KAUNAS UNIVERSITY OF TECHNOLOGY

VAIDA ŠULNIŪTĖ

PHYTOCHEMICAL COMPOSITION AND ANTIOXIDANT PROPERTIES OF EXTRACTS ISOLATED FROM VARIOUS SPECIES OF THE GENUS *SALVIA*

Doctoral dissertation
Physical Sciences, Chemistry (03P)

2017, Kaunas

This doctoral dissertation was prepared at Kaunas University of Technology, Faculty of Chemical Technology, Department of Food Science and Technology during the period of 2013–2017. The studies were supported by Research Council of Lithuania.

Scientific Supervisor:

Prof. Dr. Petras Rimantas VENSKUTONIS (Kaunas University of Technology, Physical Sciences, Chemistry, 03P).

Doctoral dissertation has been published in:
http://ktu.edu

Editor:

Armandas Rumšas (Publishing Office “Technologija”)

ISBN 978-609-02-1356-8

The bibliographic information about the publication is available in the National Bibliographic Data Bank (NBDB) of the Martynas Mažvydas National Library of Lithuania

KAUNO TECHNOLOGIJOS UNIVERSITETAS

VAIDA ŠULNIŪTĖ

ĮVAIRIŲ *SALVIA* GENTIES AUGALŲ RŪŠIŲ EKSTRAKTŲ FITOCHEMINĖ SUDĖTIS IR ANTIOKSIDACINĖS SAVYBĖS

Daktaro disertacija
Fiziniai mokslai, chemija (03P)

2017, Kaunas

Disertacija rengta 2013–2017 metais Kauno technologijos universiteto Cheminės technologijos fakultete Maisto mokslo ir technologijos katedroje katedroje. Mokslinius tyrimus rėmė Lietuvos mokslo taryba.

Mokslinis vadovas:

Prof. dr. Petras Rimantas VENSKUTONIS (Kauno technologijos universitetas, Fiziniai mokslai, chemija, 03P).

Interneto svetainės, kurioje skelbiama disertacija, adresas:
http://ktu.edu

Redagavo:

Armandas Rumšas (leidykla „Technologija”)

ISBN 978-609-02-1356-8

Leidinio bibliografinė informacija pateikiama Lietuvos nacionalinės Martyno Mažvydo bibliotekos Nacionalinės bibliografijos duomenų banke (NBDB)

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AAPH	2,2'-Azobis(2-amidinopropane) dihydrochloride
ABTS	2,2'-Azinobis(3-ethylbenzthiazoline-6-sulphonic acid)
ASE	Accelerated Solvent Extraction
DPPH	2,2-diphenyl-1-picrylhydrazyl
DWE	Dry weight of an extract
DWP	Dry weight of a plant
EOs	Essential Oils
ESR	Electron Spin Resonance spectroscopy
FRAP	Ferric Reducing Antioxidant Power
GAE	Gallic Acid Equivalents
GRAS	Generally Recognised As Safe
KI	*Kováts* retention Indices
LOD	Limit Of Detection
LOQ	Limit Of Quantification
nd	Not detected
NMR	Nuclear Magnetic Resonance
ORAC	Oxygen Radical Absorbance Capacity
PLE	Pressurised Liquid Extraction
RSC	Radical Scavenging Capacity
RT	Retention Time
QENCHER	QUick, Easy, New, CHEap and Reproducible
SDE	Simultaneous Distillation-Extraction
SAm-C	CO_2 extract of *S. amplexicaulis*
SAm-E	Ethanolic extract of *S. amplexicaulis*
SAm-W	Water extract of *S. amplexicaulis*
SAu-C	CO_2 extract of *S. austriaca*
SAu-E	Ethanolic extract of *S. austriaca*
SAu-W	Water extract of *S. austriaca*
SFE	Supercritical Fluid Extraction
SF-C	CO_2 extract of *S. forsskaolii*
SF-E	Ethanolic extract of *S. forsskaolii*
SF-W	Water extract of *S. forsskaolii*
SG-C	CO_2 extract of *S. glutinosa*
SG-E	Ethanolic extract of *S. glutinosa*
SG-W	Water extract of *S. glutinosa*
SN-C	CO_2 extract of *S. nemorosa*
SN-E	Ethanolic extract of *S. nemorosa*
SN-W	Water extract of *S. nemorosa*
SO-C	CO_2 extract of *S. officinalis*
SO-E	Ethanolic extract of *S. officinalis*
SO-W	Water extract of *S. officinalis*
SP-C	CO_2 extract of *S. pratensis*
SP-E	Ethanolic extract of *S. pratensis*

SP-W	Water extract of *S. pratensis*
SSc-C	CO_2 extract of *S. sclarea*
SSc-E	Ethanolic extract of *S. sclarea*
SSc-W	Water extract of *S. sclarea*
SSt-C	CO_2 extract of *S. stepposa*
SSt-E	Ethanolic extract of *S. stepposa*
SSt-W	Water extract of *S. stepposa*
SV-C	CO_2 extract of *S. verticillata*
SV-E	Ethanolic extract of *S. verticillata*
SV-W	Water extract of *S. verticillata*
TE	Trolox Equivalents
TEAC	Trolox Equivalent Antioxidant Capacity
TPC	Total Phenolic Content
TPTZ	Ferric tripyridyltriazine
Trolox	6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid
TQ-S	Tandem Quadrupole mass Spectrometer
UPLC	Ultra Performance Liquid Chromatography
Q/TOF	Quadrupole Time Of Flight mass spectrometer

1. Introduction

Botanicals are among the most important sources of natural antioxidants and other valuable phytochemicals which may find applications as novel food additives or bioactive ingredients for functional foods and nutraceuticals. In addition, more than 25% of the pharmaceutical drugs prescribed worldwide are derived from the plant sources (Schmidt et al., 2008). Moreover, 'naturalness' has become one of the most important factors for the consumer's preferences in choosing various products for human consumption. Therefore, the interest in a more systematic and comprehensive characterisation of less studied plant species has been systematically increasing. Such studies are necessary for developing new natural preparations which might impart double benefits to foods, i.e. by not only enhancing their health benefits but also by providing antioxidant and antimicrobial protection; in the latter case, they may be considered as promising alternatives for such currently widely used synthetic food additives as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), benzoates and others which are prominently becoming more and more refused by the consumers due to safety concerns.

Numerous studies have demonstrated that dietary antioxidants may protect human cells from the negative effects of excessive free radicals which are linked to the development of many chronic diseases; if present in foods, such constituents may retard lipid oxidation and rancidity. Oxidation of lipids in foods reduces the sensory quality and nutritional value of the food products (Kristinová, Mozuraityte, Storrø, and Rustad, 2009), while in living organisms under conditions of excess of reactive oxygen species (ROS), they may damage cell membranes, accelerate their ageing, and induce the development of a number of diseases. Therefore, the application of natural antioxidants in foods is considered as a promising means for increasing their shelf-life, reducing waste and nutritional losses (Tsuda, Osawa, Ohshima, and Kawakishi, 1994). All the above-mentioned factors as well as the presence of a vast number of poorly investigated plant species foster search and valorisation of new sources of valuable and applicable natural substances, including dietary antioxidants.

Lamiaceae family herbs are among the most popular aromatic, medicinal and spicy plants, many of which have been extensively studied and reported to accumulate high amounts of essential oils, strong phenolic antioxidants and other valuable constituents. Some species of 'sage' (the common name used for many genus *Salvia* plants) have also been widely used in cosmetics, perfumery, soft drinks and various foods. *S. officinalis* and *S. sclarea* are the most thoroughly studied *Salvia* species, whereas the information about many other *Salvia* species is rather scarce. To fill this gap, 10 different *Salvia* spp. plants, namely *S. amplexicaulis*, *S. austriaca*, *S. forsskaolii*, *S. glutinosa*, *S. nemorosa*, *S. officinalis*, *S. pratensis*, *S. sclarea*, *S. stepposa* and *S. verticillata* have been selected.

In order to obtain valuable compounds from 10 different *Salvia* spp. plants, various extraction techniques and different polarity solvents were used. Scholarly practice has proven that only by consecutively employing different techniques can the best results be actually achieved. In this study, the antioxidant properties as well as the composition of phenolic and volatile compounds were also evaluated for the

valorisation of *Salvia* spp. as new raw materials for the isolation of functional ingredients for human nutrition.

The Aim of the Research

The aim of this work was to investigate phytochemical composition of volatile and polyphenolic compounds and antioxidant potential of various plant species of the genus *Salvia* using different extraction methods and different polarity solvents for their processing and to assess theoretically the potential of their wider application in the preparation of functional ingredients with health benefits.

The following objectives were outlined in order to achieve this aim:

1. To evaluate the applicability and effectiveness of various extraction methods and solvents for the separation of different polarity soluble fractions from 10 *Salvia* spp. plants and to assess their yields.

2. To evaluate the antioxidant potential of soluble fractions isolated from 10 *Salvia* spp. plants by various methods using radical scavenging and other antioxidant capacity measurement assays.

3. To determine the content of total phenolics in soluble fractions isolated from 10 *Salvia* spp. plants by employing various methods and to evaluate their composition by using the chromatographic and mass spectrometric methods.

4. To determine the variations of the main compounds identified in *Salvia* spp. plants and to evaluate the species in terms of their possible uses for the recovery of valuable natural compounds.

5. To determine the composition of tocopherols in lipophilic CO_2 extracts of *Salvia* spp.

6. To determine the composition of volatile compounds in volatile fractions of different *Salvia* spp. plants isolated by employing simultaneous solvent extraction-distillation and supercritical fluid extraction methods.

7. On the basis of the obtained experimental results, to theoretically evaluate the prospects of application of *Salvia* extracts in the preparation of functional ingredients with health benefits.

Scientific Novelty

The following scientific novelty was achieved by fulfilling the above outlined tasks:

1. Systematic studies on the variations of antioxidant properties and total phenolic compounds in various extracts isolated from different *Salvia* spp. plants were performed for the first time.

2. The phytochemical composition of the majority of plants of *Salvia* spp. selected in our study has not been reported previously. Only the common sage (*Salvia officinalis*) has been studied to some extent; although it is used in our study mainly for comparison purposes, comprehensive evaluation of its fractions isolated by using green high pressure extraction and fractionation technologies has not been reported previously.

3. We applied comprehensive and complex approach in the fractionation of *Salvia* spp. (separation into lipophilic and other fractions by different polarity green solvents, determination of the phytochemical concentration in the extracts and in the whole plant material). This approach has never been applied previously to any of the *Salvia* plants selected for our research; it has even never been applied to the more frequently studied *S. officinalis* and *S. sclarea*. Moreover, this concept has been rather scarcely applied to other plants as well.

4. Some phytochemicals (ethyl gallate, 3',4',5,7-tetraxydroxy-3-methoxyflavone, hyperoside and isorhamnetin-glucoside) are reported in *Salvia* spp. plants for the first time.

5. The composition of the volatile compounds of the majority of the *Salvia* spp. plants selected in our study has never been reported previously. Only *Salvia officinalis* has been studied, which is predominantly used in our study for comparison purposes only.

Practical Significance

It was established that ethanolic extracts of *S. officinalis*, *S. sclarea*, *S. amplexicaulis*, *S. verticillata*, *S. nemorosa*, *S. forsskaolii* and *S. pratensis* are rich in bioactive compounds; these results are practically important for further valorisation of the use of plant extracts as a source of natural antioxidants in the food industry as they may serve for food enrichment with bioactive compounds. The chromatographic data of *S. amplexicaulis*, *S. austriaca*, *S. forsskaolii*, *S. glutinosa*, *S. nemorosa*, *S. officinalis*, *S. pratensis*, *S. sclarea*, *S. stepposa* and *S. verticillata* extracts obtained by using different extraction methods provide important information on the variations in their phytochemical composition. This information is useful for the industry in terms of the selection of the proper *Salvia* spp. and extraction solvent thus ensuring the highest recovery of bioactive compounds.

Structure and Outline of the Dissertation

The dissertation is written in English. It consists of a list of abbreviations, an introduction, a review of the most relevant scholarly literature, a section on the employed materials and methods, a chapter covering the results and discussion, conclusions, literature references (in total, 231 references were used), and a list of the author's publications on the theme of this dissertation. The final work contains 98 pages including 9 tables and 18 figures.

Publication of the Research Results

The results of the research have been presented in 3 publications delivered in journals covered in the list of *Clarivate Analytics* (formerly, *Thompson Reuters*) *Web of Science* database and reported at 5 international conferences.

Key Points Presented for the Defence:

1. Application of consecutive extraction with supercritical CO_2 and pressurised liquid extraction with higher polarity solvents enables to obtain from *Salvia* spp.

plant material whose valuable fractions possess strong antioxidant capacity and high concentrations of bioactive compounds.

2. Some underinvestigated *Salvia* spp. plants, namely, *S. amplexicaulis*, *S. verticillata*, *S. nemorosa*, *S. forsskaolii* and *S. pratensis*, may represent promising material for the recovery of valuable natural compounds.

3. The composition of volatile constituents of *Salvia* spp. plants isolated by simultaneous solvent extraction-distillation and supercritical fluid extraction methods is significantly different.

2. Literature Review

2.1. Antioxidants in Plants

2.1.1. Classification of Antioxidants

During the recent years, the most widely used definition of antioxidants was proposed by Halliwell and Gutteridge (1995): “any substance that, when present at low concentrations compared with that of an oxidizable substrate, significantly delays or inhibits oxidation of that substrate.” The most common strategy is to classify antioxidants into primary and secondary ones and to describe the origin of antioxidants as natural or synthetic. Primary antioxidants are free radical scavengers that inhibit or delay oxidation, while secondary ones function as metal chelators, converting hydroperoxydes to non-radical species, deactivating singlet oxygen, absorbing ultraviolet radiation, or acting as oxygen scavengers (Pukalskas, 2008).

Synthetic Antioxidants

The best known synthetic antioxidants are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), butylhydroquinone (TBHQ) and gallic acid esters (propyl, octyl, dodecyl) (Mikalauskas, 2006). All of them have been widely used as antioxidants in foods. Synthetic antioxidant TBHQ is the most effective antioxidant used by the food industry (Yanishlieva and Marinova, 2001). Synthetic antioxidants are efficient, easily available, and relatively cheap, but, on the other hand, they may have negative side effects which are related to their toxicity. Thus the importance of searching for and exploiting natural antioxidants, especially those of plant origin, has greatly increased in recent years. Besides, there is also a growing interest in natural additives as potential antioxidants.

Natural Antioxidants

The majority of natural antioxidants are phenolic compounds, and they can be classified into the lipophilic group (mainly tocopherols) and the hydrophilic group (phenolic acids and flavonoids) (Maestri, Nepote, Lamarque, and Zygadlo, 2006).

Tocopherols and tocotrienols are collectively known as tocols, each of which contains four main isomers (α, β, γ, δ). Tocopherols and tocotrienols consist of a polar chromanol ring and a hydrophobic side chain, phytyl in tocopherols and isoprenyl with three double bonds in tocotrienols (Ryynänen, Lampi, Salo-Väänänen, Ollilainen, and Piironen, 2004) (Fig. 2.1.). Tocopherols and tocotrienols are amphipathic and lipid-soluble compounds. α-Tocopherol is the major vitamin E form which is present in green plant tissues, whereas γ-tocopherol and tocotrienols tend to accumulate at higher levels in seeds (Munné-Bosch and Falk, 2004).

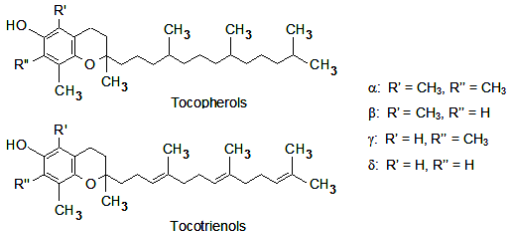


Fig. 2.1. Chemical structures of tocopherols and tocotrienols

Flavonoids are one of the major subgroups of phenolic compounds derived from higher plants, and are subdivided into anthocyanins, flavanols, flavanones, flavones, flavonols, and isoflavones (Yao et al., 2004) (Fig. 2.2.). The common characteristic of flavonoids is the basic 15 carbon atoms flavan structure (C_6–C_3–C_6) where atoms are arranged in 3 rings labelled *A*, *B*, and *C*. Individual compounds within the class differ in the substitution pattern of the *A* and *B* rings (Wojdyło, Oszmiánski, and Czemerys, 2007).

Flavonoids are a major coloring component of flowering plants and they are found in all plant foods. Approximately 90% of flavonoids in various plants occur as glycosides. Flavonoids in food are responsible for its taste, colour, protection of enzymes and vitamins, and prevention of fat oxidation (Yao et al., 2004).

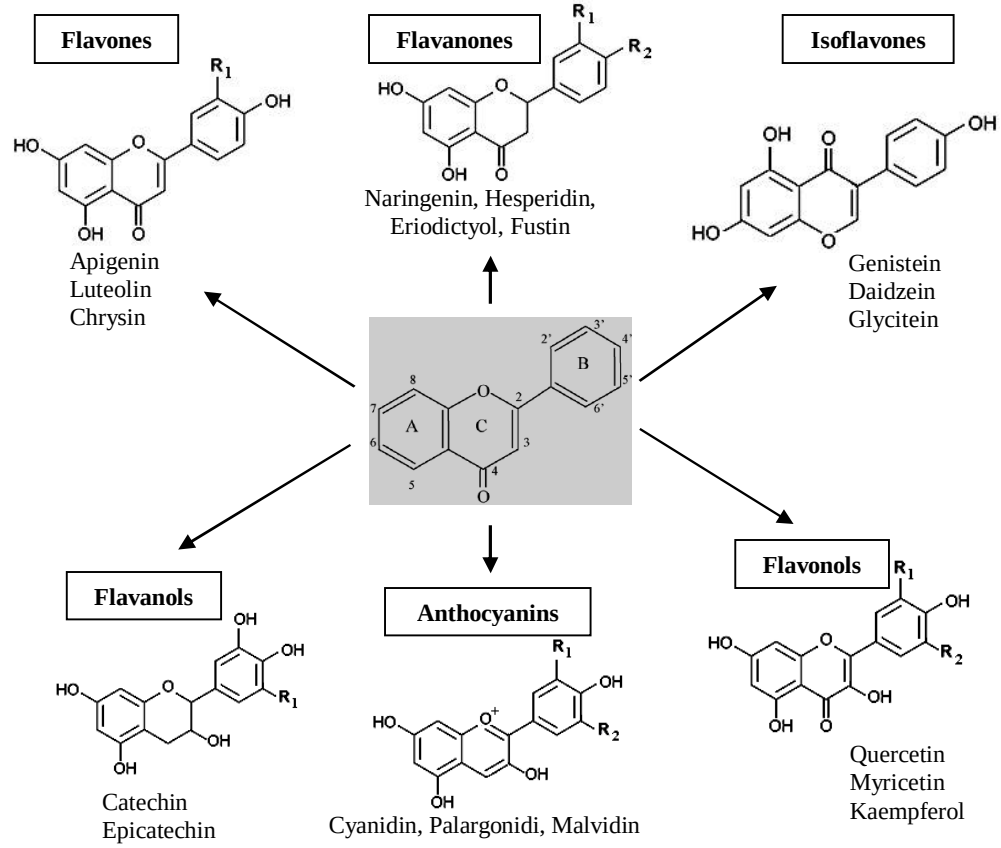
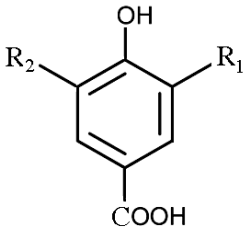


Fig. 2.2. Structures of flavonoids and related compounds isolated from various plants

Phenolic acids are another very important subgroup of phenolic compounds broadly distributed throughout the plant kingdom. In plants, phenolic acids occur as substituted benzoic and cinnamic acid derivatives which usually exist as glycosides, or esters of organic acids (Fig. 2.3.). Ferulic acid and other cinnamic acids (derivatives of *p*-coumaric and caffeic acids) have been found to show a good antioxidant activity (Andreasen, Kroon, Williamson, and Garcia-Conesa, 2011; Emmons, Peterson, and Paul, 1999). The group of the CH=CH–COOH in

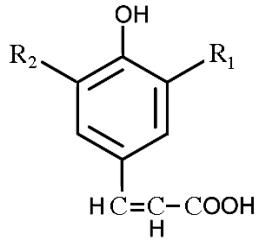
hydroxycinnamic acids is considered to be key for the significantly higher antioxidative efficiency than the COOH group in hydroxybenzoic acids (White and Xing, 1997).

