSD, inhibition zone 6.8 ± 0.4 mm). None of the tested arcobacters were suppressed by the hydrolates obtained by the distillation of lavender, fennel, and laurel. The obtained results show that the prepared non-concentrated hydrolates do not have significant antimicrobial potential against the tested microorganisms.

In contrast, concentrated hydrolates exhibited significant antimicrobial with inhibition zones up to 23.5 mm in diameter and minimal inhibitory (bactericidal) concentration up to 0.1 %. The inhibitory effect of concentrated hydrolates may be partially due to the presence of extraction reagent (ethanol). The influence of ethanol on the resulting inhibitory activity of the concentrated hydrolates was, of course, also evaluated. The antimicrobial activity of ethanol presents inhibition zones in the range of 6.3–9 mm (MIC in the range 1.6–12.5 %), depending on the strain tested. Significant inhibition was observed in the presence of concentrated hydrolates HHD_SPE and HSD_SPE. Overall, the minimum inhibitory concentrations of all

monitored samples in the range of 0.1–6.3 % were found. In most cases, the MIC and MBC values were the same or lower. However, in general, most of the hydrolates obtained by steam distillation showed higher antimicrobial activities compared to hydrolates obtained by hydrodistillation. Both concentrated clove hydrolates exhibited the highest antimicrobial activity of all the matrices tested. Clove is a very rich source of various antimicrobials [40]. In addition, according to our results, a significant content of eugenol and eugenyl acetate was observed in clove hydrolates. The lowest minimum inhibitory concentrations, in the range of just 0.1–0.8 %, were measured for clove hydrolate, and can be considered essentially the most antimicrobial effective.

### *3.2 Summary*

This study focuses on the chemical analysis of hydrolates prepared by the hydrodistillation and steam distillation of *Lavandula angustifolia Mill.*, *Syzygium aromaticum L.*, *Foeniculum vulgare Mill*., and *Laurus nobilis L*., as well as on assessing their antimicrobial potential on planktonic and biofilm cells. The MIC and MBC values of the tested hydrolates were recorded in the range of 0.1–12.5 %. The highest antimicrobial activity was observed especially in the case of clove hydrolates. As far as we know, the antimicrobial activity of these hydrolates against especially Arcobacter-like bacteria has not yet been monitored. The significant antimicrobial activity of the above-mentioned hydrolates against the microorganisms tested is also described for the first time, especially after their concentration by SPE. Moreover, concentrated hydrolates did not show any cytotoxic effect against human A549 cells. Thus, it can be concluded that hydrolates could be used as, for example, natural antimicrobial substances or food preservatives, but further testing will be needed.

# *4* Chemical Composition and Antibacterial Activity of Essential Oils in Liquid and Vapor Phases Isolated from *Houttuynia cordata* and *Persicaria odorata*

In this research essential oils from two Asian herbs, *Houttuynia cordata* and *Persicaria odorata*, were obtained by hydrodistillation and thereafter analysed by standard techniques GC-MS and GC-FID. Antimicrobial activity was determined by modern recently developed method of Houdkova *et al*. called broth microdilution volatilization method. It is a simple and rapid simultaneous determination of antibacterial potential of plant volatile compounds in the liquid and the vapour phase at different concentrations [41]. In this chapter results and discussion of this research is described as published in the article ŘEBÍČKOVÁ, K. *et al*., Chemical Composition and Determination of the Antibacterial Activity of Essential Oils in Liquid and Vapor Phases Extracted from Two Different Southeast Asian Herbs-Houttuynia cordata (Saururaceae) and Persicaria odorata (Polygonaceae).

## *4.3 Results and discussion*

### 4.3.1 Extraction yield and chemical composition

#### *4.3.1.1 Essential oil of Houttuynia cordata*

Hydrodistillation in Clevenger-type aperture of *Houttuynia cordata* produced a pale-yellow liquid with a fishy scent. The essential oil content of distilled aerial parts of dried plant was 0.34 %. The extraction yield is higher in comparison with those previously published by R. S. Verma et al. [42] who achieved only a yield of 0.06 – 0.14 %. A total of 41 compounds were identified that made up 90.6 % of the essential oil composition. The essential oil contained higher amount of terpenoid compounds (75.5 %), followed by non-terpenoid compounds (15.1 %) such as derivates of phenylpropene, aldehydes, ketones, esters and fatty acids. The major group of substances was monoterpenes with a content of 59.4 %, followed by the group of other compounds with a content of 14.8 %, oxidized monoterpenes with a content of 7.2 % and sesquiterpenes with a content of 6.6 %. Other groups were oxidized sesquiterpenes and derivates of phenylpropene. Major compounds of the essential oil were myrcene (51.6 %), 2-undecanone (6.7 %), tridecan-2-one (6.1 %), *cis*-*β*-ocimene (5.7 %), geranyl acetate (3.1 %), bornyl acetate (2.9 %) and *cis*-caryophyllene (2.6 %). The other compounds were present at less than 2 %. These results are similar with the results of previous reports [42-44]. Only a few fluctuations from other reports were found and probably are attributed to the origin of the plant

samples or different extraction method. For the characteristic fishy scent and flavouring of *H. cordata* essential oils is responsible compound houttuynin (decanoyl acetaldehyde). This compound was not identified in our essential oil due to its instability. It is usual that decanoyl acetaldehyde is during the process of distillation easily oxidized into 2-undecanone [42]. This compound had the second highest concentration in our essential oil. Therefore, the amount of 2-undecanone is the primary indicator for the quality of *Houttuynia cordata* essential oil [42, 45].