Benzoic acid derivatives



Compound	R_1	R_2
p-Hydroxybenzoic acid	H	H
Syringic acid	OCH_3	OCH_3
Vanillic acid	H	OCH_3
Dihydroxybenzoic acid	OH	H
Gallic acid	OH	OH

Cinnamic acid derivatives



Compound	R_1	R_2
p-Coumarinic acid	H	H
Sinapic acid	OCH_3	OCH_3
Ferulic acid	H	OCH_3
Caffeic acid	OH	H

Fig. 2.3. Structures of the naturally occurring phenolic acids in plants

2.1.2. Sources of Phenolic Antioxidants

There is currently great worldwide interest in discovering new safe antioxidants from natural sources which could minimise the oxidative damage to living cells and to prevent oxidative deterioration of foods. It should be mentioned that the major sources of naturally occurring antioxidants are fruits and berries, vegetables, cereals, coffee, black and green tea, herbs and spices.

Fruits and berries contain high levels of phytochemicals with the phenolic structure (flavonoids, phenolic acids, stilbenes, and tannins) that can act as antioxidants and perform health-promoting activities (He and Liu, 2006; Leahy, Speroni and Starr, 2002; Yan, Murphy, Hammond, Vinson and Neto, 2002). The main subgroups of flavonoids in fruits and berries are anthocyanins, flavonols, and flavanols (catechins). Flavonoids usually accumulate in the leaves and bark of plants, because their synthesis is stimulated by light (Price, Breen, Valladao and Watson, 1995). The phenolic acids which are present in fruits and berries are hydroxylated derivatives of benzoic and cinnamic acids (Paredes-López, Cervantes-Ceja, Vigna-Pérez and Hernández-Pérez, 2010). Stilbenes are small naturally occurring phenolic compounds also found in plants; berries are a good source of them as well. Pterostilbene, resveratrol, and piceatannol are compounds of stibenes which are found in bilberry, cowberry, blueberry, and lingonberry (Rimando, Kalt, Magee, Dewey and Ballington, 2004; Lyons et al., 2003; Wang, Catana, Yang, Roderick and van Breemen, 2002). Antioxidants (including phenolic compounds) in fruits and berries have anti-inflammatory, anti-carcinogenic and anti-atherosclerotic effects as a result of their antioxidant activity (Pan, Lai and Ho, 2010).

Among vegetables, broccoli (Zhang and Hamauzu, 2004), garlic (Bhatt and Patel, 2013), mushroom (Smolskaitė, Venskutonis and Talou, 2015), white cabbage and cauliflower (Gazzani, Papetti, Massolini and Daglia, 1998), beans, beet and corn (Kahkonen et al., 1999) have been reported to show high antioxidant activity. Other vegetables, such as spinach, Brussels sprout, kale, onion, cauliflower, alfalfa sprouts, beets, red bell pepper, corn, cucumber and eggplant are also rich sources of antioxidants (Prior and Cao, 2000). High levels of quercetin have been found in kale, tomato, onion and certain varieties of lettuce whereas high levels of kempferol were observed in in kale, broccoli and endive (Justesen, Knuthsen and Leth, 1998). A daily diet rich in vegetables can thus reduce the risk of heart disease and stroke, lower the risk of eye and digestive problems, prevent some types of cancer, and exert a positive effect upon blood sugar levels.

Cereals are also a good source of phytochemicals. Cereals contain a significant amount of phenolic acids such as caffeic, ferulic, *p*-hydroxybenzoic, *p*-coumarinic, vanillic and syringic acids (White and Xing, 1997). Cereal grains are also a good source of catechins. The highest amount of catechins is found in buckwheat seeds, rye, wheat and oats (Holasova et al., 2002; Peterson, Emmons and Hibbs, 2001).

Tea and coffee are the most commonly consumed beverages in the world. Coffee polyphenols, such as chlorogenic acids, are a very important source of natural antioxidants (Clifford, 2000; Svilaas et al., 2004) in our daily life, and high consumption of antioxidants from coffee can contribute to the reduction of risks of morbidity and mortality. In black and green tea, the major antioxidants are catechins (Huang and Frankel, 1997). (–)-Epigallocatechin 3-gallate, (–)-epigallocatechin, (–)-epicatechin 3-gallate, (–)-epicatechin, (+)-gallocatechin and (+)-catechin were also identified as an important source, and their antioxidant activity has been investigated (Atoui, Mansouri, Boskou and Kefalas, 2005).

Aromatic herbs and spices are one of the most important targets in the research of natural antioxidants from the point of view of safety. Herbs and spices are potential sources of invaluable antioxidants. Their antioxidant activity has been attributed to the presence of essential oils and polar phenolic compounds, particulary phenolic acids, such as gallic, caffeic, vanillic, ferulic, *p*-hydroxybenzoic, *p*-coumarinic and rosmarinic acids (Demo, Petrakis, Kefalas and Boskou, 1998). Aromatic herbs and spices represent one of the simplest way to increase the phenolic content and the antioxidant capacity of the daily diet, with possible health benefits (Ninfali, Mea, Giorgini, Rocchi and Bacchiocca, 2005).

Based on scholarly literature, the family *Lamiaceae* (*Labiatae*) is one of the largest and most important distinctive families of flowering plants, with about 220 genera and almost 4000 species, which are widespread throughout the world (Naghibi, Mosaddegh, Mohammadi Motamed and Ghorbani, 2005). The family *Lamiaceae* has been attracting the attention of researchers due to its high content of polyphenolic compounds, and a large number of them are well known for their antioxidant properties (Özgen et al., 2006; Tepe, Sokmen, Akpulat and Sokmen, 2006). Among the herbs of the *Lamiaceae* family, rosemary has been more extensively studied, and its extracts are the first ever marketed natural antioxidants (Yanishlieva and Marinova, 2001). Sage, peppermint, basil, oregano, lemon balm,

marjoram and thyme, which belong to the same family, have been gaining interest as potential antioxidants. Apart from herbs and spices of the *Lamiaceae* family, there are many others which represent rich sources of polyphenols. The phenolic antioxidants of some widely investigated plants are summarised in Table 2.1.

Table 2.1. Antioxidants isolated from various herbs and spices

Family/ Common name/ Botanical name	Major phenolic antioxidants	References
Lamiaceae		
Lemon balm (*Melissa officinalis)*	Caffeic, rosmarinic, and *m*-coumaric acids, eriodictyol-glucoside, naringin, hesperidin, naringenin, hesperetin.	Dastmalchi et al., 2008.
Basil (*Ocymum basilicum)*	Rosmarinic, caffeic, vanillic, coumarinic, syringic, and ferulic acids.	Lee and Scagel, 2009; Jayasingne, Gotoh, Aoki and Wada, 2003.
Marjoram (*Origanum majorana)*	Rosmarinic, caffeic, gallic, *p*-coumaric, and ferulic acids, rutin, apigenin, eriodyctiol.	Zgórka and Głowniak, 2001.
Oregano (*Origanum vulgare)*	Rosmarinic, caffeic, and protocatechuic acids, apigenin, eriodictyol, dihydroquercetin, dihydrokaempferol.	Embuscado, 2015.
Rosemary (*Rosmarinus officinalis)*	Rosmarinic and carnosic acids, carnosol, rosmanol, rosmadial.	Yanishlieva and Marinova, 2001; Kontogianni et al.,2013.
Sage (*Salvia officinalis)*	Rosmarinic and carnosic acids, rosmanol, rosmadial, carnosol, methyl carnosate.	Cuvelier, Berset and Richard, 1994; Kontogianni et al., 2013; Miura, Kikuzaki and Nakatani, 2001.
Thyme (*Thymus vulgaris)*	Rosmarinic acid, eriodictyol, taxifolin, luteolin glucuronide.	Dapkevicius et al.,2002.
Apiacee		
Parsley (*Apium petroselinum)*	Apigenin, luteolin, apigenin glycosides.	Justesen and Knuthsen, 2001.
Coriander (*Coriandrum sativum)*	Vanilic, *p*-coumaric, and ferulic acids, quercetin, kaempferol, acacetin flavanoids.	Nambiar, Daniel and Guin, 2010.
Cumin (*Cuminum cyminum)*	Vanilic and *p*-coumaric acids, quercetin.	Bettaieb et al., 2010.
Zingiberaceae		
Turmeric (*Curcuma longa)*	Curcumins	Aggarwal and Sung, 2009.
Ginger (*Zingiber officinale)*	Gingerol, shogaol, paradols.	Prasad and Tyagi, 2015.

2.1.3. Assessment of Antioxidant Activity *in Vitro*

For the assessment of antioxidant properties and radical scavenging of various foods, numerous *in vitro* methods have been developed (Moon and Shibamoto, 2009; Niki, 2010). The selection of methods for the evaluation of the antioxidant potential should be based on recommendation of well-known experts in the relevant area, namely Huang, Ou and Prior (2005). They recommend that for comprehensive *in vitro* evaluation, TPC, ORAC and one method based on single electron transfer (DPPH, ABTS or FRAP) should be applied.

The total phenolic content (TPC) is one of the oldest methods designed to determine the total content of phenolics by using Folin-Ciocalteu's reagent (Singleton and Rossi, 1965). This colorimetric method is based on oxidation/reduction reactions between phenolic compounds and Folin-Ciocalteu's reagent forming a blue colour complex that can be quantified by visible light spectrophotometry.

The main disadvantage of this method is that various substances, especially aromatic amines, ascorbic acid, sugars, sulphur dioxide and other nonphenolic organic substances readily react with the Folin-Ciocalteu's reagent and can distort the results. However, the TPC method is precise, sensitive and simple and has been widely used for studying phenolic antioxidants (Roginsky and Lissi, 2005; Singleton and Rossi, 1965). Numerous studies on antioxidants present in plants have been conducted by using the TPC assay including vegetables, fruits, seeds, cereals, and herbs (Kamath, Arunkumar, Avinash and Samshuddin, 2015).

The oxygen radical absorbance capacity (ORAC) is a very popular method of measuring antioxidant capacities in plasma, serum or other biological samples *in vitro* (Prior et al., 2003). This method is based upon inhibition of the peroxyl-radical-induced oxidation of fluorescein in the presence of an antioxidant. The source of the peroxyl radical is the thermal decomposition of azo-compounds, such as 2,2'-azobis(2-amidinopropane) dihydrochloride (AAPH). The fluorescence decay kinetic curve is obtained by measuring the fluorescence intensity at the conditions (pH=7, 37°C) which are the most relevant to human biology. This method is standardised (it allows for data comparison across laboratories) and integrates both the degree and the time of the antioxidant reaction. However, normally, it requires the use of expensive equipment showing pH-sensitivity as well as long times to quantify the results (Zulueta, Esteve and Frígola, 2009). The ORAC method has been proposed as a standard method for the evaluation of the food antioxidant capacity (Prior, Wu and Schaich, 2005).

2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay has become very popular in the natural antioxidant research. One of the main reasons of this phenomenon is the fact that this method is sensitive and fairly simple. DPPH assay is based on the theory that a hydrogen donor is an antioxidant. Fig. 2.4. shows the mechanism developing between $DPPH^{\bullet}$ and the antioxidant (RH). The antioxidant effect is proportional to the disappearance of $DPPH^{\bullet}$ in samples (Moon and Shibamoto, 2009). Different methods of monitoring the amount of $DPPH^{\bullet}$ have been reported: electron spin resonance spectroscopy (ESR)/plant powders (Calliste, Trouillas, Allais, Simon and Duroux, 2001), UV spectrophotometry/polyphenols (Chaillou and Nazareno, 2006), and nuclear magnetic resonance NMR/catechins (Sawai and Sakata, 1998). $DPPH^{\bullet}$ shows a very strong absorption maximum at λ=517 nm (the colour is purple). The colour turns from purple to yellow followed by the formation of DPPH upon absorption of hydrogen from an antioxidant. Thus the antioxidant effect can be quite easily evaluated by following the decrease of UV absorption at λ=517 nm (Moon and Shibamoto, 2009). This method is easy, simple and rapid; hence, it is a very popular choice for the screening of antioxidants. However, it is difficult to test compounds (i.e. carotenoids) that have spectra overlapping $DPPH^{\bullet}$ at λ=515 nm

(Prior, Wu and Schaich, 2005). DPPH assay has been applied to the investigation of the antioxidant activities of herbs and spices (Kulišić, Dragović-Uzelac and Miloš, 2006; Mata et al., 2007), cereals (Choi et al., 2007; Wang, Zhao, Zhao and Jiang, 2007), tea and leaves (Saito et al., 2007; Su, Duan, Jiang, Duan and Chen, 2007; Amarowicz, Pegg, Rahimi-Moghaddam, Barl and Weil, 2004), fruits and vegetables (Abdille, Singh, Jayaprakasha and Jena, 2005; Kanatt, Chander, Radhakrishna and Sharma, 2005; Miller, Rigelhof, Marquart, Prakash and Kanter, 2000).

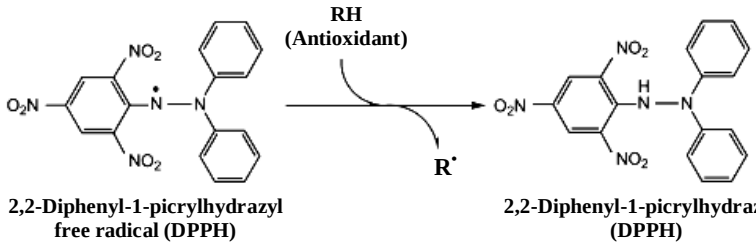


Fig. 2.4. Reaction between DPPH• and antioxidant forming DPPH (adapted from Moon and Shibamoto, 2009)

2,2'-azinobis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) assay has been widely used to evaluate the antioxidant activities of various components in foods and beverages due to its applicability in lipid and aqueous phases (MacDonald-Wicks, Wood and Garg, 2006). The ABTS decolourisation assay is based on the production of a radical cation by the reacting ABTS (it has blue-green chromophore absorption) with potassium persulfate solution ($K_2S_2O_8$) prior to the addition of antioxidants as shown in Fig. 2.5. (Re et al., 1999). The antioxidant activity of various natural products (i.e. phenolic compounds or carotenoids) is determined by the decolourisation of the ABTS by measuring the reduction of the radical cation as the percentage inhibition of absorbance at λ=734 nm (Biglari, AlKarkhi and Easa, 2008). This method is inexpensive, easy to use, quick and stable in relation to pH, hence, it can be used to study the pH effect on antioxidant activity. However, the extra step is required to generate a free radical from ABTS salt; what is more, this method is not standardised, thus it is hard to compare values across laboratories (Zulueta, Esteve and Frígola, 2009).

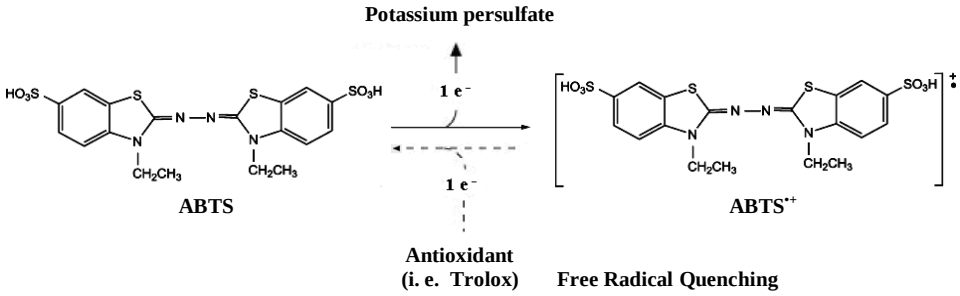


Fig. 2.5. Formation of the stable ABTS radical from ABTS with potassium persulfate

The ferric reducing antioxidant power (FRAP) assay is a recently developed direct test of the 'total antioxidant power'. FRAP assay in based on the ferric ion Fe^{3+}-TPTZ complex reduction to the ferrous Fe^{2+} form by an antioxidant under acidic (pH=3.6) conditions (Fig. 2.6.). Fe^{2+}-TPTZ is denoted by an intensive blue colour that can be monitored spectrophotometrically at λ=593 nm (Moon and Shibamoto, 2009). FRAP assay is fast, simple and sensitive, and its results are highly reproducible. FRAP assay is based on the hypothesis that redox proceeds so rapidly that the reactions are complete within 4 or 6 minutes. However, this is not always the case in practice. Some polyphenols react more slowly and require longer reaction times for detection (approx. 30 minutes). Besides, FRAP cannot detect species that act by radical quenching (e.g. H transfer), particularly, the SH group containing antioxidants (i.e. thiols, such as proteins or glutathione) (Phipps, Sharaf and Butterweck, 2007). As well as other assays, FRAP assay has been used in many studies dealing with fruits and vegetables (Szeto, Tomlinson and Benzie, 2002), essential oils (Chizzola, Michitsch and Franz, 2008) and cereals (Venneria et al., 2008).

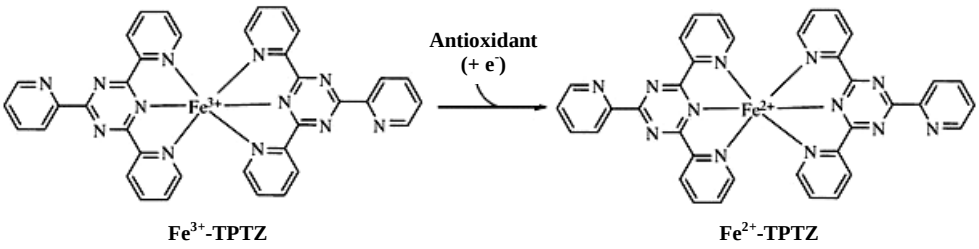


Fig. 2.6. Formation of the (Fe^{2+}-TPTZ) complex from the (Fe^{3+}-TPTZ) complex by an antioxidant

QUENCHER procedure. Although solvent extraction is the most frequently used technique for the isolation of plant antioxidant compounds, some antioxidatively active constituents may be strongly bound to other components in the plant matrix and cannot be extracted by various solvents. Yet, they can be released in the human intestinal tract during digestion. Several years ago, the QUENCHER (QUick, Easy, New, CHEap and Reproducible) method was developed which determines the antioxidant activity of the whole plant material (Serpen, Capuano, Fogliano and Gökmen, 2007). The values obtained by employing the QUENCHER method for insoluble food components demonstrated significant antioxidant capacity values which in some cases were remarkably higher than those determined by the traditional extraction procedures. Thus free functional groups on the surface of insoluble particles may also quench with the radicals (Gökmen, Serpen and Fogliano, 2009). The QUENCHER procedure has been applied for the evaluation of antioxidant activities of insoluble matter in different foods including nuts, vegetables, fruits, cereals and cereal-based products (Serpen, Gökmen and Fogliano, 2012).