#### *4.3.1.2 Essential oil of Persicaria odorata*

Hydrodistillation in Clevenger-type apparature of *Persicaria odorata* produced a deep yellow liquid with a strong spicy coriander-like aroma. Due to its aroma it is also called Vietnamese coriander [46]. The essential oil content of distilled aerial parts of dried plant was 0.41 %. The extraction yield is lower in comparison with those previously published by A. A. Almarie et al. [47] who achieved a yield of 0.64 %. A total of 41 compounds were identified that made up 90.4 % of the essential oil composition. In comparison with other reports we identified more compounds [48, 49]. N. X. Dung *et al*. [48] used steam distillation for isolation of essential oil and they identified 28 compounds and the most abundant were *β*-caryophyllene, dodecanal and caryophyllene oxide. M. V. Hunter *et al*. [49] identified only 17 compounds using steam distillation as an extraction technique for isolation of essential oil from *P. odorata* where the most abundant compounds were *α*-humulene, decanal and dodecanal.

Our essential oil contained higher amount of non-terpenoid compounds (72.7 %) followed by terpenoid compounds (17.7 %). Carbonyls and alcohols, especially C10 and C12 made up 68.8 % of essential oil composition, followed by the group of sesquiterpenes with a content of 11.5 % and oxidized sesquiterpenes with a content of 5.7 %. Other groups (monoterpenes, oxidized monoterpenes and oxidized diterpenes) made up less than 1% of essential oil constitution. Major compounds of the essential oil were *n*-dodecanal (37.1 %), *n*-decanal (18.1 %), 1-decanol (5.4 %), 1-dodecanol (4.8 %), *α*-humulene (4.5 %), *cis*-caryophyllene (3.9 %) and *n*-undecane (2.5 %). The other compounds were present at less than 2 %. These results in relative percent content are similar with the results of previous reports. As it is obvious essential oil from *Persicaria odorata* is rich for C10 and C12 carbonyls. Dodecanal and decanal are the main compounds of *Persicaria odorata* essential oil in all previous published reports and ours [46-49].

### 4.3.2 Antimicrobial activity

The antimicrobial activity of *H. cordata* and *P. odorata* essential oils is reported in Table 1. Both essential oils showed antimicrobial efficiency but in different concentrations. *H. cordata* and *P. odorata* essential oil expressed various antimicrobial activity in the range of 126 – 1024 $\mu g \cdot ml^{-1}$, 512 – 1024 $\mu g \cdot ml^{-1}$ in broth and 1024 $\mu g \cdot ml^{-1}$, 512 – 1024 $\mu g \cdot ml^{-1}$ in agar, respectively. In liquid phase, the lowest MIC was showed for *H. cordata* (126 $\mu g \cdot ml^{-1}$) against *Ent. faecalis* and for *P. odorata* (512 $\mu g \cdot ml^{-1}$) against *Str. pyogenes, Ent. faecalis and B. subtilis.* In vapour phase, the lowest MIC was observed for *H. cordara* (1024 $\mu g \cdot ml^{-1}$) against *Ent. faecalis* and *E. coli* and for *P. odorata* (512 $\mu g \cdot ml^{-1}$) against *E.coli*. There are observable differences between the efficiency of the vapor and liquid phases of observed essential oils. In most cases, the higher MIC reached the liquid phase, except in the case of EO from *P. odorata* on *E.coli*, where the vapor phase was twice as effective as the liquid phase. As far as authors know there are no previous reports about *Persicaria odorata* and *Houttuynia cordata* essential oils and its antimicrobial activity in liquid and vapor phase, so it is not possible to further compare those results with other publications.

**Table 1** Antimicrobial activity of tested essential oils and antibiotic ampicillin against Gram negative and Gram positive bacteria.

| | Sample/Growth/MIC ($\mu g \cdot ml^{-1}$) | | | | | |
|---|---|---|---|---|---|---|
| | *Houttuynia cordata* | | *Persicaria odorata* | | *Ampicillin* | |
| | Agar | Broth | Agar | Broth | Agar | Broth |
| Gram negative bacterium | | | | | | |
| *Escherichia coli* | 1024 | 512 | 512 | 1024 | >4 | 0.50 |
| *Pseudomonas aeruginosa* | >1024 | >1024 | >1024 | 1024 | >4 | 1.00 |
| *Klebsiella pneumoniae* | >1024 | 1024 | >1024 | 1024 | >4 | >4.00 |
| *Seratia marcescens* | >1024 | 1024 | >1024 | >1024 | >4 | 4.00 |
| Gram positive bacterium | | | | | | |
| *Staphylococcus aureus* | >1024 | 1024 | >1024 | >1024 | >4 | 0.50 |
| *Enterococcus faecalis* | 1024 | 126 | >1024 | 512 | >4 | 0.25 |
| *Streptococcus pyogenes* | >1024 | 512 | 1024 | 512 | >4 | 1.00 |
| *Bacillus subtilis* | >1024 | >1024 | >1024 | 512 | >4 | 2.00 |

## *4.4 Summary*

This research showed interesting new knowledge about essential oils distilled from two Asian herbs – *Persicaria odorata* and *Houttuynia cordata*. The chemical composition of essential oils corresponds to previous studies with minor deviations that may be caused by agronomic factors, sample storage or sample preparation and other factors. As far as we know, we were the first who describe the antimicrobial properties of those essential oils in both vapor and liquid phase on 8 selected bacteria. Both essential oils showed antimicrobial activity in different concentrations to different bacteria. Due to great antibacterial activity along with the

composition of essential oils we see a great potential for future usage of these oils, like natural antimicrobials or food preservatives. Further it is necessary to study possible cytotoxicity of these oils. The disadvantage is that both oils contain *cis*-caryophyllene that causes allergic reactions and skin irritation. It would be necessary to find the balance in concentrations of beneficial antimicrobial active compounds and potentially toxic compounds.

# 5 Evaluation of Structurally Different Ionic Liquid-Based Surfactants in a Green Microwave-Assisted Extraction for the Flavonoids Profile Determination of *Mangifera sp*. and *Passiflora sp.* Leaves

The aim of this study is to evaluate the influence of the IL structure in a MA-SLE extraction method of flavonoids from plant leaves (*Mangifera sp*. And *Passiflora sp*., intending significant improvements in the greenness of the entire method compared to previous IL-based approaches [22]. Thus, six ILs-based surfactants are assessed, containing different cation moieties (imidazolium, guanidinium and pyridinium) and with different structural characteristics (monocationic versus multicationic or with different alkyl chain lengths). The method integrates high-performance liquid chromatography and photodiode array detector (HPLC-PDA) for determining three target flavonoids (rutin, quercetin and apigenin) in both Passiflora sp. and Mangifera sp. leaves. The method was thoroughly optimized using the Box-Behnken experimental design, and the optimum IL-based surfactant was used to determine flavonoids profiles in several lines and cultivars of the selected plant leaves. In this chapter results and discussion of this research are described as published in the article MOUČKOVÁ, K. *et al*., Evaluation of Structurally Different Ionic Liquid-Based Surfactants in a Green Microwave-Assisted Extraction for the Flavonoids Profile Determination of Mangifera sp. and Passiflora sp. Leaves from Canary Islands, Molecules, 2020, vol. 25, no. 20, s. 4734. ISSN: 1420-3049.

## *5.1 Results and Discussion*

### 5.1.1 Optimization of IL-MA-SLE method by RSM

Considering the promising results obtained in our previous study related to the extraction of phenolic compounds from *Vitis vinifera* leaves by an IL-MA-SLE method using [$C_{16}C_4Im^+$][$Br^-$] [22], this IL was selected to optimize the extraction procedure in the current study. Ultimately, the purpose was to use this IL as a screening solvent but intending the further use of ILs with lower toxicity and higher analytical performance. Three flavonoids (apigenin, rutin, quercetin) were selected as target analytes given their significant presence in passion fruit and mango leaves, as it has been previously reported in the literature [50-54]. The method optimization was performed using an experimental design to reduce the number of experiments, save reagents and plants material, and to determine the interactions among variables that affect the extraction efficiency towards the target analytes. Amongst all the samples, the *Passiflora flavicarpa* (PS032) sample was used as a representative matrix to carry out the optimization.

According to preliminary tests and other methods reported in the literature dealing with MA-SLE methods [55-59], four main factors were selected for optimization: MW irradiation time, MW irradiation temperature, liquid to solid ratio (l/s), and concentration of IL-based surfactant. MW irradiation power was fixed at 50 W in order to save energy. Besides, high MW power produces a high temperature inside the plant material, which may destroy some of the target compounds thus reducing the extraction efficiency [60]. The sample amount was fixed to 50 mg, to meet green requirements, while the IL-based surfactant aqueous solution volume was varied to evaluate the effect of l/s ratio.

The Box-Behnken statistical design was used to optimize the method. The limit values of the factors were selected according to previous studies [55-60] and a few preliminary tests, while the dependent variable used to evaluate the extraction efficiency in the experimental design was the peak area of the three target analytes. Thus, the ranges assessed for the different variables were 5-30 minutes for extraction time, 30–80 °C for extraction temperature, 10–50 $mL \cdot g^{-1}$ for l/s ratio, and 0.9–45 mM (from the CMC to 50 times the CMC) for the $[C_{16}C_4Im^+][Br^-]$ IL-based surfactant concentration.

The effects of chosen parameters on the extraction efficiency of target compounds, and interactions between them, can be estimated from the shape of the three-dimensional response surface. For example, as it can be observed in the representative response surfaces (Figure 1), the peak area of rutin and quercetin decreased with the increase of l/s ratio. However, the behaviour is totally different for apigenin since the extraction efficiency increases as the l/s ratio increases, and then it starts to decrease.

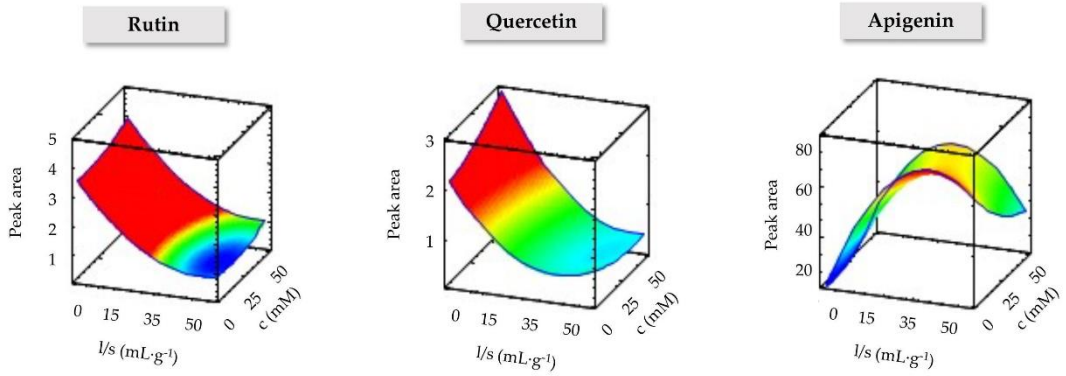


**Figure 1** Representative response surfaces described by the second order multivariate regression equation, obtained for each target flavonoid, showing the dependency of the peak area with the IL concentration and l/s ratio.