2.2. Essential Oils in Plants

2.2.1. Biosynthetic Pathways of Essential Oils

There are two main groups of metabolites in nature: primary and secondary. Primary metabolites are compounds such as proteins, carbohydrates, lipids, and nucleic acids which are found in all living organisms. Secondary metabolites are found only in some species and are classified into terpenes, shikimates, polyketides, and alkaloids (Zuzarte and Salgueiro, 2015). Although terpenes are the most abundant in essential oils, certain plant species contain high quantities of shikimates, namely phenylpropanoids, which provide indispensable and significant odour and flavour to the plants.

Terpenes are a large and diverse class of naturally occurring organic compounds derived from the branched five-carbon isoprene (C_5) units; therefore, they have the general formula $(C_5H_8)_n$. They can be classified by the homologous chains of isoprene units '*n*' in their structure: monoterpenes ($C_{10}H_{16}$), sesquiterpenes ($C_{15}H_{24}$), diterpenes ($C_{20}H_{32}$), sesterpenes ($C_{25}H_{40}$), troterpenes ($C_{30}H_{48}$), tetraterpenes ($C_{40}H_{64}$) and polyterpenes $(C_5H_8)_n$ (Wu et al., 2012).

Terpenes in aromatic plants are usually synthesised via the mevalonate pathway and the non-mevalonate or deoxyxylulose phosphate pathway (Fig. 2.7.) (Eisenreich, Bacher, Arigoni and Rohdich, 2004).

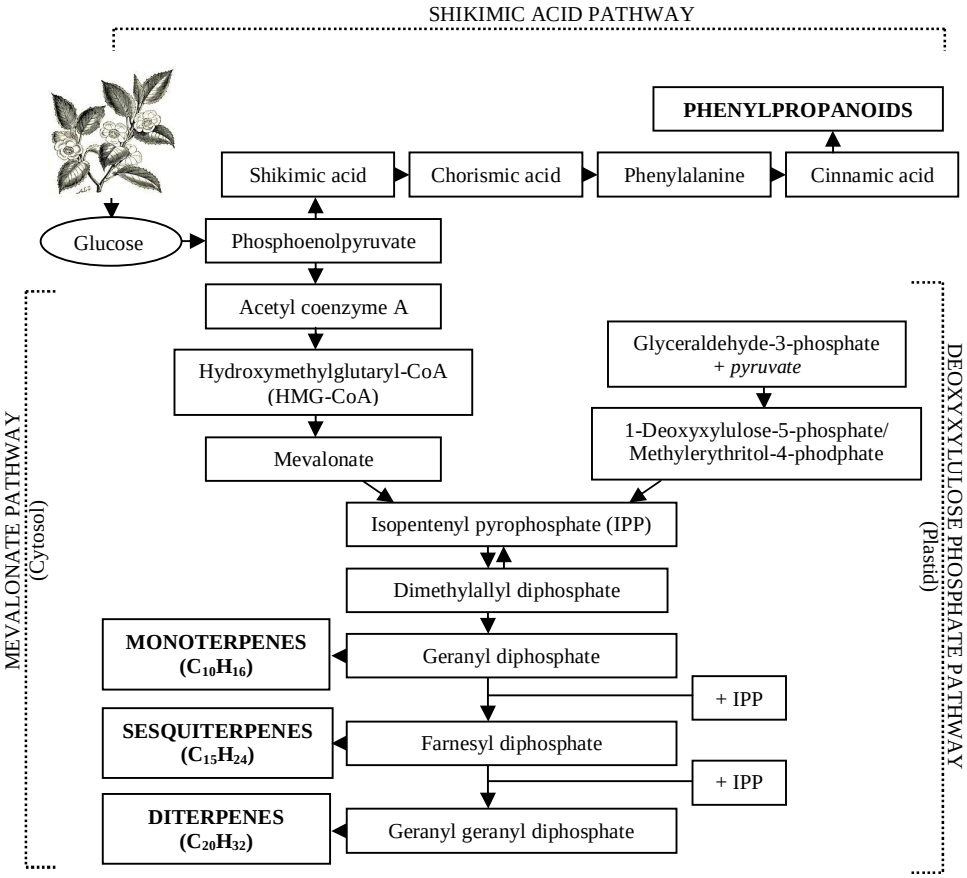


Fig. 2.7. Biosynthetic pathways of terpenoids and phenylpropanoids (Zuzarte and Salgueiro, 2015)

The biosynthesis of terpenes involves two main universal precursors: isopentenyl pyrophosphate (IPP) and dimethylallyl diphosphate (DMAPP). In the mevalonate pathway, IPP is formed through mevalonic acid which results from the condensation process of acetyl coenzyme A moieties. In the non-mevalonate or deoxyxylulose phosphate pathway, methylerythritol-4-phosphate (MEP) and 1-deoxyxylulose-5-phosphate (DOXP) are involved resulting from the condensation process of glyceraldehyde phosphate and pyruvate (Zuzarte and Salgueiro, 2015). The action of prenyltransferases then generates geranyl diphosphate (GPP), farsenyl diphosphate (FPP), and geranyl geranyl diphosphate (GGPP) which are the main precursors of monoterpenes ($C_{10}H_{16}$), sesquiterpenes ($C_{15}H_{24}$), and diterpenes ($C_{20}H_{32}$), respectively. Monoterpenes and sesquiterpenes are the main compounds found in essential oils. Although phenylpropanoids are not common constituents of plant essential oils, certain plant species contain abundant proportions of such compounds (Sangwan, Farooqi, Shabih and Sangwan, 2001). Phenylpropanoids always contain one or more C_6–C_3 units, the C_6 being a benzene ring. Many of the phenylpropanoids found in essential oils are phenols or phenol ethers such as

eugenol, methyl eugenol, myristicin, methyl cinnamate, chavicol, dillapiole, anethole, estragole, etc. Phenylpropanoids are synthesised via the shikimic acid pathway (Fig. 2.7.) (Dixon et al., 2002). In the shikimic acid pathway, shikimic acid is synthesised from phosphoenolpyruvate and erythrose 4-phosphate. The elimination of one shikimic acid alcohol ring and the reaction with phosphoenol pyruvate yields chorismic acid. Chorismic acid forms the skeleton of phenylpropionic acid. Amination and reduction of the ketone function produces phenylalanine while the reduction and elimination leads to cinnamic acid. Therefore, the main precursors of the biosynthesis of phenylpropanoids are cinnamic and *p*-hydroxycinnamic acids originating from phenylalanine and tyrosine, respectively (Zuzarte and Salgueiro, 2015).

2.2.2. Sources of Essential Oils

The worldwide demand of EOs has recently increased. Presently, EOs are being produced from more than 2000 species of various plants. However, only about 100 species of plants have economic significance in the world of EOs. Plant EOs are usually produced commercially from several botanical sources, many of which are members of the mint family *Lamiaceae*, *Apiaceae* and *Rutaceae* (Devi, Chakrabarty, Ghosh and Bhowmick, 2015).

EOs are aromatic oily liquids obtained from different parts of a plant, for instance, buds, flowers, seeds, twigs, leaves, bark, herbs, fruits, fruits rinds, roots and wood. EOs are usually accumulated in secretary cells, channels, cavities, and epidermic cells (Burt, 2004). The *Lamiaceae* herb family constitutes one of the richest EO bearing plant family (Pandey, Singh and Tripathi, 2014). The EO is generally composed of complex mixtures of monoterpenes and sesquiterpenes. The most abundant components of many sage species EOs are 1,8-cineole (6.0–14.0%), β-thujone (2.0–10.0%) and α-thujone (4.0–5.0%). 1,8-Cineole (43.6%) and camphor (12.3%) are the major constituents of EOs from rosemary, menthol (35.0–45.0%) and menthone (10.0–30.0%) from peppermint, thymol (45–75%) from garden thyme, carvacrol (37.7%) and p-cymene (25.9%) from savory, linalyl acetate (50.3%) and linalool (35.5%) from lavender, methyl chavicol and 1,8-cineole from sweet basil (Ramasubramania Raja, 2012; Benchaar et al., 2008; Koul, Walia and Dhaliwal, 2008; Burt, 2004; Chao, Young and Oberg, 2000). Although toxic components have been described as having presence in some *Lamiaceae* plants, the importance of many *Lamiaceae* family members to the EO industries and the culinary world has been explored for more than 70 years (Manosroi, Dhumtanom and Manosroi, 2006; Martins et al., 1999). For instance, EOs recovered from the family of *Lamiaceae* species have been used against different diseases, such as bronchitis and intestinal disorder.

Angelica, coriander, dill and cumin belonging to the family of *Apiaceae* are also a good source of EOs. The most abundant components of angelica are α-pinene (24.7%) and limonene (12.9%). Coriander contains a high amount of linalool (up to 72.0%), while dill is the best source of limonene (50.9%), dillapiole (36.6%) and carvone (20.3%). Cumin EO also contains numerous chemical compounds, and the major ones among them are *p*-mentha-l,4-dien-7-al (34.3%) and cuminaldehyde

(23.8%) (Chao, Young and Oberg, 2000). The health benefits of cumin EO include its ability to aid digestion, improve immunity and treat insomnia, asthma and bronchitis.

EOs of the genus *Citrus* belonging to the family *Rutaceaea* develop another large sector of the world production of essential oils. *Citrus* EOs contain 85–99% volatile and 1–15% non-volatile components. Monoterpenoids make up 97% of the citrus EOs composition with aldehydes, alcohols, and esters being the lowest percentage components ranging from 1.8% to 2.2% (Fisher and Phillips, 2008). The major chemical component of citrus EOs is monoterpene limonene, ranging from 32 to 98%. Sweet orange usually contains 68–98% of limonene, tangerine reaches 85–93%, grapefruit contains 88–95%, lemon features 45–76% and bergamot contains 32–45%, respectively (Svoboda and Greenaway, 2003). Although linalool and citral are thought to be the most potent aroma compounds in citrus fruits, yet they do not exceed 3% (Blanco Tirado, Stashenko, Combariza and Martinez, 1995).

Apart from the aromatic plants of *Lamiaceae, Apiaceae* and *Rutaceae* families there are many others which contain a high content of EOs (Table 2.2).

Table 2.2. Other aromatic plants and their main components of EOs

Family/ EO	Main components	References
Cupressaceae		
Cypress	α -Pinene (40.9%), δ-3-carene (15.2%).	Chao, Young and Oberg, 2000.
Juniper	α -Pinene (33.7%), sabinene (27.6%).	Benchaar et al., 2008.
Lauraceae		
Cinnamon	(E)-Cinnamaldehyde (77.1%), eugenol (7.2%).	Benchaar et al., 2008.
Myrtaceae		
Clove	Eugenol (83.63%), β -caryophyllene (12.39%).	Murbach Teles Andrade, Barbosa, Probst and Fernandes Júnior, 2014.
Eucalyptus	Citronellal (72.8%), citronellol (14.5%).	Benchaar et al., 2008.
Nutmeg	Sabinene (27.1%), α -pinene (26.0%) β-pinene (15.0%).	Chao, Young and Oberg,2000.
Tea tree	Terpinene-4-ol (40.1%), γ-terpinene (23.0%), α-terpinene (10.4%).	Benchaar et al., 2008.
Pinaceae		
Cedar	Widreno (27.75%), α -cedrol (22.14%), α-cedrenus (19.84%).	Murbach Teles Andrade, Barbosa, Probst and Fernandes Júnior, 2014.
Pepper	Sabinene (19.4%), limonene (17.5%), β-caryophyllene (1 4.7%)	Chao, Young and Oberg, 2000.
Pine	α-Pinene (38.4%), δ-3-carene (21.6%), β-pinene (12.5%).	Chao, Young and Oberg, 2000.
Rosaceae		
Rose	Citronellol and nerol (52.4%), nonadecane and nonadecene (15.9%), geraniol and neral (11.4%).	Chao, Young and Oberg, 2000.
Zingiberaceae		
Cardamon	α-Terpinyl acetate (44.8%), 1,8-cineole (33.7%).	Chao, Young and Oberg,2000.
Ginger	β-Bisabolene (22.1%), α-curcumene (14.5%), camphene (14.1%).	Benchaar et al., 2008.

2.2.3. Uses of Essential Oils

The interest in EOs and their application has been amplified during the recent years by the increasingly negative consumer perception of synthetic preservatives. Moreover, 'naturalness' has become one of the most important factors pertaining to the consumer's preferences in choosing various products for human consumption. Essential oils (EOs) are considered to be secondary volatile metabolites which are characterised by a strong odour and are formed by aromatic plants (Bakkali, Averbeck, Averbeck and Idaomar, 2008). Flavor and fragrance industries consume approximately 90% of the global production of EOs, which are mostly used in the cosmetics, perfumery, medicine, soft drinks and various foods (Adams, 2007). In addition, more than 25% of the pharmaceutical drugs prescribed worldwide are derived from the plant sources (Ahmadi and Mirza, 1999).

The genus *Salvia* (sage) is one of the largest and most important aromatic genera of the *Lamiaceae* family. *Salvia* species are widespread plants in many countries. Clary sage (*S. sclarea*) is usually cultivated for its aromatic properties and the widely used essential oil (Lawrence, 1979); meadow sage (*S. pratensis*) is usually used in cosmetics and perfumery and possesses some medicinal properties. Some species of *S. glutinosa* were tested in enzyme-dependent and enzyme-independent systems of lipid peroxidation and found to be effective (Zupkó et al., 2001). However, reports on the antioxidant properties of these plants are very scarce (Tepe, Sokmen, Akpulat and Sokmen, 2006). Lavender is an important source of the thoroughly studied EO which has long been used in the production of perfume and in aromatherapy as a pleasant fragrance or as an antimicrobial agent. Basil, thyme and rosemary are also aromatic herbs whose EOs can be used as aroma additives in food, pharmaceuticals, and cosmetics (Javanmardi, Khalighi, Kashi, Bais and Vivanco, 2002). Traditionally, EOs of basil and rosemary have been used for the treatment of headaches, coughs, constipation, worms, and kidney malfunction. The EO of thyme also possesses antioxidative, antiseptic and antimicrobial properties (Baranauskienė, Venskutonis, Viškelis and Dambrauskienė, 2003). It is usually derived from leaves of *Mentha piperita* L. Peppermint oil is extensively used in medicine, especially in phytotherapy for the external treatment of various human diseases e.g. various pain conditions including headache syndromes or mild bacterial or fungal infections of the skin (Schuhmacher, Reichling and Schnitzler, 2003). Recently, strong *in vitro* evidence was obtained that the EO of peppermint can act as an antibacterial agent against gram-positive and gram-negative bacteria along with antifungal properties (Ali et al., 2015).

The EOs of angelica, coriander, dill and cumin are also denoted by application. The EO of angelica is used for treatment of anemia, hypertension, asthma, and cardiovascular diseases. These effects are thought to be due to a number of components of the EO, particularly ligustilide. Ligustilide, butyl phthalide and butylene phthalide are the major components of the angelica EO (Chen et al., 2004). Dill EO is used in the food industry for flavouring and seasoning. The seed EO of dill is extensively used in perfumery and to aromatise soaps as well as a substitute for caraway oil (Lawless, 1995). The EOs of coriander and cumin are extensively used as flavoring agents in all types of food products including alcoholic beverages,

candy, pickles, meat sauces and seasonings (Allahghadri et al., 2010; Burdock and Carabin, 2009). Coriander and cumin EO is reported to possess antimicrobial properties against selected pathogenic and saprophytic microorganisms (Burdock and Carabin, 2009).

The genus *Citrus* of the family *Rutaceae* includes more than 17 species distributed throughout the tropical and temperate regions. Although their fruits are mainly used for dessert, they are still renowned for the economic value of their EOs due to their aromatic compounds (Minh Tu, Thanh, Une, Ukeda and Sawamura, 2002). Many authors have reported antimicrobial, antifungal, antioxidant and radical-scavenging properties of *Citrus* EOs and, in some cases, referred to direct food related application as well (Soković and Griensven, 2006; Madsen and Bertelsen, 1995). For instance, the EO of orange is used to add the orange aroma to such products such ice creams, cakes, carbonated drinks and air-fresheners. Recently, other applications of limonene, major component of orange EO, as a green solvent for extraction of fats and oils from olive seeds in combination with microwave energy have found their use in practice (Virot, Tomao, Ginies, Visinoni and Chemat, 2008). The EOs of tangerine and bergamot are mainly used as antiseptics, antispasmodics and sedative diuretics. The antiseptic qualities of tangerine, bergamot and other *Citrus* fruits have been actually recognised since antiquity (Ali et al., 2015).

2.3. Techniques for the Extraction of Bioactive Compounds

The extraction of bioactive compounds from various plant materials is the first step in the utilisation of phytochemicals in the food, fragrance and drug industries. Usually, fresh, frozen or dried plant material can be used as a source of plant material for the isolation of various bioactive compounds. Before the extraction process, plant material is usually treated by freeze-drying, air-drying or oven-drying. These procedures allow higher diffusion of extracts inside the extracted samples. Sejali and Anuar (2011) indicated that higher amounts of phenolics are extracted from air-dried plant material than from oven-dried material. Dried plant materials are ground or milled so that to obtain a certain particle size, whereas liquid samples are treated by centrifugation, filtration and purification (Khoddami, Wilkes and Roberts, 2013). Higher extraction yields of bioactive compounds are achieved by milling the sample into smaller particle sizes, thereby improving the extraction process (Gião, Pereira, Fonseca, Pintado and Malcata, 2009).

Complete extraction of bioactive compounds is the next step after sample preparation. Different extraction techniques such as solid-phase microextraction, microwave-assisted extraction or solid-phase extraction can be used; however, solvent extraction is the most commonly used procedure of the preparation of extracts from plant materials due to their ease of use and the wide applicability. The range of the extracted compounds depends on the conditions and solvents of the extraction process. For instance, lipophilic compounds are better extracted with non-polar solvents, such as hexane and dichlormetane, while for the extraction of hydrophilic compounds, methanol, ethyl acetate and acetone could be used. It should also be noted that essential oils are also a good source of bioactive compounds. An

essential oil is a volatile mixture of organic compounds obtained from various plant materials by physical means including the method of steam distillation or simultaneous steam distillation-extraction (SDE) most commonly used for the production of essential oils (Al-Reza, Rahman, Parvin, Rahman and Rahman, 2011). However, nowadays, there is growing interest in natural bioactive compounds obtained by using 'green' processes. The application of high pressure extraction techniques such as supercritical fluid extraction (SFE) and pressurised liquid extraction (PLE) by using environmentally and food-friendly solvents (e.g. carbon dioxide, ethanol and water) also provide additional benefits in processing botanicals for isolating valuable functional ingredients.

Pressurised Liquid Extraction

Pressurised liquid extraction (PLE), also known as accelerated solvent extraction (ASE) (Fig. 2.8.), is a relatively new technology applied for the extraction of phytochemicals by using solvents at temperatures above their boiling points (usually between 50°C and 200°C, and at pressures between 10 and 15 MPa). When 100% water is used as a solvent, and it is heated up to 200°C, PLE is generally called subcritical water extraction (SWE) (Pronyk and Mazza, 2009). Thus PLE and SWE methods are developed at elevated temperature and pressure conditions operating above the normal boiling point of the relevant liquids. The high temperature of the extraction process increases the diffusion rate, the solubility and the mass transfer of analyte, and decreases the viscosity and the surface tension of the solvent thus allowing better penetration into the matrix. The high pressure forces the solvent into the matrix pores and hence should facilitate the extraction of analytes (Kaufmann and Christen, 2002). PLE and SWE have been developed as novel methods thus serving as an alternative to the current extraction methods, such as Soxhlet, maceration or percolation and offering advantages with respect to solvent consumption, time, yields and the reproducibility of the extraction.

A main drawback of PLE is its requirement of special instrumentation in order to get relatively high pressure together with high temperature. Furthermore, there is no available data on the solubility of compounds in the solvent at the temperature and pressure employed in PLE (Raut et al., 2015). Another main drawback of PLE for all the classes of analytes is that wet samples always require the drying step prior to analysis. Sodium sulfate usually serves this purpose, but the amount of the drying agent that can be used is often limited by the volume of the extraction cell (Schantz, 2006). However, PLE has been successfully and extensively applied to the extraction of bioactive compounds from rye and wheat bran (Povilaitis, Šulniūtė, Venskutonis and Kraujalienė, 2015), herbs and spices (Hossain, Barry-Ryan, Martin-Diana and Brunton, 2011), fruit and vegetables (Jäpelt and Jakobsen, 2016).

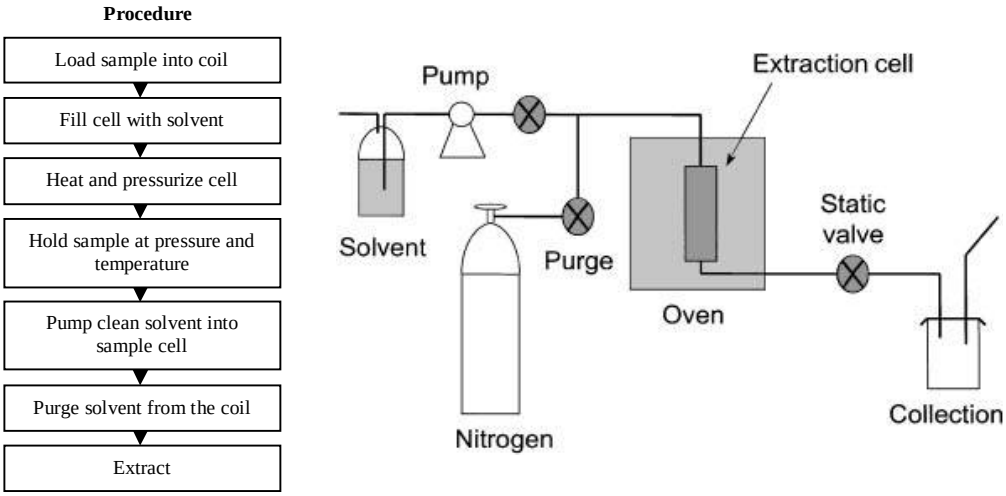


Fig. 2.8. Scheme of the accelerated solvent extraction (ASE) system (Richter, Jones, Ezzell and Porter, 1996)

Supercritical Fluid Extraction

Supercritical fluid extraction (SFE) has become one of the most popular 'green' extraction techniques (Fig. 2.9.) using supercritical fluid (SF) as the extracting solvent above its critical pressure and temperature. The most commonly used extracting agent is carbon dioxide due to its low toxicity, low cost and favourable critical parameters (P_c=74.8 atm and T_c=31.1°C) (Herrero, Cifuentes and Ibañez, 2006). A review of recent literature reveals that SFE-CO_2 is a highly attractive method for the extraction of essential oils and other nonpolar thermo labile compounds such as tocols, terpenoids, carotenoids, fatty acids and triglycerides from various plant materials (Lang and Wai, 2001). The extraction efficiency of polar compounds with CO_2 can be improved by the addition of small quantities of polar organic solvents used as modifiers. The modifiers increase the solubility of analytes preventing them from adsorption on the active sites of the sample matrix. Ethanol is the most commonly preferred modifier because it is comparatively cheap and has the 'GRAS' (Generally Recognised As Safe, according to the classification of *American Food and Drug Administration*) status. SFE is always performed in the absence of both air and light; processes of degradation and oxidation are significantly reduced in comparison with other extraction techniques (Dai and Mumper, 2010). However, the main drawback of SFE is that the requirement for a high pressure increases the cost compared to the conventional liquid extraction. Moreover, solvents which are usually used to extract compounds are non-polar, so they can dissolve only non-polar compounds. One disadvantage of using a modifier (mostly ethanol) is that it can cause poor selectivity, i.e. a higher amount of impurities containing, such as waxy material and chlorophylls, may be extracted with the desired compounds (Lang and Wai, 2001). Another main drawback of SFE is that the equipment needs the clean-up step prior the extraction, which takes several hours (Luque de Castro and Jiménez-Carmona, 2000).

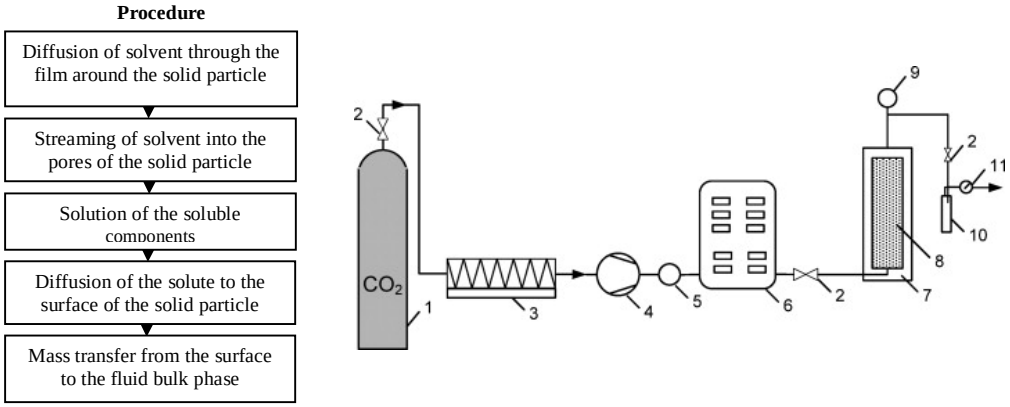


Fig. 2.9. Scheme of the supercritical fluid extraction (SFE) system: 1 – CO_2 tank; 2 – valve; 3 – cooler; 4 – high pressure pump; 5 – pressure gauge; 6 – controller of temperature and flow rate; 7 – heating jacket; 8 – extraction vessel; 9 – temperature gauge; 10 – extract containing vial; 11 – flow meter (Kemzūraitė, Venskutonis, Baranauskienė and Navikienė, 2014)

Simultaneous Distillation-Extraction

Among the several techniques which have been developed to isolate volatile compounds from various plant materials, simultaneous distillation-extraction (SDE) introduced in 1964 by Likens and Nickerson is one of the most widely employed methods (Fig. 2.10.). The Likens-Nickerson method has been successfully applied for the extraction of aroma compounds (Blanch, Reglero and Herraiz, 1996), essential oils (Eikani, Golmohammad, Rowshanzamir and Mirza, 2005; Stashenko, Jaramillo and Martínez, 2004) and other volatile products (Barták, Frnková and Čáp, 2000) from different matrices. Simultaneous distillation-extraction in the Likens-Nickerson apparatus (L-N) is usually considered to be superior to the classical methods, such as distillation or solvent extraction, because this technique combines steam distillation together with continuous extraction with a low-boiling solvent (petroleum ether or diethyl ether) (Chaintreau, 2001). Moreover, this one-step extraction technique requires low volume of the extracting solvent (thereby minimising the cost), allows rapidly concentrating the volatiles and carrying out the analysis without the sample clean-up step. The required time, the fact that organic solvents are used, the artefacts generation as well as the possibility of thermal degradation of compounds are the most prominent disadvantages of the SDE approach. Moreover, the loss of valuable water-soluble volatile compounds is also a major drawback (Teixeira, Mendes, Alves and Santos, 2007).

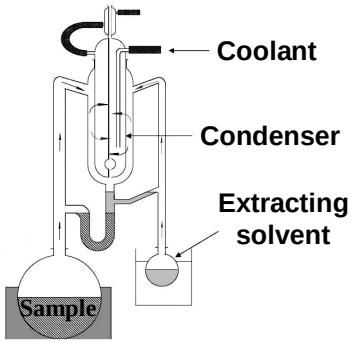


Fig. 2.10. Likens-Nickerson simultaneous distillation-extraction apparatus

2.4. Botanical Characterisation, Phytochemistry and Health Benefits of the Genus *Salvia*

2.4.1. Botanical Description

Botanicals are among the most valuable sources of bioactive compounds which could be used for the development and commercialisation of high added value functional ingredients, such as dietary antioxidants. However, considering the abundance of plant species, many of them inevitably remain under-investigated, and this fact encourages the search of new sources and the evaluation of new natural substances including dietary antioxidants. Therefore, the interest in a more systematic and comprehensive characterisation of less extensively studied plant species has been regularly increasing.

The genus *Salvia* (sage) is one of the most important and largest medicinal and aromatic genera of the *Lamiaceae* or mint family with about 900 species which is widespread throughout the world: in North America (20 species), Central (300 species) and South America (220 species), in Asia (90 species), throughout Europe, especially around the Mediterranean (250 species), and is also represented by a few examples in South Africa (30 species) (Whittlesey, 2014). The genus name, *Salvia*, is variously attributed to derivation from the Latin *salvus*, meaning "safe", or *salveo*, meaning "to heal", or *salvo*, meaning "to save".

Salvia is a perennial plant native to the Mediterranean region, especially in the region of the Adriatic Sea. In Europe, *Salvia* was introduced into cultivation during the 19th century. It is hard to describe *Salvia* as an industrial crop because its worldwide production is less than 25,000 kg per year (Kintzios, 2000). The plants in the genus *Salvia* are aromatic, with soft, greyish green leaves. Their flower colours vary depending on the species; they may be purple, pink, blue, or white. Sage usually blooms from May to July. This plant prefers full sun and well-drained soils. Sage grows up fairly rapidly to a height of 60–90 cm. The exclusivity of *Salvia* is that the corolla tube of the petals is two-lipped, the lower and the upper lip being significantly different from each other. Moreover, the arrangement of the two stamens is also a key identifier of the genus *Salvia* (Whittlesey, 2014).

Since ancient times, the *Salvia* species has been used as medicinal, aromatic and spicy plants, and many of them have been widely studied and reported to

accumulate high amounts of essential oils, strong phenolic antioxidants as well as other valuable constituents. Some species of the genus *Salvia* are also denoted by economic importance because they have been used as flavouring agents in cosmetics and perfumery. For example, clary sage (*S. sclarea*) and common sage (*S. officinalis*) are commercially cultivated, and their essential oils are widely used as a flavouring. Meadow sage (*S. pratensis*) is used in cosmetics and has some medicinal properties (Wu et al., 2012). *Salvia* has also been traditionally used in the food, drug and fragrance industry (Capecka, Mareczek and Leja, 2005); they were reported as biosynthesising various useful natural constituents, including terpenoids and flavonoids (Topçu and Ulubelen, 2007) and other phenolic compounds (Lu and Foo, 2002). Although culinary and medicinal uses of several *Salvia* spp. plants, e.g. *S. fruticosa* (Greek sage or Greek oregano), *S. officinalis* (garden or common sage) and *S. pomifera* (fruit or apple sage) can be traced back to the Ancient Greece, there is still remarkable interest concerning more detailed knowledge regarding their phytochemicals (Kintzios, 2000).

2.4.2. Bioactive Compounds in Selected *Salvia* spp. Plants

The flowering plant family of *Lamiaceae* is very important since it is a highly diverse and rich source of bioactive compounds which can be divided into essential and non-essential compounds (polyphenols) (Gryszczynska et al., 2015). Usually, these compounds occur in nature, are part of the food chain, and can be shown to exert an effect on human health (Biesalski et al., 2009). This chapter focuses on the characterisation of bioactive compounds of 10 different *Salvia* spp. plants, namely *S. amplexicaulis*, *S. austriaca*, *S. forsskaolii*, *S. glutinosa*, *S. nemorosa*, *S. officinalis*, *S. pratensis*, *S. sclarea*, *S. stepposa* and *S. verticillata* (Fig. 2.11.). *S. officinalis* and *S. sclarea* are the most thoroughly studied *Salvia* spp. plants, whereas information on other *Salvia* spp. plants selected in this study is rather scarce.

S. officinalis *S. pratensis* *S. sclarea* *S. verticillata*

Fig. 2.11. Examples of plants used in the present study*

Volatile Compounds

The chemical composition of *S. officinalis* (Delamare, Moschen-Pistorello, Artico, Atti-Serafini and Echeverrigaray, 2007; Raal, Orav and Arak, 2007; Mirjalili, Salehi, Sonboli and Vala, 2006; Santos-Gomes and Fernandes-Ferreira, 2003; Perry et al., 1999; Chalchat, Michet and Pasquier, 1998) and *S. sclarea* (Kuźma et al., 2009; Džamić et al., 2008; Schmiderer, Grassi, Novak, Weber and

*Copyright of photographs belongs to Vilnius University Botanical Garden.

Franz, 2008; Carrubba, la Torre, Piccaglia and Marotti, 2002; Pitarokili, Couladis, Petsikos-Panayotarou and Tzakou, 2002) essential oils (EOs) has been widely studied. The essential oil composition of *S. officinalis* was found to be the richest in oxygenated monoterpenes, with its range varying from 59.43 to 70.68% (Said-Al Ahl, Hussein, Gendy and Tkachenko, 2015). The main constituents identified in *S. officinalis* were *cis*-thujone (17.4%), *α*-humulene (13.3%), 1,8-cineole (12.7%), *β*-caryophyllene (8.5%) and borneol (8.3%) (Lima et al., 2004). These findings are also in agreement with the previously reported data by Marino, Bersani and Comi (2001), except for manool. Previously, manool was reported in relatively large percentage in *S. officinalis* originating from Cuba (15%) (Pino, Estarrón and Fuentes, 1997).

The chemical composition of *S. sclarea* EO has also been reported. It was discovered that *S. sclarea* EO accumulates a high amount of linalool (24.5%), linalyl acetate (20.9%), geranyl acetate (6.3%), (E)-*β*-ocimene (5.7%), and caryophyllene oxide (5.3%) (Fraternale et al., 2005). A different distribution of the main constituents in EOs isolated from different anatomical parts of *S. sclarea* was determined by Farakaš, Hollá, Tekel, Mellen and Štefánia (2005): linalool (18.9%), sclareol (15.7%), and linalyl acetate (13.7%) were the most abundant in the EO distilled from inflorescences, whereas gemacrene D (28.8%), bicyclogermacrene (12.5%), spathulenol (10.1%) and β-caryophyllene (6.2%) were the main constituents in the EO of the leaf. These differences may stem from genetic and environmental factors as well as post-harvest and storage conditions.

The reports on many other *Salvia* spp. plants are relatively scarce. For instance, only one scholarly article is available on the EO composition of *S. pratensis*: Anačkov et al. (2009) reported that the main constituents identified in the *S. pratensis* EO were *E*-caryophyllene (26.4%), Z-*β*-farnesene (6.0%), *β*-cubebene (5.6%), *epi*-bicyclo sesquiphellandrene (5.6%) and germacrene B (3.4%). The EO composition of *S. verticillata* was found to be the richest in β-pinene (21.4%) and 1,8-cineole (16.1%) (Askun, Baser, Tumen and Kurkcuoglu, 2010). Veličković et al. (2012) reported the EO composition of *S. austracia* and *S. amplexicaulis* from Serbia; different main constituents were identified. Spathulenol (17.1%) was the main compound in *S. austriaca,* whereas germacrene D (21.0%) was dominant in *S. amplexicaulis*. The chemical composition of *S. glutinosa* originating from the Southeast Region of Serbia has been also studied by Velickovic, Ristic, and Velickovic (2003). The main constituents identified in *S. glutinosa* were caryophyllene oxide (28.9% in leaf), humulene epoxide II (13.8% in leaf), β-caryophyllene (9.0% in flower) and α-humulene (5.9% in flower). The volatiles present in *S. nemorosa* were also investigated by Chizzola (2012). It was found that the leaf of *S. nemorosa* in terms of EO accumulates a high amount of *β*-caryophyllene (14–41%), germacrene D (14–38%) and caryophyllene oxide (5–20%). To the best of our knowledge, there have been no publications covering the composition of volatiles in *S. forsskaolii* and *S. stepposa*.

Phenolic Compounds

Plants produce different phenolic compounds through the mevalonate, shikimate and phenyl propanoid pathways. Numerous chemical studies have

revealed that *Salvia* spp. plants are an outstandingly rich source of phenolic compounds, with an excess of 160 phenolic compounds identified, some of which are unique to the genus (Lu and Foo, 2002). *S. officinalis* is the most widely studied sage species. Rosmarinic acid and phenolic diterpenes (e.g. carnosic, rosmarinic and caffeic acids) were reported in it as the main components possessing strong antioxidant, radical-scavenging and antibacterial activities capacity (Farhat, Chaouch-Hamada, Sotomayor, Landoulsi and Jordán, 2014; Upadhyay and Mishra, 2014; Cvetkovikj et al., 2013; Walch, Tinzoh, Zimmermann, Stühlinger and Lachenmeier, 2011; Zimmermann, Walch, Tinzoh, Stühlinger, and Lachenmeier, 2011). However, reports on many other *Salvia* spp. plants selected for our study are rather scarce. The main polyphenol carboxylic acids (rosmarinic, caffeic *p*-coumaric and chlorogenic) and flavonoids (luteolin, luteolin-7-glucoside, apigenol and apigenin 7-glucoside) were determined in *S. austriaca*, *S. glutinosa*, *S. nemorosa*, *S. officinalis*, *S. pratensis*, and *S. verticillata* collected from spontaneous and cultivated populations; *S. officinalis*, *S. verticillata* and *S. glutinosa* exhibited the highest content of these compounds (Coisin et al., 2012). Bandoniene, Murkovic and Venskutonis (2005) reported rosmarinic acid as the main radical scavenger in polar extracts isolated from the leaves of *S. officinalis*, *S. glutinosa* and *S. sclarea*; the highest content of rosmarinic acid was found in *S. glutinosa* and *S. sclarea*. However, the previously published results on *Salvia* spp. are difficult to compare due to different extraction or sample preparation procedures. To the best of our knowledge, so far, there have been no publications on the phytochemical composition of *S. amplexicaulis*, *S. forsskaolii* and *S. stepposa*.

2.4.3. Application of *Salvia* in Medicine and Food

The positive benefits of *Salvia* (sage) to health are reputed throughout the times of Ancient Rome and the Middle Ages. Old English provebs such as "*He that would live for aye [ever], must eat sage in May*" or "*Why should a man die whilst sage grows in his garden?*" epitomise the impact of the sage on the society at the time. Apart from general scientific curiosity, the understanding of the chemistry of *Salvia* plants is very important for several commercial industries because these plants are broadly used in cosmetics, aromatherapy, medicine and food preparations mainly as aromatic ingredients.

Sage is one of the most appreciated herbs for its rich EO and its plethora of biologically active compounds extensively used in folk medicine. Sage has been traditionally used for the treatment of digestive issues, bronchitis, cough, asthma, depression, mouth and throat inflammations, angina, excessive sweating, skin diseases, and many other diseases and health issues. Some of these diseases have been recently extensively reviewed by Miroddi et al. (2014). The EOs of *Salvia* are used in the treatment of a wide range of diseases including the nervous system, heart and blood circulation, the digestive system, the respiratory system, metabolic and endocrine diseases (Baricevic and Bartol, 2000). Moreover, the EO of sage has been shown to possess antispasmodic, antiseptic, and astringent properties. Sage is also one of the most important sources of natural antioxidants which play a very important role in protecting the body against the oxidative stress and free radical

induced damages causing various ailments such as heart diseases, brain dysfunction, diabetes or weakened immune system (Yadav and Mukundan, 2011).