With the aim of finding trends, and particularly, trying to understand which of the studied factors significantly affect the IL-MA-SLE extraction efficiency, an analysis of variance (ANOVA) was performed. The determination coefficients ($R^2$) of the regressions indicate that the fitted models explain 89% of the variability in rutin, 58% in quercetin, and 37% in apigenin. Thus, the model is well fitted for rutin, but less for quercetin and apigenin. According to the P-values, the major statistically significant factor influencing the peak area was l/s ratio in the linear ($\beta_3$) and quadratic terms ($\beta_{13}$, $\beta_{23}$, $\beta_{33}$ and $\beta_{34}$) for all the analytes, except for apigenin, as previously observed from Figure 1. According to the P-values, the extraction time is an important factor influencing the extraction yield of apigenin ($\beta_1$ and $\beta_{11}$). For all the analytes, the extraction yield firstly increases with increasing extraction times. However, with further increase of time, the extraction yield decreases, which is particularly significant for apigenin, maybe due to the decompositions of analytes.

In general, rutin and quercetin present similar optimum conditions: low extraction times, temperature, and l/s ratio, and the maximum IL-based surfactant concentration (50 times the CMC). In the case of apigenin, best results were obtained with higher extraction times and l/s ratios, the highest extraction temperature, and the lowest IL-based surfactant concentration (CMC value). In order to benefit the highest number of analytes, a compromise solution was proposed, and the following optimum conditions were chosen for further extractions: 10 minutes of extraction time, 30.0 °C for the extraction temperature, 10.5 $mL \cdot g^{-1}$ of l/s ratio, and 50 times the CMC for the IL-based concentration. The l/s ratio was kept at this value to favour rutin and quercetin, for which this variable was especially significant. The extraction time, which affected apigenin the most, was set at an intermediate value amongst the optimum times, which were very similar. Due to the non-significant influence of temperature, it was set at the minimum value to save energy, while the highest IL-based surfactant concentration was used due to its positive effect at low temperatures for rutin and quercetin.

### 5.1.2 Analytical performance of IL-MA-SLE-HPLC-PDA method

Once the IL-MA-SLE method was optimized, and the target compounds were correctly identified in the chromatograms, the quantification of the analytes was required in order to determine their concentration in the different samples. The external calibration method was used for the quantification of the analytes in the samples. Linear range, calibration sensitivity (determined as the calibration slope), inter-day precision, determination coefficients ($R^2$), limits of detection (LODs), and limits of quantification (LOQs) of the chromatographic method were determined. The method exhibits good linearity with $R^2$ values higher than 0.9988, within the

concentration range of 0.1–500 $mg \cdot L^{-1}$ for rutin, 0.05–500 $mg \cdot L^{-1}$ for quercetin and 0.03–500 $mg \cdot L^{-1}$ for apigenin. LODs were experimentally obtained by decreasing the concentration of the analytes in the standard until a signal to noise ratio (S/N) of 3 was obtained, while the LOQs were estimated as 10/3 times the LODs and then experimentally verified. Thus, chromatographic LODs were 40, 20 and 10 $\mu g \cdot L^{-1}$ for rutin, quercetin and apigenin, respectively. The chromatographic LOQs ranged from 30 to 100 $\mu g \cdot L^{-1}$. In order to assess the precision of the method, standard solutions at 30 $mg \cdot L^{-1}$ were analysed by HPLC-PDA three consecutive times in the same day, and in three non-consecutive days. The proposed method presents good intermediate precision, with RSD values lower than 3.22 %.

### 5.1.3 Evaluation of different IL-based surfactants in the IL-MA-SLE-HPLC-PDA method

The effect of the IL structure in the extraction of the target flavonoids from plant matrices was evaluated using six IL-based surfactants. All of them were compatible with HPLC analysis due to their solubility in the mobile phase, thus not requiring a back-extraction of the IL extract before the injection in the instrument. They were selected considering different factors. Imidazolium ILs have been widely used for the extraction of different analytes from different samples [56, 60-64]. The effect of the secondary alkyl chain was assessed by using $[C_{16}MIm^+][Br^-]$ and $[C_{16}C_4Im^+][Br^-]$. In the case of multicationic IL-based surfactants, despite their interesting characteristics, they have been scarcely evaluated in extraction procedures, thus $[(C_8Im)_3Bn^+][3Br^-]$ was selected. Considering the recent concern on the toxicity of most common imidazolium ILs [65], other cationic cores were evaluated, including pyridinium $[C_{16}Py^+][Br^-]$ and alkyl guanidinium chloride IL-based surfactants. In the case of guanidinium ILs, which have been recently reported as low cytotoxic ILs [66, 67], different alkyl chain were also studied ($[C_8Gu^+][Cl^-]$ and $[C_{10}Gu^+][Cl^-]$). It is also important to highlight the environmentally friendliness in the synthetic procedure for the guanidinium ILs in comparison with that of imidazolium ILs, the latter requiring the use of toxic organic solvents (e.g., chloroform).