In a study conducted on the antioxidant activity of many plant extracts including sage (*S. officinalis*), it was found that terpenoids, flavonoids and other phenolic compounds are mainly responsible for the antioxidant and free radical scavenging effects of this plant (Bandonienė, Venskutonis, Gruzdienė and Murkovic, 2002). Various phenolic compounds, such as rosmarinic, carnosol, carnosic acids, rosmadial, and methyl carnosate, can stimulate endogenous antioxidant defense systems or scavenge reactive species (Sá et al., 2009). Sage is also used extensively in the food industry as flavoring agents and spices, especially in meat, soups and sauces of baked fish and salad. Numerous studies have demonstrated that plant antioxidants may play a role of natural additives preventing the oxidation of food components, particularly unsaturated fats, and health beneficial compounds acting in various ways, e.g. as scavengers of the excessive reactive oxygen species which may damage biologically important molecules (Lobo, Patil, Phatak and Chandra, 2010; Pham-Huy, He and Pham-Huy, 2008).

3. Materials and Methods

3.1. Research Objects

Dried *Salvia* spp. plant materials were obtained from Kaunas Botanical Garden of Vytautas Magnus University (Kaunas, Lithuania). The plants were collected in 2013 at the beginning of plant flowering. 10 different *Salvia* spp. plants, namely *S. amplexicaulis*, *S. austriaca*, *S. forsskaolii*, *S. glutinosa*, *S. nemorosa*, *S. officinalis*, *S. pratensis*, *S. sclarea*, *S. stepposa* and *S. verticillata* have been selected for further analysis. *S. officinalis* and *S. sclarea* are the most thoroughly studied *Salvia* spp. plants, whereas information about other *Salvia* spp. plants selected for this study is rather scarce.

3.1.1. Reagents

Ethanol (96%) was purchased from Stumbras MV Group Production (Kaunas, Lithuania); diatomaceous earth was acquired from Dionex (Sunnyvale, CA, USA); methanol was sourced from Chempur (Piekary Śląskie, Poland); Trolox, ABTS, DPPH, AAPH, Folin-Ciocalteu's reagent (2 M), gallic acid, microcrystalline cellulose (20 μm), acetonitrile, dichloromethane, formic acid, methanol, caffeic, carnosic and rosmarinic acids, carnosol, diosmetin, ethyl gallate, rhamnetin, rutin hydrate (95%) and quercetin 3-glucuronide were acquired from Sigma-Aldrich (St. Louis, MO, USA); acid-free fluorescein was obtained from Fluka Analytical (Steinheim, Switzerland); carbon dioxide (99.9%) was sourced from Gaschema (Jonava, Lithuania); apigenin-7-*O*-β-D-glucuronide and luteolin-7-*O*-β-D-glucuronide were produced by HWI Analytik GmbH (Ruelzheim, Germany); 3',4',5,7-tetraxydroxy-3-methoxyflavone was acquired from Extrasynthese (Genay, France); apigenin, hyperoside and luteolin were sourced from Carl Roth GmbH (Karlsruhe, Germany); DL-α-tocopherol (99.9%), rac-β-tocopherol (90+%), γ-tocopherol (99%) and δ-tocopherol (95.5%) were bought from Supelco Analytical (Bellefonte, PA, USA); diethyl ether (ACS, ≥99.9%) and Na_2SO_4 (≥99.0%, anhydrous) were purchased from Lach Ner (Neratovice, Czech Republic); pentane (for residue analysis, ≥99.0%) was acquired from Sigma-Aldrich (Steinheim, Germany).

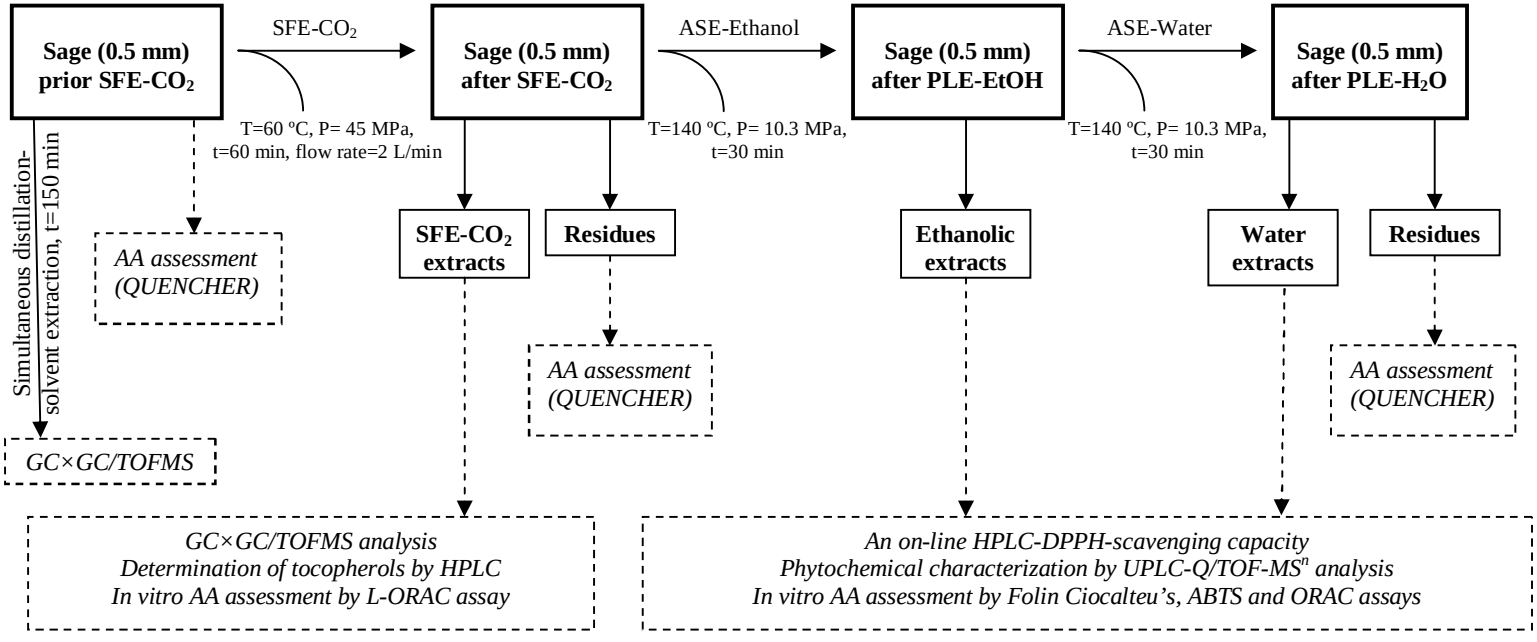


Fig. 3.1. Thesis research scheme

3.2. Sample Preparation and Extraction

Dried plants were ground in an ultra centrifugal mill *ZM 200* (Retsch, Haan, Germany) by using a 0.5 mm hole size sieve. SFE-CO_2 was performed with 99.9% CO_2 in the Helix system (Applied Separation, PA, USA) from 10 g of ground plant placed in a 50 cm^3 cylindrical extractor of 14 mm inner diameter and 320 mm length. The conditions for extraction were set as follows: time: 60 minutes, pressure: 45 MPa, temperature: 60°C, flow rate of CO_2: 2 L/min. A static time of 10 minutes was included in the total extraction time. These parameters were selected based on previous reports demonstrating high extract yields (Kemzūraitė, Venskutonis, Baranauskienė and Navikienė, 2014; Al-Asheh, Allawzi, Al-Otoom, Allaboun and Al-Zoubi, 2012). The CO_2 extracts were collected in glass vials, weighed, transferred into opaque bottles and kept refrigerated until further handling.

After SFE-CO_2, 5 g of plant material residue was mixed with diatomaceous earth (3:1) in a 10 mL stainless-steel cell and further extracted in a Dionex accelerated solvent extractor *ASE 350* (Dionex, Sunnyvale, CA, USA) at 140°C temperature and 10.3 MPa pressure for 30 min. Diatomaceous earth was used both as dehydrating or/and dispersing agent during the extraction. The extraction was performed sequentially by using the solvents of increasing polarity, ethanol (96%) and water. Ethanol was removed in a *Rotavapor R-210* instrument (Büchi, Flawil, Switzerland), whereas the water extracts were freeze-dried. The extracts isolated with CO_2, ethanol and water are further referred by the abbreviations composed of the first letters of the plant species and first letter of the solvent, *C*, *E*, and *W*, respectively: *S. amplexicaulis* Lam. (SAm-C, E, W), *S. austriaca* L. (SAu-C, E, W), *S. forsskaolii* L. (SF-C, E, W), *S. glutinosa* L. (SG-C, E, W), *S. nemorosa* L.(SN-C, E, W), *S. officinalis* L. (SO-C, E, W), S. *pratensis* L. (SP-C, E, W), *S. sclarea* L. (SSc-C, E, W), *S. stepposa* Des-Shost. (SSt-C, E, W), *S. verticillata* L. (SV-C, E, W).

3.2.1. Isolation of Volatiles by Simultaneous Distillation-Extraction

Volatile compounds were isolated in a *Likens-Nickerson* simultaneous distillation-solvent extraction apparatus (further referred to as L-N) from 10 g of dried herb placed in a 500 mL round-bottomed flask with ~250 mL distilled water during 2.5 h. Distilled volatile compounds were extracted with 20 mL of the pentane:ether (1:1) mixture. The L-N extracts were dried over Na_2SO_4, concentrated under a stream of nitrogen to 2 mL and kept in sealed vials at –18°C temperature until GC-FID and GC-MS analysis.

3.3. Evaluation of Antioxidant Activity

Salvia extracts, the initial material and residues remaining after extractions were analysed by using the total phenolic content (TPC), the trolox equivalent antioxidant capacity (TEAC in ABTS reaction) and the oxygen radical absorbance capacity (ORAC) assays by applying conventional and QUENCHER analysis procedures to the extracts and solids, respectively.

3.3.1. Total Phenolic Content (TPC)

For TPC, 10 μL of appropriate dilutions of methanolic extract or gallic acid solutions (for calibration) were oxidised with 190 μL Folin-Ciocalteu's reagent solution in deionised water (1:13) (Singleton and Rossi, 1965). The reagents were mixed, allowed to stand for 3 minutes and then neutralised with 100 μL of 7% Na_2CO_3. The absorbance was measured at 765 nm in a *FLUOstar Omega Reader* (BMG Labtech, Offenburg, Germany). The TPC was calculated by using the gallic acid calibration curve and expressed in mg gallic acid equivalents per g of dry weight plant material (DWP) and extract (DWE), mg GAE/g DWP or DWE.

3.3.2. $ABTS^{\bullet+}$ Scavenging Assay

TEAC assay was used to determine RSC of $ABTS^{\bullet+}$ as described by Re et al. (1999). The working solution of $ABTS^{\bullet+}$ was produced by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulphate; the two stock solutions were mixed in equal quantities and stored for 14–16 h. It was further diluted with phosphate buffer solution (PBS) in order to obtain the absorbance of 0.80±0.03 at 734 nm. Samples or trolox solutions (3 μL) were reacted with 300 μL of the $ABTS^{\bullet+}$ solution for 30 minutes, and the absorbance was read at 734 nm in a *FLUOstar Omega* reader. A series of trolox solutions (750–3000 μmol L^{-1}) were used for calibration. The percentage of the scavenged $ABTS^{\bullet+}$ was calculated by the formula $[(Abs_{control}–Abs_{sample})/(Abs_{control})]\times100$ where $Abs_{control}$ and Abs_{sample} are the absorbance values of the control mixture with methanol and the analysed sample, respectively. The TEAC values were calculated from the calibration curve and expressed in μmol trolox equivalents (TE), μmol TE/g DWP and DWE.

3.3.3. ORAC Assay

The advantage of ORAC assay is that it uses a biologically relevant radical source (Prior et al., 2003). The reaction was carried out in 75 mM phosphate buffer (pH=7.4); a stock solution of fluorescein was prepared according to Prior et al. (2003), the samples were prepared by dissolving the extracts in methanol. The prepared samples or trolox (25 μL) and fluorescein (120 μL; 14 μmol L^{-1}) solutions were placed in the 96 well black opaque microplates with a transparent flat-bottom. The microplates were sealed and incubated for 15 minutes at 37°C. After incubation, the AAPH solution as a peroxyl radical generator (25 μL; 240 mM) was added manually with a multichannel pipette. The microplate was immediately placed in a *FLUOstar Omega* fluorescent reader. The plate with the samples was shaken prior to each reading. Fluorescence measurements (excitation wavelength 485 nm; emission wavelength 510 nm) were read every 66 s, in total, 90 cycles were performed. The raw data was analysed by using software *Mars* (BMG Labtech GmbH, Offenburg, Germany). Methanol solutions of trolox were used for calibration (6–250 μmol L^{-1}). Antioxidant curves (fluorescence versus time) were normalised, and the area under the fluorescence decay curve (AUC) was calculated

as $AUC = 1 + \sum_{i=1}^{i=120} \frac{f_i}{f_0}$, where f_0 is the initial fluorescence at 0 min and f_i is the fluorescence at time *i*. The final ORAC values were calculated by using a regression equation between the trolox concentration and the net area under the curve (AUC). The antioxidant activity was expressed in μmol TE/g DWP and DWE.

The ORAC assay was adapted to measure lipophilic CO_2 extracts when using 7% randomly methylated β-cyclodextrin (RMCD) solution in acetone:water (50:50 v/v) to solubilise the antioxidants in oils (Tikekar, Ludescher and Karwe, 2008). For L-ORAC assay, 1 mg of CO_2 extract was dissolved in 1 mL of 7% RMCD solution. The 7% RMCD solution was used as a blank in measurements which were performed as described above.

3.3.4. Assessment of Antioxidant Capacity by QUENCHER Assay

Measurements of the total antioxidant capacity by using modified $ABTS^{\bullet+}$, ORAC and TPC methods were applied directly to the solid ground plant material of *Salvia* spp. plants as described by Pastoriza, Delgado-Andrade, Haro and Rufián-Henares (2011). In principle, all the assays were carried out in the same way as described for *Salvia* extracts. In $ABTS^{\bullet+}$ scavenging assay, 0.01 g of the powdered sample was weighed in a test tube and diluted with 40 μL of PBS. The reaction was initiated by adding 2 mL of $ABTS^{\bullet+}$ reagent. The mixture was vortexed for 27 min, centrifuged at 4800 rpm for 3 min, and 300 μL of an optically clear supernatant was transferred to the microplate. In ORAC assay, 0.01 g of the powdered sample was transferred to a test tube and diluted with 40 μL of methanol. The reaction was started by adding 2 mL of fluorescein. The mixture was vortexed for 15 min at 37°C, and then centrifuged at 4800 rpm for 3 min. Then, 150 μL of prepared solution was transferred to the microplate, kept at 37°C for 15 min, and 25 μL of AAPH solution was added. For TPC, 0.01 g of the sample was transferred to a test tube with 40 μL of ethanol and 1.4 mL of Folin-Ciocalteu's reagent solution (1:13). As the next step, the mixture was neutralised with 0.6 mL of 7% Na_2CO_3, vortexed for 77 min and centrifuged at 4800 rpm for 3 min; the absorbance of the supernatant was measured at 765 nm. In all the methods, when the samples exerted excessively high antioxidant activity, they were diluted with microcrystalline cellulose as an inert material. The mixtures of samples of microcrystalline cellulose and the reagent were used as controls in all measurements. Trolox solutions were used to prepare the calibration curve by using microcrystalline cellulose as well. The results are expressed in μmol TE/g DWP.

3.4. Chromatographic Analysis

3.4.1. Determination of Tocopherols by High Performance Liquid Chromatography (HPLC)

Tocopherols were analysed by HPLC in the saponified sample according to the methodology developed by Gruszka and Kruk (2007) with a slight modification. *Perkin Elmer Series 200* HPLC system was equipped with a C30 reverse-phase

column (particle size 5 µm, 250×4.6 mm) (Waters, Milford, MA, USA) by applying the isocratic elution with acetonitrile: methanol: dichloromethane (72/22/6, v/v/v) at 30°C. The injection volume was 20 µL whereas the flow rate was 1 mL/min. Tocopherols were detected by using a fluorescence detector at 290 nm excitation and 330 nm emission; the analytes eluted in 20 min: at 12.2, 10.6, 10.2, 8.9 min for α, β, γ, and δ -tocopherols, respectively. They were identified by comparing retention times to the pure standards, which ware prepared in a mobile phase at 0–10 µg/mL concentrations for drawing the calibration curves for quantification. The analyses were performed in triplicate.

3.4.2. An On-Line HPLC-DPPH$^{\bullet}$-Scavenging Assay

The on-line method was applied by using a *Waters HPLC* system with a *Waters 1525* binary pump (Milford, MA, USA), a *Rheodyne 7125* manual injector (Rheodyne, Rohnert Park, CA, USA) with a 20 µL injection loop and a *Discovery HS C18* 250×0.46 cm (5 µm) analytical column (Supelco Analytical, Bellefonte, PA, USA). The linear binary gradient was formed at a constant flow rate of 0.8 mL/min while using solvent A (0.1%, v/v, formic acid solution in ultra pure water) and solvent B (methanol) with the following gradient program: 0–30 min 10% B, 30–50 min 40% B, 50–55 min 100% B, 55–56 min 10% B, 56–60 min 10% B. The applied gradient enabled to achieve good resolution and a good peak shape. The compounds eluted from the column were detected with a *Waters 996* photodiode array detector in the range from 210 to 450 nm. After the UV detection, the freshly prepared 5×10^{-6} M DPPH$^{\bullet}$ solution in methanol was added to the main eluent at a flow rate of 0.6 mL/min by using an *Agilent 1100* series quaternary pump (Agilent Technologies, Inc., Santa Clara, CA, USA). The mixture was introduced into a 15 m×0.25 mm i.d. reaction coil made of PEEK (polyetheretherketone) tubing (Interchim, Frankfurt, Germany). The decrease of absorbance after the reaction of radical scavengers with DPPH$^{\bullet}$ was detected photometrically as a negative peak at 515 nm with a variable-wavelength *Shimadzu SPD-20A* UV detector (Shimadzu Corporation, Kyoto, Japan). For the identification of phenolic compounds, the HPLC system was coupled to a quadrupole mass detector *Micromass ZQ* (Waters) operating in the ESI negative ionisation mode at the following parameters: scanning range 100–1000 m/z; capillary voltage 3 kV; source temperature 120°C; cone voltage 30 V; cone gas flow 80 L/h; desolvation temperature 350°C; desolvation gas flow 400 L/h. The flow rate was set at 0.8 mL/min, the injection volume of 1% extract solutions was 20 µL, and the gradient was as described previously.