Given the different structures of the tested ILs, they present different CMC values, which range from 0.61 mM for $[C_{16}MIm^+][Br^-]$ to 44.6 mM for $[C_8Gu^+][Cl^-]$. Therefore, imidazolium IL-based surfactants present the lowest CMC values and lower amounts of IL are required to take advantage of their surface-active properties in comparison with guanidinium ILs. However, the ILs with guanidinium moieties present safer toxicological profiles. Apart from these properties, several studies have pointed out that the structure of ILs has a significant effect on the extraction

efficiency of target analytes [57, 60, 68, 69]. In general, the anion as well as the length of IL alkyl chain affects water miscibility, hereby it affects the extraction efficiency of target compounds [60, 70-72]. Thus, ILs with halide anions, such as [$Cl^-$] or [$Br^-$], are miscible with water in any proportion, but those ILs containing [$PF_6^-$] are mostly hydrophobic. On the other hand, increasing the alkyl chain length of the ILs also increases the hydrophobicity and viscosity of the ILs, whereas densities and surface tension values decrease [73].

A screening study was carried out to evaluate the extraction performance of the selected IL-based surfactants in the optimized IL-MA-SLE method and using PS032 and Sweet tart samples as representative leaves matrices for passion fruit and mango, respectively. The optimum conditions previously obtained were used for the remaining IL-based surfactants. Therefore, the extraction time, temperature, and l/s ratio, were the same, while the IL-based surfactant concentration was different for each IL since it depends on their CMC value. Thus, 30.5 mM was used for [$C_{16}MIm^+$][$Br^-$], 45 mM for [$C_{16}C_4Im^+$][$Br^-$], 36 mM for [$C_{16}Py^+$][$Br^-$], 2.23 M for [$C_8Gu^+$][$Cl^-$], 930 mM for [$C_{10}Gu^+$][$Cl^-$], and 115 mM for [$(C_8Im)_3Bn^+$][$3Br^-$], in all cases being 50 times higher than their respective CMC values.

The obtained results are included in Figure 2, showing the concentration obtained for each flavonoid when using the different IL-based surfactants as extractants. It is important to highlight the good precision in the determination of the analytes when using all the IL-based surfactants, with RSD values lower than 2.48%. As it can be observed, within the same cationic core, the longer the alkyl chain length, the better extraction efficiency for all the analytes. Thus, [$C_{10}Gu^+$][$Cl^-$] provided best results than its analogue with a chain of 8 carbon atoms for both samples, while the imidazolium IL-based surfactant with the longest substituents exhibited better extraction performance for all the analytes in the *Passiflorora sp.* sample, and for rutin in the *Magnifera sp*. sample. In the case of the tricationic IL-based surfactant, it provided the lowest extraction efficiency when analysing the *Passiflorora sp.* leaves and, indeed, it was not able to extract apigenin. However, this multicationic IL-based surfactant presented the best results for the extraction of apigenin and quercetin in the *Magnifera sp*. samples, particularly for quercetin. In the case of the pyridinium IL, the extraction efficiency was slightly lower in comparison with the remaining IL-based surfactants, except for apigenin in both samples, for which they presented similar results. Therefore, in general, it is clear that [$C_{16}C_4Im^+$][$Br^-$] and [$C_{10}Gu^+$][$Cl^-$] are the most efficient extractants for passion fruit leaves, while the tricationic IL and [$C_{10}Gu^+$][$Cl^-$] were the best for mango leaves, in comparison with the remaining IL-based surfactants evaluated. Given the significantly different behaviour observed for both samples

(regardless their different flavonoids content), it is difficult to determine that a single or a specific type of IL-based surfactant will provide the best results for this application. When comparing plant extracts it must be considered that they have a quite complex composition. Therefore, the origin of the leaves must be considered when evaluating different IL-based surfactant characteristics to enhance the extraction of flavonoids from any type of plant material.

Considering these results and with the aim of and favouring the best extraction performance for both type of samples and improving the sustainability of the method, $[C_{10}Gu^+][Cl^-]$ was selected as optimum extraction IL-based surfactant in further research. This IL presents low cytotoxicity in comparison with the imidazolium ILs, as it has been previously reported [66]. Despite higher amounts of IL are required for $[C_{10}Gu^+][Cl^-]$ due to its higher CMC value, the amount of this IL-based surfactant for each extraction is only 488 μL, which is still really low. Moreover, $[C_{16}C_4Im^+][Br^-]$ and $[(C_8Im)_3Bn^+][3Br^-]$ are solids at room temperature, while $[C_{10}Gu^+][Cl^-]$ is a liquid, which facilitates its manipulation and the preparation of aqueous solutions.

With the purpose of evaluating the performance of $[C_{10}Gu^+][Cl^-]$ in the proposed method, a comparison with a more conventional extraction method previously reported [74] was also carried out. The same samples with the same conditions of l/s ratio as the proposed method were extracted three times by a UA-SLE method using methanol. The extracts were further analysed by HPLC-PDA to obtain the average concentrations of the target flavonoids. The obtained results are also included in Figure 2 for both samples. It can be observed that both methods provided similar relative composition (percentage of each flavonoid concentration with respect to the total content of flavonoids) of rutin, quercetin and apigenin, but showing different concentrations.

The conventional UA-SLE method yielded much lower concentrations of the analytes, except for apigenin in *Passiflorora sp*., for which the results were slightly higher. Indeed, in general, in comparison with studies reported in the literature regarding the use of other extraction methods for the isolation of flavonoids from plant material, the proposed method with IL-based surfactants exhibited higher or comparable yields of rutin, quercetin and apigenin [54, 75]. Apart from the differences in the extraction performance of both methods, it is important to highlight other advantages of the proposed IL-MA-SLE method over the UA-SLE used for the same application. The proposed method has fewer steps, making the process less tedious and faster due to the elimination of the clean-up step. Our previous study demonstrated that the use

of IL-based surfactant aqueous solutions as extraction solvent avoids the co-extraction of green chlorophylls that could compromise the analytical performance of the chromatographic column [22]. In the current study, the entire IL-MA-SLE consumes less time, 15 minutes compared to the 45 minutes required in the UA-SLE method. Moreover, MW power is energetic enough to deal with the plant matrix while allowing a faster diffusion of the target compounds to the solvent [76, 77]. Another important advantage of the method proposed in this study in comparison with the more conventional method and our previous study [22] is the use of an IL of low cytotoxicity, thus gaining in greenness over the organic solvent required in the UA-SLE method, and the imidazolium IL utilized in our previous MA-SLE method [22].

Flavonoids present in fruits have also been determined through a number of extraction methods using different extraction solvents, including imidazolium-based ILs in combination with HPLC and UV [57-60, 65] or MS [50, 78] detection. Conventional extractions using solvents with different polarity such as ethanol [50, 54, 78], methanol [79], and chloroform [75], are one of the most used extraction techniques for isolation of flavonoids from fruits. Conventional Soxhlet extraction is another option to extract flavonoids from fruits but it is far more time and energy consuming [59, 79]. In comparison with these strategies, our proposed method uses low toxicity IL and requires relatively low or similar volumes of ILs solutions as extraction solvent (~500–2500 μL for 50 mg of plant material) [57-60]. Therefore, the method proposed in the current study is much more efficient for extraction of flavonoids from plants and it is characterized by its greenness in comparison with other methods, mainly in terms of toxicity of the extraction medium as well as time and energy consumption.