3.4.3. Identification of Phenolic Compounds by Using UPLC-Q/TOF

An *Acquity UPLC* system with a binary solvent delivery system, an autosampler with a 10 µL sample loop, a photodiode array (PDA) detector, a column manager, and a data station running the Compass acquisition and data software (Waters, Milford, MA, USA) combined with a *Bruker maXis UHR-TOF* mass spectrometer (Bruker Daltonics, Bremen, Germany) were used. An *Acquity BEH C18* column (1.7 µm, 100 mm×2.1 mm, i.d.) (Waters, Milford, MA, USA) was used

for the separation of compounds at 40°C. The mobile phase was initially composed of 100% eluent A (0.1%, v/v, formic acid solution in ultra pure water) and 0% B (acetonitrile) while maintaining these conditions for 1 min. Afterwards, a linear gradient was shifted from 0 to 40% of eluent B in 5 min, and, later on, to 100% B during the following 3 min. It was kept under these conditions during the following 1 min. After the analysis, the initial conditions were re-introduced over 1 min and preserved for 1 min. Before each new run, the column was equilibrated for additional 2 min. The flow rate was 0.4 mL/min, and the effluents were monitored at 254 nm. The effluents from the PDA detector were introduced directly into the UHR-Q/TOF mass spectrometer equipped with an ESI source. Instrument control and data acquisition were achieved by using the *Compass 1.3* (HyStar 3.2 SR2) software. MS experiments were performed in the negative ionisation mode, the capillary voltage was maintained at +4500 V with the end plate offset at −500 V. Nitrogen was used as the drying and nebulising gases at a flow rate of 10.0 L/min and a pressure of 2.0 bar, respectively. Mass spectra were recorded within the range from 100 to 1200 m/z, at a rate of 2.5 Hz. Peak identification was carried out by comparing the retention times with those of the corresponding peaks in chromatograms of standards or by the obtained accurate masses.

3.4.4. Quantitative Analysis of Phenolic Compounds by Using UPLC-TQ-S

Quantitative UPLC-MS analysis was performed by using a Waters Acquity UPLC™ H-Class from Waters (Milford, MA) equipped with a *Xevo TQ-S* tandem quadrupole mass spectrometer (Waters, Milford, MA) operating in the negative electrospray ionisation (ESI) mode; the capillary voltage was set at 2 kV, the cone voltage was 20 V, whereas the source offset was 50 V. The desolvation temperature was 350°C, the desolvation gas flow measured 800 L/h, the cone gas flow was set at 150 L/h, and the nebuliser pressure was set at 0.7 MPa. The recorded mass range was from 100 to 1000 m/z. Chromatographic separations were performed by using the above described column and gradient. MassLynx 4.1 software was used for the instrument control and data collection. All the samples were run in triplicates. Quantitative analysis was performed by using *TargetLynx* (Waters, Milford, MA) software. The concentration of phytochemicals was measured both in the dry weight of extracts (DWE) and in the dry weight of the whole plant material (DWP). In the latter case, the amount of the compounds isolated with CO_2, ethanol and water was summed up by taking into account the extract yields.

3.4.5. Method Validation

The validation of the analytical method for quantification of *Salvia* spp. plant extracts included the limit of detection (LOD) and the limit of quantification (LOQ) according to Shrivastava and Gupta (2011). For a linear calibration curve, it is assumed that the instrument response *y* is linearly related to standard concentration *x* for a limited range of concentration. It can be expressed in a model such as y=a+bx. Thus the LOD and LOQ can be expressed as $LOD=3\times S_a/b$; $LOQ=10\times S_a/b$, where S_a is the standard deviation of the response and *b* is the slope of the calibration curve.

3.4.6. Gas Chromatographic (GC) Analysis

L-N and CO_2 extracts (the latter was diluted in pentane at the concentration of 10 mg/mL) were analysed with a *PerkinElmer Clarus500* gas chromatograph (Shelton, USA) equipped with a flame ionisation detector (FID) and an *Elite-5* (5% diphenyl, 95% dimethylpolysiloxane) fused silica capillary column, 30 m length, 0.25 mm i.d., 0.25 μm film thickness (PerkinElmer, Shelton, USA). The carrier gas was helium at the inlet pressure of 15 psi at 50°C, which was equivalent to a 1.3 mL/min volumetric flow; the detector temperature was 300°C, the oven temperature was programmed from 50°C (2 min) to 280°C (10 min) at the rate of 5°C/min. A split/splitless injector was used at 260°C in a split mode at a ratio of 1:10; the injection volume was 1 μL. The obtained quantitative data was calculated according to the methodology developed by Cachet et al. (2016) using the formula $m_i = RRF_i^{Pred} m_{MD} \frac{A_i}{A_{MD}}$, where m_i is the mass of compound i to be quantified, expressed in mg in kg of plant dry weight (DWP); RRF_i^{Pred} is the predicted relative response factor of compound i, m_{MD} is the mass of decane (internal standard, ISTD), A_i and A_{MD} represent the peak area of the analyte and the ISTD, respectively. The concentrations of the compounds are calculated for the DWP. The mean values were calculated from duplicate extractions and triplicate injections; standard deviations (SD) did not exceed 5% in any of the conducted measurements.

3.4.7. Gas Chromatography-Mass Spectrometry (GC-MS)

The composition of L-N and CO_2 extracts was analysed on a *GC×GC-TOFMS LECO Pegasus 4D* system consisting of an *Agilent 7890A GC* system, a *GERSTEL Multipurpose Sampler MPS* (Gerstel GmbH, Mulheim an der Ruhr, Germany), a high-speed *TOFMS* detector (LECO, St. Joseph, MI, USA) and a four jet cryogenic modulator (Zoex, Houston, TX) by comparing the 1D first dimension linear temperature programmed retention index with the peak's identities provided by the mass spectral similarity search. The column set consisted of a primary column *BPX-5* (30 m, 0.25 mm i.d., 0.25 μm film thickness) (SGE Analytical Science, Australia) connected in series to a secondary column, *BPX-50* (1.8 m, 0.10 mm i.d., 0.1 μm film thickness). The primary oven programming was 2 min at 50°C then ramped to 280°C at a rate of 5°C/min (holding for 10 min); the secondary oven programming was 2 min hold at 65°C then ramped to 295°C at a rate of 5°C/min. The transfer line temperature was 250°C. The GC injector port was maintained at 280°C with the desorption time of 5 min. The TOFMS acquisition rate was 10 spectra/s, the mass range used for identification was 35–550 *m/z* units. The detector voltage was set at 1550 V with the ion source temperature of 250 °C. The data from the GC×GC-TOFMS system was collected with *ChromaTOF* software v.4.22 (LECO) after a solvent peak delay of 500 s; the split ratio was set at 1:20; for peak detection and spectral identification against NIST, MainLib, Replib and Adams mass spectra libraries, signal-to-noise threshold was set as 50, and the minimum accepted similarity was selected as 750.

The components were identified by comparison of their Kováts retention indices (KI) relative to C_7–C_{30} *n*–alkanes obtained on the nonpolar *Elite-5* column with those provided in scholarly literature (Adams, 2007) and by comparing their mass spectra with the data provided by the NIST, Mainlib, Replib and Adams mass spectral libraries and by comparing the mass spectra with the corresponding data of the components of the reference oils.

3.5. Statistical Data Evaluation

All the extractions and analyses were carried out in triplicate, and the obtained results are expressed as the mean±standard deviation (SD). Significant differences among the means were determined by one-way ANOVA by using the statistical package *Statgraphics Plus 5.1*. Fisher's *least significant difference* (LSD) was used to determine the significant difference among the treatments at $p<0.05$. Correlation coefficients were calculated between each of the variables. The statistical difference was established at $P<0.05$.

4. Results and Discussion

4.1. Comprehensive Evaluation of Antioxidant Potential of 10 *Salvia* spp. Plants by Using High Pressure Methods for the Isolation of Lipophilic and Hydrophilic Plant Fractions

4.1.1. Antioxidant Capacity of *Salvia* spp. Plant Extracts

There are many assays for the assessment of antioxidant properties; the majority of them are based on single electron or hydrogen atom transfer reactions. It was concluded that ORAC, TPC measured with Folin-Ciocalteu's reagent and one of the electron/hydrogen transfer assays should be recommended for the representative evaluation of the antioxidant properties of foods (Huang, Ou and Prior, 2005). Following this recommendation, all these methods were applied for *Salvi*a spp. extracts and powdered materials in our study. The antioxidant potential was expressed in g of extract (DWE) but also recalculated for 1 g DWP. Both values are informative as it is beneficial in practice not only to show the activity of the extracts obtained by different solvents but also to reveal the potential of the whole plant material. For instance, the extracts may be strong antioxidants even though their yields may be very low; on the contrary, less antioxidatively active yet high-yielding extracts would isolate a larger part of antioxidants from the whole plant matrix.

It may be observed that not only extract yields but also L-ORAC values in SFE-CO_2 were highly dependent on the plant species; the extract yields ranged from 1.8±0.1% (*S. stepposa*) to 5.2±0.2% (*S. sclarea*); the L-ORAC values varied from 570±23 to 6015±11 μmol TE/g DWE and from 29.8±1.2 to 224.4±0.4 μmol TE/g DWP, respectively. The highest and the lowest L-ORAC values were demonstrated by *S. officinalis* and *S. sclarea*, respectively.

Pressurised ethanol was a highly effective solvent in terms of extraction of antioxidatively active constituents (Table 4.1.). The values of radical scavenging capacity (RSC) in $ABTS^{\bullet+}$ assay of *Salvia* spp. ethanolic extracts were from 684±5 to 1742±2 μmol TE/g DWE and from 201±2 to 515±1 μmol TE/g DWP. Particularly high amounts of radical scavengers were isolated with ethanol from *S. verticillata* and *S. sclarea*, up to 1742±2 and 1634±22 μmol TE/g DWE, respectively. $ABTS^{\bullet+}$ scavenging capacity of *S. officinalis* and *S. verbenaca* extracts isolated with methanol was reported previously (Farhat, Landoulsi, Chaouch-Hamada, Sotomayor and Jordán, 2013; Farhat, Jordán, Chaouech-Hamada, Landoulsi and Sotomayor, 2009): the TEAC values in these studies depending on the collection site of *S. officinalis* and *S. verbenaca* were 309.22–346.61 and 120.11–287.81 μM TE/mg extract, respectively. Considering that the molar mass of Trolox is 250.29 g/mol, it means that 1 g of plant extract demonstrates the $ABTS^{\bullet+}$ scavenging capacity equivalent of 77.4–86.8 g TE/g (*S. officinalis*) and 30.1–72.8 g TE/g (*S. verbenaca*) extracts, which is many times higher compared to our results as well as to other previously reported data for various botanicals.

Antioxidant properties of extracts were also evaluated by using the ORAC assay which is based on quenching peroxyl free radicals, the major oxidative products produced during lipid peroxidation in biological systems (Prior, Wu and Schaich, 2005). It may be observed that the variations in ORAC values measured for the *Salvia* extracts isolated with different solvents were not so remarkable if compared with the $ABTS^{\bullet+}$ assay. The highest scavenging capacity was demonstrated by ethanolic extracts of *Salvia* spp. (up to 4735±114 μmol TE/g DWE and 1208±29 μmol TE/g DWP) followed by water extracts (up to 1339±83 μmol TE/g DWE and up to 485±30 TE/g DWP). It should be noted that the previously reported data on ORAC values of *Salvia* spp. plants is inadequately scarce. Porres-Martínez, González-Burgos, Accame and Gómez-Serranillos (2013) reported that the essential oil of *S. lavandulifolia* Vahl. exhibited the highest ORAC values, 0.36±0.10–0.76±0.01 μmol TE/mg. The differences in the antioxidant potential between the analysed samples in their work, at least in part, were linked to the presence of 1,8-cineole in the oils.

The TPC values of ethanolic extracts were in the range of 0.48±0.03–43.65±0.67 mg GAE/g DWP; the ethanol extracts possessed the highest TPC, up to 43.65±0.67 mg GAE/g DWP, followed by the water extracts, up to 7.17±0.07 mg GAE/g DWP. Thus ethanol was the most effective solvent in extracting phenolic compounds from various *Salvia* spp. plant material. These values are in agreement with the previously reported data. For instance, Shan, Cai, Sun and Corke (2005) determined 5.32 g GAE/100 g DWP in *S. officinalis* leaves and branches, while Cosio, Buratti, Mannino and Benedetti (2006) found remarkably higher TPC values in the same species ranging from 23.2 to 26 mg GAE/g DWP, whereas Erdoğan, Karik, Hüsnü and Başer (2014) reported TPC in *Salvia* spp. plants in the range from 4.88 to 16.55 mg GAE/g DWP. The effects of various factors on the formation of secondary metabolites in plants may explain the TPC differences in the assayed *Salvia* samples (Shan, Cai, Sun and Corke, 2005).

Table 4.1. The yields and antioxidant characteristics of various *Salvia* spp. plant extracts consecutively extracted by using SFE-CO_2, ethanol and water

Samples	Yield % w/w	ABTS$^{\bullet+}$, µmol TE/g		ORAC, µmol TE/g		TPC, mg GAE/g	
		DWE	DWP	DWE	DWP	DWE	DWP
SAm-E	25.5±0.1^{d}	1177±7^{f}	300±2^{e}	4735±114^{e}	1208±29^{e}	97.41±0.79^{c}	24.85±0.20^{d}
SAm-W	32.4±0.1^{c}	79.52±0.52^{d}	25.77±0.18^{b}	676±27^{cd}	219±9^{c}	5.92±0.14^{e}	1.92±0.05^{e}
SAu-E	29.4±0.1^{f}	684±5^{a}	201±2^{a}	2009±196^{a}	591±58^{b}	72.57±0.97^{a}	21.35±0.02^{c}
SAu-W	40.7±0.1^{f}	85.04±1.43^{e}	34.62±0.58^{d}	482±33^{b}	196±13.4^{c}	4.23±0.40^{d}	1.72±0.16^{d}
SF-E	30.4±0.1^{g}	1135±9^{e}	345±3^{g}	4508±126^{e}	1370±38^{f}	116±1^{g}	35.19±0.17^{h}
SF-W	36.2±0.1^{d}	328±3^{i}	119±1^{i}	1339±83^{g}	485±30^{g}	19.80±0.19^{i}	7.17±0.07^{j}
SG-E	26.1±0.1^{e}	1072±10.2^{d}	279±3^{d}	1758±118^{a}	457±31^{a}	106±1 ^{e}	27.72±0.27^{e}
SG-W	31.0±0.1^{b}	246±2^{h}	76.26±0.64^{g}	897±33^{e}	278±10^{d}	16.86±0.16^{h}	5.23±0.05^{i}
SN-E	30.7±0.2^{g}	1005±2^{c}	308±1^{f}	2392±151^{b}	733±46^{c}	109±1^{f}	33.33±0.17^{g}
SN-W	43.7±0.1^{h}	150±0^{f}	65.57±0.13^{f}	718±14^{d}	313±6^{e}	7.60±0.25^{f}	3.32±0.11^{g}
SO-E	36.2±0.1^{h}	1080±10^{d}	391±4^{h}	2535±180^{b}	917±66^{d}	121±2^{h}	43.65±0.67^{i}
SO-W	37.7±0.1^{e}	225±1^{g}	84.98±0.29^{h}	1143±69^{f}	431±26^{f}	13.15±0.19^{g}	4.96±0.07^{h}
SP-E	25.0±0.1^{c}	973±11^{b}	243±3^{b}	1783±85^{a}	446±21^{a}	85.84±0.63^{b}	21.46±0.16^{c}
SP-W	42.5±0.2^{g}	38.85±1.71^{a}	16.52±0.73^{a}	279±23^{a}	118±10^{b}	1.12±0.07^{a}	0.48±0.03^{a}
SSc-E	18.1±0.1^{a}	1634±22^{h}	296±4^{e}	3820±193^{c}	692±35^{c}	106±1^{e}	19.26±0.10^{a}
SSc-W	30.2±0.2^{a}	153±1^{f}	46.15±0.42^{e}	867±16^{e}	262±5^{d}	7.59±0.13^{f}	2.29±0.04^{f}
SSt-E	19.6±0.2^{b}	1392±14^{g}	273±3^{c}	2480±145^{b}	487±28^{a}	103±2^{d}	20.28±0.40^{b}
SSt-W	30.2±0.2^{a}	51.82±1.38^{b}	15.63±0.42^{a}	279±30^{a}	84.13±9.03^{a}	2.13±0.09^{b}	0.64±0.03^{b}
SV-E	29.6±0.2^{f}	1742±2^{i}	515±1^{i}	4121±301^{d}	1218±89^{e}	106±1^{e}	31.35±0.44^{f}
SV-W	40.4±0.1^{f}	69.64±1.38^{c}	28.16±0.56^{c}	626±34^{c}	253±14^{d}	2.85±0.16^{c}	1.15±0.06^{c}

Values represented as mean±standard deviation (n=3); a-j: the mean values followed by different letters are significantly different (p<0.05) between one type of solvent and different *Salvia* spp. plants

4.1.2. Direct Evaluation of Antioxidant Capacity by QUENCHER Method

Some antioxidatively active constituents may be strongly bound to other components in the plant matrix and are thus hard to extract by solvents. They may be released in the human intestinal tract in the process of digestion. Recently, the QUENCHER method was developed with the capacity to determine the antioxidant activity of the whole plant material (Serpen, Capuano, Fogliano and Gökmen, 2007). The values obtained by the QUENCHER method for insoluble food components demonstrated the existence of significant antioxidant capacity values which in some cases were remarkably higher than those determined by the traditional extraction procedures. It is thus suggested that free functional groups on the surface of insoluble particles may also quench with the radicals.

Antioxidant capacity values were determined by the QUENCHER method in raw dried plant materials, the residues were calculated after SFE-CO_2 and the final residue was measured after PLE (Table 4.2.). In terms of the changes of all the measured values after SFE-CO_2, *Salvia* spp. plants may be divided into three groups: antioxidant capacity indicators significantly increased (1), significantly decreased (2) or remained almost unchanged (3). For this purpose, statistical data evaluation was applied. Fisher's least significant differences test was used to determine significant differences among the samples at the 5% probability level ($P<0.05$). For instance, $ABTS^{\bullet+}$ scavenging capacity decreased after SFE-CO_2 of SAm, SP, SSc and SV by 27, 36, 30, and 52%, respectively. Several factors may be considered as an explanation of these changes. The decrease of antioxidant capacity values may be simply explained by the partial removal of active lipophilic compounds during SFE-CO_2. For instance, the ORAC value of SAm-C (1914±13 μmol TE/g) was the second highest value among all *Salvia* spp. plants, while after SFE-CO_2 it remarkably decreased comparing to the raw material. The increase of the measured values in the SFE-CO_2 residues may be explained by the structural changes of the plant material after subjecting it to a very high pressure at an elevated temperature. Such changes may increase the availability of polar antioxidatively active groups in the matrix. For instance, the ORAC value of the SO-C extract was the highest; however, for the SFE-CO_2 residue of *S. officinalis*, it was almost two times higher compared to the initial plant material. *S. officinalis* accumulates high amounts of essential oil, which is extracted by SFE-CO_2; this *Salvia* spp. plant also contains high concentrations of polyphenolic antioxidants, such as rosmarinic acid, which are not extracted by SFE-CO_2. It may be suggested that after treatment at high pressure/elevated temperature, antioxidatively active groups in *S. officinalis* become more available for scavenging peroxyl radicals in the ORAC assay. Also, some chemical changes during high pressure/elevated temperature treatment may be considered.

PLE with high polarity solvents exhibited remarkably reduced antioxidant capacity values in all *Salvia* spp. plants (Table 4.2). It may be easily explained by the removal of strong polar antioxidants (e.g., rosmarinic acid) from the plant material. TEAC values measured in $ABTS^{\bullet+}$ assay after PLE decreased on average by 6–22 times; for instance (in μmol TE/g DWP), in *S. verticillata*, the values decreased from 879±7 to 38.47±0.13; in *S. sclarea*, the values changed from 575±3

to 32.73±0.17; in *S. pratensis*, the values fell from 510±8 to 33.75±2.27; in *S. amplexicaulis*, the values decreased from 444±12 to 32.99±0.23. The residual TPC values after all the high pressure extraction procedures were in the range of 1.14±0.02–5.10±0.13 mg GAE/g, while in the initial material they were in the range of 20.21±1.34–72.84±1.73 mg GAE/g. It shows that 2-step high pressure extraction (PLE) effectively isolates antioxidatively active constituents from the herbal material. It was suggested that the extraction type and other treatments may release various compounds from the complex product matrix containing proteins and other constituents which may demonstrate antioxidant activity *in vivo* (Pastoriza, Delgado-Andrade, Haro and Rufián-Henares, 2011). To the best of our knowledge, no evaluation of various *Salvia* spp. plant material by using the QUENCHER method at various extraction processing steps has been reported previously.