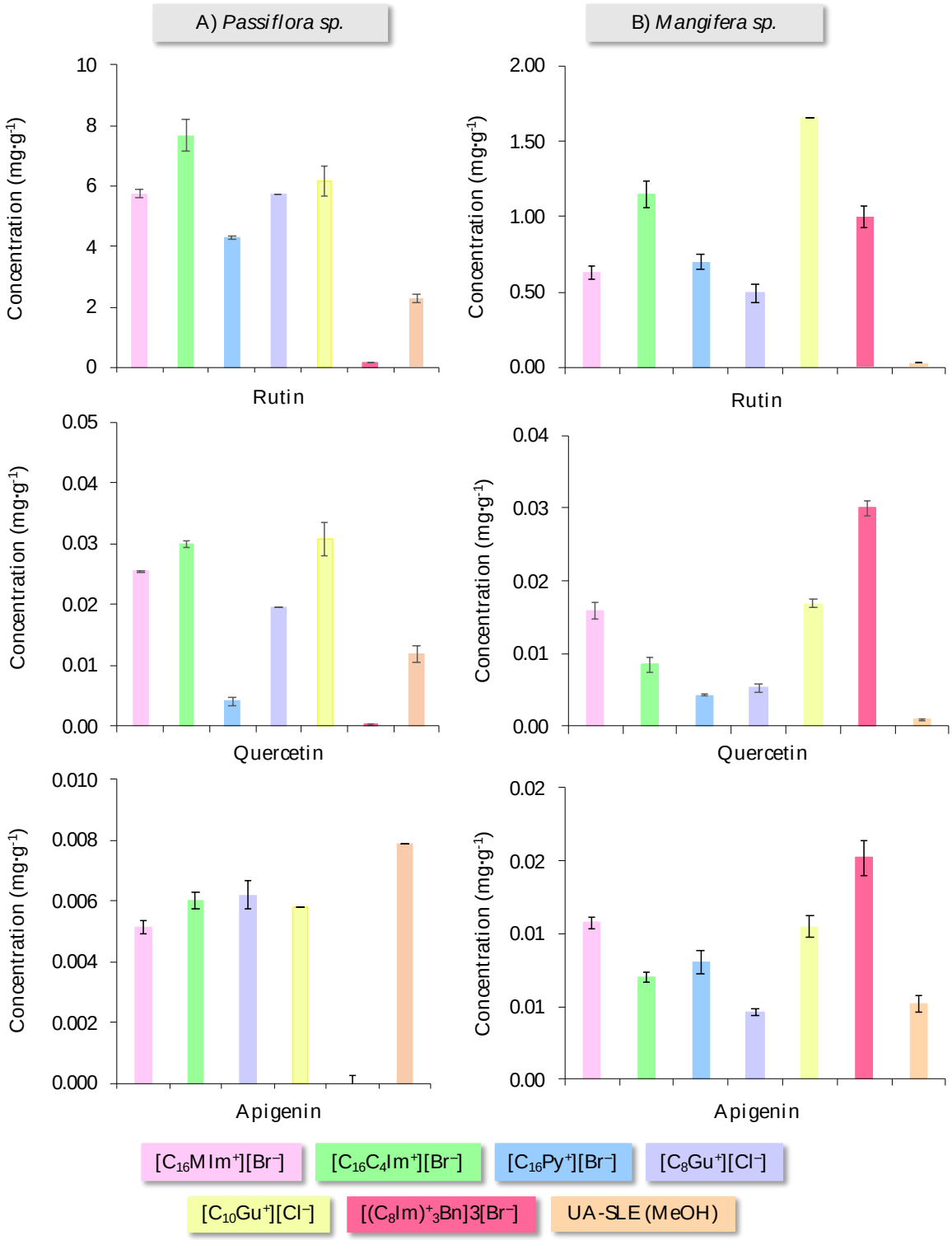


**Figure 2** Extraction performance of the IL-MA-SLE-HPLC-PDA method when using different IL-based surfactants for the determination of rutin, quercetin, and apigenin, in *Passiflora sp.* (PS032) and *Mangifera sp.* (Sweet tart), respectively. Extractions were performed in triplicate under the optimum experimental conditions.

### 5.1.4 Analysis of fruit and plant samples under optimum IL-MA-SLE-HPLC-PDA conditions

The optimized IL-MA-SLE-HPLC-PDA method using the $[C_{10}Gu^+][Cl^-]$ IL was applied to the determination of the three target flavonoids in fruit leaves samples from *Passiflora sp*. and *Mangifera sp*. Initially, seven analytes (rutin, quercetin, apigenin, myricetin, kaempferol, naringin and ellagic acid) were considered, but only the three flavonoids previously studied were detected in all samples. The results are included in the Table 2. Rutin, quercetin, and apigenin, were detected in all types of passion fruit and mango fruit leaves samples. The concentration of rutin, quercetin, and apigenin, varied in all types of passion fruit leaves ranged from 2.35 to 6.15 $mg \cdot g^{-1}$, from 0.021 to 0.090 $mg \cdot g^{-1}$, and from 0.006 to 0.017 $mg \cdot g^{-1}$ for the five *Passiflora sp.* types. Also, the concentration of rutin, quercetin, and apigenin, varied in all four types of mango leaves from 0.082 to 0.239 $mg \cdot g^{-1}$, from 0.006 to 0.044 $mg \cdot g^{-1}$, and from 0.007 to 0.015 $mg \cdot g^{-1}$, respectively. It is clear, that concentrations of flavonoids among types of fruit species are different. The phenolic content of the plant extract is influenced by several factors such as environmental (e.g. temperature, rainfall, day length [80]), harvesting (e.g. season, geographical location growth stage, daily harvest period [81-83]), and post-processing of plant material (e.g. drying [84, 85]).

The richest type of *Passiflora sp.* for flavonoids was PS032, while the richest type of *Mangifera sp.* for flavonoids was Mun. Extracts from passion fruit were much richer in rutin than extracts from mango (30 times higher), while content of quercetin and apigenin was very similar in both fruit leaves. Furthermore, the extracts from passion fruit 18PS003 had the lowest concentration of flavonoids among all types of passion fruit. Mango types Gomera 1 and 3 extracts were very similar due to their same origin (Gomera island). Mango Sweet Tart had lower content of flavonoids compared to Sweet tart but higher compared to Gomera 1 and 3. Considering the total concentration of flavonoids determined, *Passiflora sp.* leaves exhibit a great potential to be exploited for its valorisation, particularly due to its high content of rutin. Rutin was previously determined in 3 species of passion fruit samples from Brazil in concentrations from 0.57 to 3.48 $mg \cdot g^{-1}$ [54], which is similar or slightly lower in comparison with those analysed in the present study. Apart from quercetin and apigenin, it has been previously reported that *Passiflora sp*. is also rich in orientin, isoorientin, vitexin, and isovitexin [51, 54]. El-Hawary *et al.* determined the flavonoid content of 8 species of mango leaves from Egypt [86]. They determined 9 flavonoids in extracts, including rutin, quercetin and apigenin, which were quantified at concentrations from 0.67 to 6.99 $mg \cdot g^{-1}$, from 0.04 to 0.14 $mg \cdot g^{-1}$, and from 0.005

to 0.17 mg·g$^{-1}$, respectively. In that particular case, the content of all analytes was higher than in the samples from Canary Islands analysed in this study.

**Table 2** Flavonoids content (in mg·g$^{-1}$) of passion fruit and mango leaves from Canary Islands, analysed by the proposed IL-MA-SLE-HPLC-PDA method using an aqueous solution of the [$C_{10}Gu^+$][$Cl^-$] IL-based surfactant as extractant.

| Type | Rutin (RSD*) | Quercetin (RSD*) | Apigenin (RSD*) |
|---|---|---|---|
| ***Passiflora sp.*** | | | |
| PS032 | 6.15 (8.0 %) | 0.031 (9.0%) | 0.006 (5.0 %) |
| 17PS009 | 4.15 (8.0 %) | 0.046 (7.0 %) | 0.006 (0.5 %) |
| PS003 | 4.51 (5.0 %) | 0.021 (9.0 %) | 0.010 (8.0 %) |
| 17PS008 | 2.59 (7.0 %) | 0.090 (2.0 %) | 0.017 (2.0 %) |
| 18PS003 | 2.35 (3.0 %) | 0.036 (6.0 %) | 0.008 (6.5 %) |
| ***Mangifera sp.*** | | | |
| Sweet tart | 0.163 (2.0 %) | 0.031 (1.5 %) | 0.015 (8.5 %) |
| Mun | 0.239 (0.3 %) | 0.044 (3.0 %) | 0.011 (9.0 %) |
| Gomera 1 | 0.082 (2.0 %) | 0.006 (1.7 %) | 0.007 (1.2 %) |
| Gomera 3 | 0.082 (1.2 %) | 0.011 (2.0 %) | 0.008 (2.0 %) |