Table 4.2. Antioxidant activity and total phenols content of various *Salvia* spp. plant material obtained by the QUENCHER procedure

Samples	ABTS$^{\bullet+}$, μmol TE/g DWP			ORAC, μmol TE/g DWP			TPC, mg GAE/g DWP		
	Raw	After SFE-CO_2	After PLE	Raw	After SFE-CO_2	After PLE	Raw	After SFE-CO_2	After PLE
SAm	$444\pm12^{d,z}$	$326\pm1^{a,y}$	$32.99\pm0.23^{a,x}$	$1020\pm5^{e,z}$	$324\pm17^{a,y}$	$48.47\pm0.33^{e,x}$	$55.31\pm2.32^{e,z}$	$36.94\pm0.83^{bc,y}$	$2.40\pm0.16^{d,x}$
SAu	$334\pm3^{a,y}$	$370\pm10^{b,z}$	$36.77\pm0.66^{b,x}$	$605\pm11^{c,y}$	$965\pm14^{c,z}$	$47.77\pm3.30^{de,x}$	$20.21\pm1.34^{a,y}$	$52.51\pm1.57^{f,z}$	$2.73\pm0.15^{e,x}$
SF	$414\pm24^{c,y}$	$556\pm5^{g,z}$	$32.68\pm0.17^{a,x}$	$1173\pm7^{g,z}$	$1559\pm16^{g,y}$	$42.80\pm3.34^{c,x}$	$66.68\pm2.47^{g,y}$	$67.77\pm0.88^{h,y}$	$2.75\pm0.09^{e,x}$
SG	$333\pm1^{a,y}$	$440\pm10^{e,z}$	$37.15\pm0.77^{bc,x}$	$517\pm3^{b,y}$	$840\pm22^{b,z}$	$52.14\pm0.30^{e,x}$	$25.48\pm0.40^{b,y}$	$46.11\pm0.55^{d,z}$	$4.41\pm0.23^{g,x}$
SN	$374\pm18^{b,y}$	$480\pm7^{f,z}$	$32.82\pm0.06^{a,x}$	$854\pm16^{d,y}$	$1443\pm34^{d,z}$	$33.12\pm2.42^{a,x}$	$45.26\pm2.16^{d,z}$	$36.16\pm0.48^{b,y}$	$1.96\pm0.10^{c,x}$
SO	$335\pm25^{a,y}$	$331\pm9^{a,y}$	$51.56\pm1.16^{d,x}$	$817\pm40^{d,y}$	$1565\pm5^{g,z}$	$41.22\pm1.39^{bc,x}$	$53.87\pm1.93^{e,z}$	$49.44\pm0.84^{e,y}$	$5.10\pm0.13^{h,x}$
SP	$510\pm8^{e,z}$	$326\pm1^{a,y}$	$33.75\pm2.27^{a,x}$	$418\pm28^{a,y}$	$851\pm13^{b,z}$	$33.97\pm1.06^{a,x}$	$33.48\pm1.24^{c,y}$	$47.25\pm0.78^{d,z}$	$1.60\pm0.14^{b,x}$
SSc	$575\pm3^{f,z}$	$402\pm8^{c,y}$	$32.73\pm0.17^{a,x}$	$1050\pm38^{e,y}$	$1486\pm37^{ef,z}$	$36.90\pm0.26^{ab,x}$	$42.80\pm2.19^{d,z}$	$37.95\pm0.64^{c,y}$	$1.14\pm0.02^{a,x}$
SSt	$331\pm2^{a,y}$	$483\pm15^{f,z}$	$32.61\pm0.39^{a,x}$	$1535\pm47^{f,y}$	$1529\pm32^{fg,y}$	$37.42\pm0.19^{ab,x}$	$63.15\pm1.10^{f,z}$	$26.22\pm0.75^{a,y}$	$1.74\pm0.06^{b,x}$
SV	$879\pm7^{g,z}$	$419\pm12^{d,y}$	$38.47\pm0.13^{c,x}$	$1552\pm3^{f,y}$	$1535\pm13^{fg,y}$	$43.74\pm2.00^{cd,x}$	$72.84\pm1.73^{h,z}$	$62.94\pm0.95^{g,y}$	$3.46\pm0.09^{f,x}$

a–h: the mean values followed by different letters are significantly different ($P<0.05$) between the types of *Salvia* spp. plants. *x*, *y*, *z*: the mean values followed by a different letter are significantly different ($P<0.05$) between the types of extraction.

4.1.3. Total Yield, Overall Antioxidant Potential and Correlation Between Different Values

The total yields and the overall antioxidant potential of *Salvia* spp. plants expressed as a sum of TPC, TEAC and ORAC values of all the extracts obtained with different solvents are presented in Fig. 4.1. The extraction yields of *Salvia* spp. plants obtained by consecutive application of SFE-CO_2 and PLE with different polarity solvents, namely, ethanol and water, are also shown in Fig. 4.1(a). The yields obtained with water were 1.04–1.70 times higher comparing to the yields isolated with ethanol. In general, the total yield of extracts was higher from *S. officinalis* (77.6%) and *S. nemorosa* (76.8%) than from the other *Salvia* spp. plants (51.6–72.9%), most likely, due to a higher content of polar substances in *S. officinalis* and *S. nemorosa*. It is obvious that *S. forsskaolii* and *S. officinalis* possess higher TPC; the values varied from 42.36 to 48.61 mg GAE/g DWP, respectively. The variations in TPC between other *Salvia* spp. plant samples ranged from 20.93 to 36.65 mg GAE/g DWP (Fig. 4.1(b)).

S. forsskaolii and *S. officinalis* possess high overall RSC in $ABTS^{\bullet+}$ scavenging assay: their TEAC values were 463.71 and 475.70 μMTE/g DWP, respectively (Fig. 4.1(c)). However, the highest TEAC value for this assay was found for *S. verticillata*, specifically, 543.01 μM TE/g DWP. It may be observed that the overall TEAC calculated by summing the values obtained in $ABTS^{\bullet+}$ scavenging assay of ethanol and water extracts differ from the TEAC values determined by the QUENCHER procedure; however, these differences are not so remarkable. For instance, for *S. verticillata*, the TEAC in QUENCHER assay was 1.62 times higher than the overall TEAC in the extract assay, whereas for *S. forsskaolii* and *S. officinalis*, on the contrary, the overall TEAC of the extracts was higher than the TEAC measured by the QUENCHER method.

The highest overall ORAC value was found for *S. forsskaolii* (1872 μmol TE/g DWP*)* followed by *S. officinalis* (1573 μmol TE/g DWP) and *S. verticillata* (1493 μmol TE/g DWP) (Fig. 4.1(d)). In this assay, the overall antioxidant capacity values were of the same order as in the QUENCHER assay. The ORAC values were previously reported only for *S. lavandulifolia* Vahl. (Porres-Martínez, González-Burgos, Accame and Gómez-Serranillos, 2013), while such data for other *Salvia* spp. plants has not been found in any available scholarly literature sources.

Comprehensive evaluation of antioxidant activity indicators by using three assays applied to 10 *Salvia* spp. plants and by using three different solvents resulted in obtaining numerous content values. Therefore, it was interesting to assess if there are correlations between different assay methods. For instance, multiple studies employing traditional and QUENCHER procedures demonstrated strong positive correlations between TPC values and RSC of $ABTS^{\bullet+}$ and ORAC. Correlation coefficients for different antioxidant assays are summarised in Fig. 4.2. It may be observed that there are strong correlations between different antioxidant activity assays of *Salvia* spp. plant extracts (0.830–0.938) and the ground plant material in the QUENCHER assay (0.812–0.852) when the values are expressed in DWP. When antioxidant capacity values are expressed in g of DWE, the correlations are also strong. For instance, the correlation coefficient for TPC-$ABTS^{\bullet+}$ is 0.942, for TPC-

ORAC, it reaches 0.847 and for $ABTS^{\bullet+}$-ORAC, it stands at 0.884. These findings reveal the complexity of the composition of antioxidatively active constituents of different *Salvia* spp. plants. Comprehensive studies of individual constituents as well as their antioxidative properties are required for obtaining more exhaustive information on the properties of different *Salvia* spp. extracts.

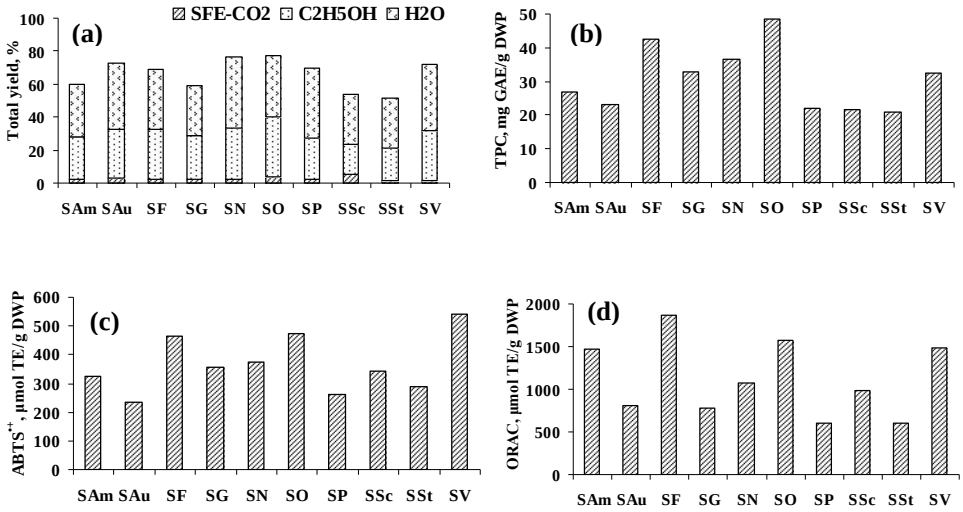


Fig. 4.1. The total yield (a) and the overall antioxidant activity of *Salvia* spp. plants expressed as a sum of the total phenolic content (TPC, b); $ABTS^{\bullet+}$ scavenging capacity (c); oxygen radical absorbance capacity ORAC (d)

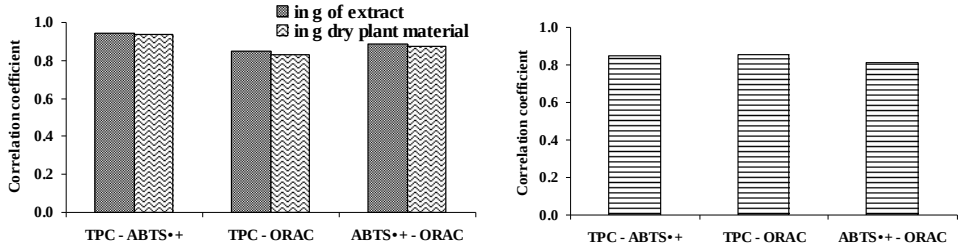


Fig. 4.2. Correlation coefficients between different antioxidant measurement assays of different *Salvia* spp. plants; on the right – as determined by the QUENCHER procedure in solid material; on the left – as determined by the traditional procedure in extracts

4.2. Phytochemical Composition of Fractions Isolated from 10 *Salvia* spp. Plants by Supercritical Carbon Dioxide and Pressurised Liquid Extraction Methods

4.2.1. Determination of Tocopherols

Botanicals biosynthesise various classes of organic compounds which may be isolated by using different polarity solvents; therefore, the yields of extracts as well as their composition highly depend on the solvent and the applied procedure. The application of high pressure extraction and fractionation technique, such as supercritical fluid extraction with carbon dioxide (SFE-CO_2) and pressurised liquid extraction (PLE) by using environmentally- and food-friendly solvents may provide additional benefits while processing botanicals with the intention of isolating valuable functional ingredients. To the best of our knowledge, such a concept has not been previously applied to *Salvia* spp. plants; on the other hand, comprehensive studies of plant biorefining into fine phytochemicals have been recently initiated, and their benefits have already been demonstrated for raspberry pomace (Kryževičiūtė, Kraujalis and Venskutonis, 2016) and chokeberry pomace (Grunovaitė, Pukalskienė, Pukalskas and Venskutonis, 2016).

Tocopherols exerting vitamin E activity in living organisms are strong lipophilic antioxidants and therefore they can be effectively isolated by the nonpolar SFE-CO_2. HPLC separation of tocopherols is achieved by using reversed phase which is based on the structure of the side chain and the number of methyl substituents (Gruszka and Kruk, 2007). The dependence of the peak area and various tocopherol concentrations demonstrated excellent linear relationships (R^2=0.99–1.00) for tocopherol isomers. It may be observed (Table 4.3) that the total yield of tocopherol isomers in the researched *Salvia* spp. plants varied from 2,360 to 10,071 μg/g DWE and from 52.64 to 221 μg/g DWP. α-Tocopherol was the dominating vitamin E isomer in 10 different *Salvia* species (Table 4.3); its concentration in the extracts and plant material were from 2242 to 8473 μg/g in the dry weight of extracts (DWE) and from 50.01 to 179 μg/g in the dry weight of the whole plant material (DWP), respectively. The concentrations of γ- and δ-tocopherols were remarkably lower, whereas the β isomer was not detected in any of the extracts. It should be noted that α-tocopherol is the most biologically active isomer. Thus the total amount of the extracted tocopherols from dry plant material ranged from 52.64 μg/g DWP (*S. amplexicaulis*) to 221 μg/g DWP (*S. nemorosa*). The antioxidant potential of CO_2 extracts isolated from *S. officinalis* at different parameters were tested previously in rapeseed oil (Daukšas, Venskutonis, Povilaitytė and Sivik, 2001), and tocopherols may play an important role in increasing the oxidative stability of food lipids. To the best of our knowledge the content of tocopherols in different *Salvia* spp. plants has not been reported previously.

Table 4.3. The yield of tocopherol isomers isolated from *Salvia* spp. plants obtained by SFE-CO_2. The results are expressed as µg/g DWE and µg/g DWP

Samples	α-tocopherol		γ-tocopherol		δ-tocopherol		Total tocopherols	
	DWE	DWP	DWE	DWP	DWE	DWP	DWE	DWP
SAm-C	2242±17[a]	50.01±0.37[a]	118±1[a]	2.63±0.02[a]	nd	nd	2360	52.64
SAu-C	4538±63[g]	128±2[d]	429±4[d]	12.19±0.12[d]	318±7[b]	9.03±0.20[b]	5285	149
SF-C	8473±57[i]	172±1[g]	599±43[e]	12.17±0.87[d]	419±2[c]	8.50±0.04[b]	9491	193
SG-C	2436±22[b]	56.28±0.52[b]	262±12[b]	6.04±0.28[b]	87.63±0.91[a]	2.02±0.02[a]	2786	64.34
SN-C	6095±109[h]	148±3[e]	2403±60[g]	58.15±1.46[g]	616±21[d]	14.91±0.51[d]	9114	221
SO-C	3914±45[e]	146±2[e]	246±3[b]	9.17±0.13[c]	nd	nd	4160	155
SP-C	4074±11[f]	78.23±0.22[c]	161±3[a]	3.09±0.07[a]	nd	nd	4235	81.32
SSc-C	3429±58[d]	179±3[h]	476±7.3[d]	24.85±0.38[f]	nd	nd	3905	204
SSt-C	3169±6[c]	58.31±0.12[b]	321±3[c]	5.90±0.05[b]	nd	nd	3490	64.21
SV-C	8367±65[i]	155±1[f]	1110±15[f]	20.53±0.27[e]	594±9[d]	10.99±0.17[c]	10071	187

Values represented as mean±standard deviation (n=3); a–i: the mean values followed by different letters are significantly different (P<0.05); nd: not detected; β-tocopherol was not detected in any of the samples.

4.2.2. Identification of *Salvia* Phytochemicals by Using UPLC-Q/TOF

Usually, botanical extracts are extremely complex mixtures of various classes of compounds which may be present across an outstandingly wide concentration range. In addition, the antioxidant capacity as well as other bioactivities of plant constituents may also differ significantly depending on their chemical structures and isomerisation (Kintzios, 2000). Therefore, for a more comprehensive characterisation of the chemical composition of extracts isolated from 10 *Salvia* spp. plants and the whole plant material, three methods were applied, namely, UPLC-Q/TOF for compound identification, UPLC-TQ-S for their quantification, and on-line HPLC-UV-DPPH$^{\bullet}$ for the rapid monitoring of the most antioxidatively active compounds in the complex mixtures.

In total, 15 compounds were positively identified by using commercial standards, while some other compounds were identified tentatively based on the obtained fragments and on the grounds of comparison with data sourced from scholarly literature (Table 4.4). Representative chromatograms and chemical structures of the identified compounds in *Salvia* spp. plant extracts are shown in Fig. 4.3 and Fig. 4.4, respectively.

Compound **2** gave an m/z value of 197.0454, which fits molecular formula $C_9H_{10}O_5$, while its fragmentation gave the peaks of m/z 135 $[M–H–H_2O–CO_2]^-$, and 123 $[M–H–C_3H_6O_2]^-$. m/z 179 $[M–H–H_2O]^-$ can be observed at low intensity. This compound was identified as hydroxyphenylpropanoic acid (danshensu) which is the hydrated form of caffeic acid (Liu et al., 2007). Danhensu was previously reported in aqueous infusions of *S. officinalis* (Zimmermann, Walch, Tinzoh, Stühlinger and Lachenmeier, 2011). Compound **6** gave an m/z of 197.0456 fitting molecular formula $C_9H_{10}O_5$, and it was identified as ethyl gallate by using commercial standard. Compound **16** gave an m/z of 137.0242 thus fitting molecular formula $C_7H_6O_3$; its fragmentation ion of m/z 137.0242 yielded a prominent peak at m/z 93 due to the loss of CO_2 from the respective precursor ions. This pattern of fragmentation was characteristic for hydroxybenzoic acid derivatives as well as for other phenolic acids. Hence this compound was identified as hydroxybenzoic acid, which was reported previously in *Lamiaceae* family plants (Hossain, Rai, Brunton, Martin-Diana and Barry-Ryan, 2010) and in *S. officinalis* origin honey (Gašić et al., 2015). Hydroxycinnamic acids such as caffeic (**3**) and rosmarinic (**14**) acids were positively identified on the grounds of their chromatographic retention time and mass spectra in comparison with commercial standards. Rosmarinic acid exhibited two main derivatives, namely, caffeic acid (m/z 179) and the 2-hydroxy derivative of hydrocaffeic acid (m/z = 197). A similar pattern of rosmarinic acid fragmentation was reported previously (Herrero, Plaza, Cifuentes and Ibáñez, 2010) in analysing the extracts of *Lamiaceae* spices. Compound **19** gave an m/z of 373.0926 thus fitting molecular formula $C_{19}H_{18}O_8$. This structure was tentatively assigned to methyl rosmarinate. In general, our identification data is in agreement with the previously published results which reported that the majority of phenolic acids in *Salvia* spp. plants are exclusively caffeic acid derivatives predominantly occurring in the dimeric form as rosmarinic acid or monomeric caffeic acid (Lu and Foo, 2002).