* relative standard deviation (n = 3)

## *5.2 Summary*

Six IL-based surfactants containing different cation moieties (imidazolium, guanidinium and pyridinium-type ILs), alkyl chains, and even number of cationic moieties, were successfully used in a MA-SLE method in combination with HPLC-PDA to evaluate the influence of the structure and composition of the IL on the extraction performance towards flavonoids. The result showed that the structure of ILs has a significant effect on the extraction efficiency of target analytes, while the origin of the plant material was also an important factor to consider when evaluating their performance. It was observed that ILs within the same cationic core, the longer the alkyl chain length, the better extraction efficiency is for all the analytes. [$C_{16}C_4Im^+$][$Br^-$] and [$C_{10}Gu^+$][$Cl^-$] were the most efficient extractants in comparison with the other IL-based surfactants evaluated for *Passiflora sp.*, while [$C_{16}C_4Im^+$][$Br^-$] and [$(C_8Im)_3Bn^+$][$3Br^-$] were the most efficient extractants for *Mangifera sp*. All three IL-based surfactants provided similar results, but with the aim of improving the sustainability of the method, [$C_{10}Gu^+$][$Cl^-$] was selected as optimum extractant due to its low cytotoxicity.

Apart from being simpler and faster, the greenness of the IL-MA-SLE method is given by the use of MW energy and the low cytotoxicity of IL-based surfactant, together with the high extraction efficiencies achieved in the entire method. This group of advantages highlights the proposed methodology over the conventional UA-SLE method that uses methanol as extraction medium.

Regarding contents in the plant analysed, it is important to mention that 3 flavonoids were determined in 5 different cultivars of *Passiflora sp*. and in 4 different types of *Mangifera sp.* using the proposed method. Concentrations of flavonoids in both fruits were different, with *Passiflora sp*. having the highest flavonoids content. Moreover, different flavonoid content in the extracts was obtained among all varieties for each type of fruit, which was not that significant but still observable.

# 6 Conclusion

The use of plant extracts is very advantageous because their production is relatively cheap, fast and simple, for example in comparison with synthetic drugs. In addition, today's society produces a lot of organic waste, such as peals, leaves and similar unused parts of plants, which could be used to produce natural extracts. Plant´s by-products contain many beneficial bioactive substances that can be used as natural remedies or antioxidants. It should be noted that the ease of extraction of bioactive compounds can also have a negative effect, making the production and use of drugs relatively easy, as just as the extract can be very beneficial and useful to society, it can also be dangerous.

This dissertation summarizes an overview of several current trends as well as traditional methods in extraction methods used to isolate plant extracts, which are described in the theoretical part, along with their analysis and identification using chromatographic methods. At the same time, in the experimental part it was possible to isolate and analyse extracts from 11 plant matrices with different origins by different extraction methods. Hydrolates from 4 different herbs used in the Czech Republic and essential oils from 4 herbs used in Asian cuisine were isolated and analysed by hydrodistillation or steam distillation as a traditional representative of the extraction technique of bioactive substances from plants. Furthermore, extracts from 2 species of leaves of fruit trees and their several cultivars and one herb originating from the Canary Islands were obtained and analysed by extraction using ionic liquid-based surfactants as a modern representative of the extraction technique of bioactive substances from plants. Throughout the research, emphasis was placed on the greenness of the extraction methods used and the lowest possible environmental burden. In addition, for 8 out of the 11 plant matrices, the antimicrobial activity of the obtained extracts was also evaluated, and most of them showed excellent antimicrobial effects with respect to their chemical composition. For 4 matrices, the antimicrobial activity of the extracts in both liquid and gas phases was investigated and brought new unpublished findings. In this respect, the dissertation provides insight and new information about the investigated matrices and their extracts, but also brings extended knowledge about extraction using ionic liquids. Regarding the above, the dissertation fulfilled set-up goals.

The results of the research were published in 4 publications in foreign impact journals, one of which was created in foreign cooperation thanks to the Erasmus + program.

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# 8 List of Student´s Published Works related to presented dissertation

## Articles

MOUČKOVÁ, K. - PACHECO-FERNÁNDEZ, I. - AYALA, J. - BAJEROVÁ, P. - PINO, V. Evaluation of Structurally Different Ionic Liquid-Based Surfactants in a Green Microwave-Assisted Extraction for the Flavonoids Profile Determination of Mangifera sp. and Passiflora sp. Leaves from Canary Islands, Molecules, 2020, vol. 25, no. 20, s. 4734. ISSN: 1420-3049.

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ŠILHA, D. - ŠVARCOVÁ, K. - BAJER, T. - KRÁLOVEC, K. - TESAŘOVÁ, E. - MOUČKOVÁ, K. - PEJCHALOVÁ, M. - BAJEROVÁ, P. Chemical Composition of Natural Hydrolates and Their Antimicrobial Activity on Arcobacter-Like Cells in Comparison with Other Microorganisms, Molecules, 2020, vol. 25, no. 23, s. 5654. ISSN: 1420-3049.

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## Presentations (co-author)

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