Caffeic and rosmarinic acids are phenolic compounds found in various plants including such commonly used Lamiaceae herbs as basil, oregano, lemon balm, rosemary, sage and others (Lee, 2010). Rosmarinic acid is one of the best-known natural antioxidants present in various botanicals; for instance, its antioxidant capacity was shown to be more that 3-fold higher than that of trolox; it also inhibits xanthine oxidase and can be expected to scavenge the surplus free radicals in the body. In addition, rosmarinic acid reduces Mo (VI) to Mo (V) and therefore may prevent the production of free radicals caused by the polyvalent metal catalysts of oxidation (Petersen and Simmonds, 2003). Caffeic and rosmarinic acids have been extensively reported in *S. officinalis* (Hossain, Rai, Brunton, Martin-Diana and Barry-Ryan, 2010; Lu and Foo, 1999, 2001, 2002; Zimmermann, Walch, Tinzoh, Stühlinger and Lachenmeier, 2011).

Compound **10** showed an m/z value of 461.0727 (fitting molecular formula $C_{21}H_{18}O_{12}$) and fragment m/z 285 corresponding to the aglycone, luteolin. Fragment loss indicates glucuronic acid, and, therefore this compound was identified as flavone luteolin glucuronide. The identity was also confirmed by commercial standard. Luteolin 7-*O*-glucuronide was previously reported in *Salvia* spp. (Cvetkovikj et al., 2013; Lu and Foo, 2001; Zimmermann, Walch, Tinzoh, Stühlinger and Lachenmeier, 2011). Compound **13** demonstrated an m/z value of 445.0778 (fitting molecular formula $C_{21}H_{18}O_{11}$) and fragment m/z 269 corresponding to apigenin. Based on this data and by comparing it with the standard, this compound was identified as apigenin-7-*O*-β-D-glucuronide, which was also reported previously in *Salvia* spp. (Cvetkovikj et al., 2013) and *S. officinalis* tea infusions (Walch, Tinzoh, Zimmermann, Stühlinger and Lachenmeier, 2011). Compound **20** had an m/z value of 285.0406 (fitting molecular formula $C_{15}H_{10}O_6$). This compound was confirmed by comparing it with the commercial standard and identified as luteolin. Compound **21** gave an m/z value of 315.0503 fitting molecular formula $C_{16}H_{12}O_7$. Based on this data and by comparing it with the commercial standard, this compound was identified as 3',4',5,7-tetraxydroxy-3-methoxyflavone. Compound **22** had an m/z value of 269.0453 (fitting molecular formula $C_{15}H_{10}O_5$). Based on this data and by comparing it with the commercial standard, this compound was identified as apigenin. Compound **23** gave an m/z value of 299.0560 fitting molecular formula $C_{16}H_{12}O_6$. The fragmentation of m/z 299.0560 gave prominent peaks at m/z 285 corresponding to luteolin. Based on this data and by comparing it with the commercial standard, this compound was identified as luteolin derivative diosmetin, which was reported in *S. candidissima* (Topçu, Tan, Ulubelen, Sun & Watson, 1995). Compound **27** had an m/z value of 283.0611 (fitting molecular formula $C_{16}H_{12}O_5$). This structure was tentatively assigned to genkwanin.

The exact molecular mass of compound **5** was 609.1462, while the main fragment was observed at 301 $[M–H–C_{12}H_{20}O_9]^-$ in the negative ion spectrum. The suggested molecular formula $C_{27}H_{30}O_{16}$ fits rutin, which was confirmed by using the reference compound. In addition, compound **7** showed a precursor ion at m/z 593.1513 ($C_{27}H_{30}O_{15}$), and its MS/MS spectrum presented a product ion at m/z 285, which may be attributed to the elimination of rutinoside residue. Therefore, this peak was identified as kaempferol-*O*-rutinoside. Compound **8** had an m/z value of

463.0876 (fitting molecular formula $C_{21}H_{20}O_{12}$). This compound was confirmed by comparing it with the commercial standard and identified as hyperoside. Compound **11** had an m/z value of 477.1036 (fitting molecular formula $C_{22}H_{22}O_{12}$) and a fragment m/z 315. It was tentatively identified as isorhamnetin-glucoside (Carazzone, Mascherpa, Gazzani and Papetti, 2013). Compound **12** gave an m/z value of 477.0674 fitting molecular formula $C_{21}H_{18}O_{13}$. This compound had a fragment m/z of 301 corresponding to quercetin. Based on this data and by comparing it with the commercial standard, this compound was identified as quercetin 3-glucuronide (miquelianin), which was identified previously in *S. blepharophylla* (Bisio, Romussi, Ciarallo and De Tommasi, 1997). Compound **26** gave an m/z value of 315.0503 thus fitting molecular formula $C_{16}H_{12}O_7$. Based on this data and by comparing it with the commercial standard, this compound was identified as rhamnetin. Compound **25** had an m/z value of 331.0822 thus fitting molecular formula $C_{17}H_{16}O_7$. Based on this data, it was identified as 5,7,2'-trihydroxy-5',6'-dimethoxyisoflavone. This compound was identified previously in *S. plebeian* by R. Brown (Ma et al., 2014). Compounds **28** and **29** gave m/z values of 329.1759 and 331.1919 fitting molecular formulas $C_{20}H_{26}O_4$ and $C_{20}H_{28}O_4$, respectively. Based on this data and by comparing them with the commercial standard, these compounds were identified as phenolic terpenes carnosol and carnosic acid.

Some compounds present in sage extracts gave major fragment ions following a loss of CO_2 as seen in all the phenolic acids. Compound **30** gave an m/z value of 345.2075 thus fitting molecular formula $C_{21}H_{30}O_4$. This compound was identified as methyl carnosate. It produced two major fragments: m/z 301 due to the loss of CO_2 with the further loss of the methyl group producing m/z 286 ions. This fragmentation pattern was in agreement with that reported by Herrero, Plaza, Cifuentes and Ibáñez (2010) in analysing the phenolic antioxidant compounds of rosemary extracts. Carnosol, carnosic acid and methyl carnosate were reported previously in *S. officinalis* (Cvetkovikj et al., 2013; Farhat, Chaouch-Hamada, Sotomayor, Landoulsi and Jordán, 2014; Upadhyay and Mishra, 2014; Walch, Tinzoh, Zimmermann, Stühlinger and Lachenmeier, 2011; Zimmermann, Walch, Tinzoh, Stühlinger and Lachenmeier, 2011). Compound **18** with an m/z value of 493.1136 fitting molecular formula $C_{26}H_{22}O_{10}$ was tentatively identified as salvianolic acid *A* which was previously reported in *S. miltiorrhiza* roots by Li et al. (2015).

To the best of our knowledge ethyl gallate, 3',4',5,7-tetraxydroxy-3-methoxyflavone, hyperoside and isorhamnetin-glucoside have not been reported previously in *Salvia* spp. extracts. Table 4.5 shows the content of ethyl gallate and 3',4',5,7-tetrahydroxy-3-methoxyflavone isolated from *Salvia* spp. by different solvents. Hyperoside was identified in ethanolic extracts of *S. amplexicaulis* and *S. glutinosa*, and isorhamnetin-glucoside was traced in ethanolic extracts of *S. glutinosa*.

Finally, the extracts were screened by the HPLC-UV-DPPH$^{\bullet}$ on-line method in order to track which constituents possess antioxidant capacity. Combined UV (positive signals) and DPPH$^{\bullet}$ quenching (negative signals) chromatograms of

selected *Salvia* spp. extracts are presented in Fig. 4.5. Danshensu (**2**), caffeic acid (**3**), luteolin-7-*O*-β-D-glucuronide (**10**), quercetin 3-glucuronide (**12**), rosmarinic acid (**14**), hydroxybenzoic acid (**16**), methyl rosmarinate (**19**), luteolin (**20**) and carnosic acid (**29**) were determined to be the most active radical scavengers among the investigated extracts.

Table 4.4. Identification data of *Salvia* spp. compounds by UPLC-Q/TOF

Peak No.	Compound	Molecular formula	RT on UPLC	m/z, $[M–H]^-$	MS fragments
1.	Unknown	$C_9H_{18}O_8$	2.4	253.0925	133, 135, 161, 181
2.	Danshensu[b]	$C_9H_{10}O_5$	2.9	197.0454	109, 123, 135, 151, 179
3.	Caffeic acid[a]	$C_9H_8O_4$	3.8	179.0349	nd
4.	Unknown	$C_{18}H_{28}O_9$	3.9	387.1658	135, 163, 179, 207, 311
5.	Rutin[a]	$C_{27}H_{30}O_{16}$	4.3	609.1462	301
6.	Ethyl gallate[a]	$C_9H_{10}O_5$	4.4	197.0456	124, 125, 167
7.	Kaempferol-*O*-rutinoside[b]	$C_{27}H_{30}O_{15}$	4.4	593.1513	285
8.	Hyperoside[a]	$C_{21}H_{20}O_{12}$	4.5	463.0876	301
9.	Unknown	$C_{21}H_{20}O_{11}$	4.5	447.0926	285
10.	Luteolin-7-*O*-β-D-glucuronide[a]	$C_{21}H_{18}O_{12}$	4.5	461.0727	285
11.	Isorhamnetin-glucoside[b]	$C_{22}H_{22}O_{12}$	4.6	477.1036	299, 300, 315
12.	Quercetin 3-glucuronide[a]	$C_{21}H_{18}O_{13}$	4.7	477.0674	161,301, 315, 359
13.	Apigenin-7-*O*-β-D-glucuronide[a]	$C_{21}H_{18}O_{11}$	4.9	445.0778	269
14.	Rosmarinic acid[a]	$C_{18}H_{16}O_8$	5.0	359.0775	161, 179, 197
15.	Unknown	$C_{25}H_{30}O_{13}$	5.2	537.1615	133, 135, 161, 179, 295, 359
16.	Hydroxybenzoic acid[b]	$C_7H_6O_3$	5.3	137.0242	93, 108
17.	Unknown	$C_{18}H_{26}O_9$	5.3	385.1500	137, 179
18.	Salvianolic acid A[b]	$C_{26}H_{22}O_{10}$	5.5	493.1136	135, 185, 295, 313, 461
19.	Methyl rosmarinate[b]	$C_{19}H_{18}O_8$	5.6	373.0926	135, 175, 179, 197
20.	Luteolin[a]	$C_{15}H_{10}O_6$	5.7	285.0406	133,151, 197, 213
21.	3',4',5,7-Tetrahydroxy-3-methoxyflavone[a]	$C_{16}H_{12}O_7$	5.8	315.0503	297
22.	Apigenin[a]	$C_{15}H_{10}O_5$	6.2	269.0453	117, 151
23.	Diosmetin[a]	$C_{16}H_{12}O_6$	6.4	299.0560	285
24.	Unknown	$C_{29}H_{26}O_{12}$	6.4	565.1346	133, 135, 161, 359
25.	5, 7, 2`-Trihydroxy-5`,6`-dimethoxyisoflavone[b]	$C_{17}H_{16}O_7$	6.4	331.0822	135, 165, 180, 195, 316
26.	Rhamnetin[a]	$C_{16}H_{12}O_7$	7.3	315.0503	83, 145, 187
27.	Genkwanin[b]	$C_{16}H_{12}O_5$	7.4	283.0611	151, 211, 239, 268
28.	Carnosol[a]	$C_{20}H_{26}O_4$	8.3	329.1759	285
29.	Carnosic acid[a]	$C_{20}H_{28}O_4$	8.7	331.1919	244, 287
30.	Methyl carnosate[b]	$C_{21}H_{30}O_4$	9.0	345.2075	283, 286, 301

Abbreviations: RT: retention time, min; nd: not detected.
[a] Confirmed by a standard.
[b] Confirmed by the indicated scholarly literature reference

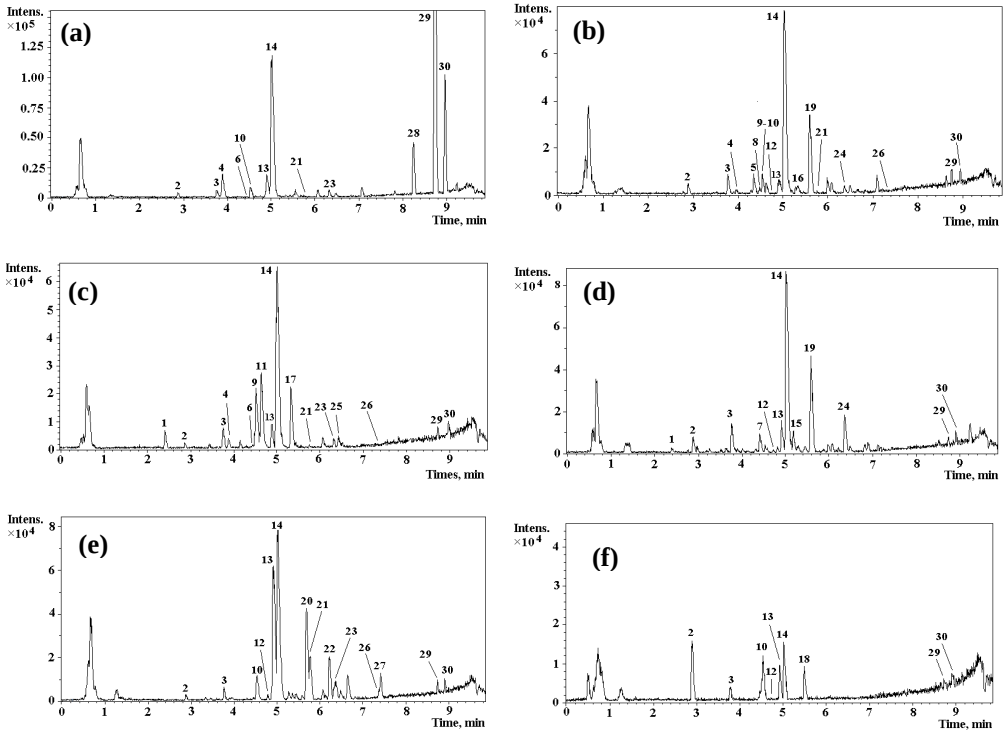


Fig. 4.3. Selected chromatograms of *Salvia* spp. extracts obtained by UPLC-Q/TOF: a) SO-E; b) SAm-E; c) SG-E; d) SN-E; e) SSc-E; f) SF-W

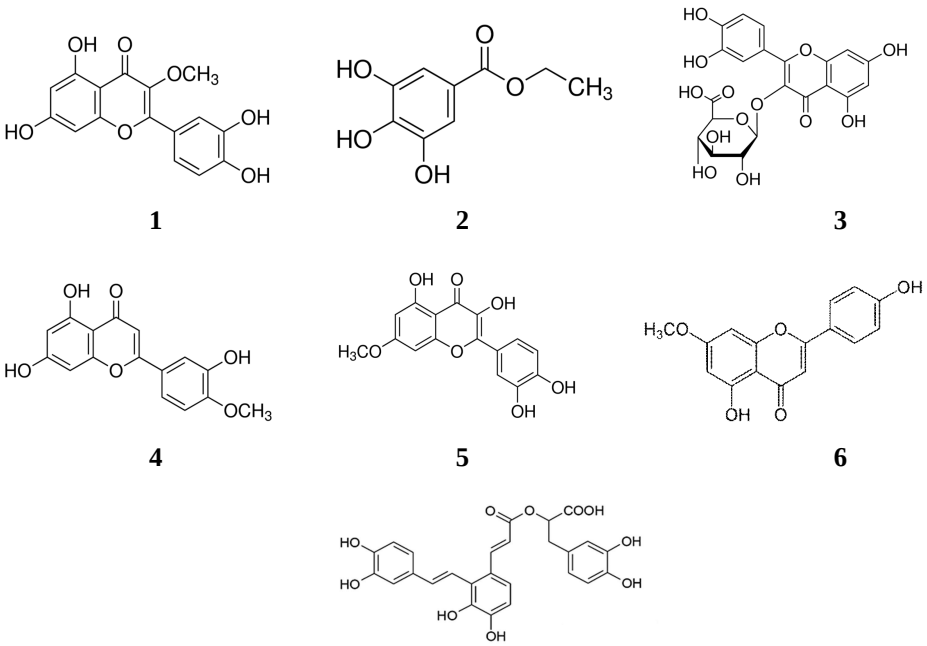


Fig. 4.4. Chemical structures of the identified compounds in *Salvia* spp. extracts: 1 – 3',4',5,7-tetraxydroxy-3-methoxyflavone; 2 – ethyl gallate; 3 – quercetin 3-glucuronide (miquelianin); 4 – diosmetin; 5 – rhamnetin; 6 – genkwanin; 7 – salvianolic acid *A*

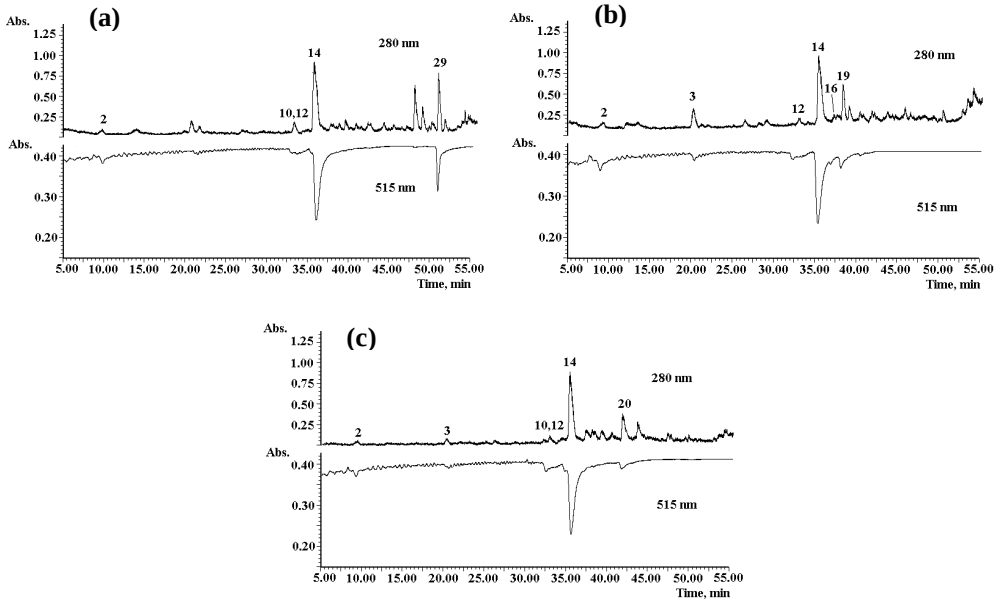


Fig. 4.5. HPLC-UV-DPPH•-scavenging chromatographic profiles of selected *Salvia* spp. extracts: a) SO-E; b) SAm-E; c) SSc-E; Abs: absorption.

4.2.3. Quantitative Analysis in Various *Salvia* spp. Plants by Using TQ-S

Quantification data of the main compounds isolated from various *Salvia* spp. plants is evaluated by using the available commercial standards, and the results are expressed in mg/g extract (DWE) (Fig. 4.6 (a–d)) whereas the concentration of other compounds is recalculated and expressed in μg of dried plant material (DWP, Table 4.5). In addition, the concentration of the main compounds isolated by all the solvents is summarised in Fig. 4.6 (e–h).

It may be evidently observed that rosmarinic acid is the major quantitative constituent (Table 4.5) in various *Salvia* spp. plants. Its concentration in *Salvia* spp. extracts varied from 3.42 to 82.99 mg/g DWE and from 5,024 to 30,017 μg/g DWP. The highest concentration of this compound was determined in *S. officinalis* extracts followed by *S. amplexicaulis*, *S. forsskaolii*, *S. verticillata* and *S. nemorosa*. The highest total concentration of rosmarinic acid isolated by CO_2, ethanol and water was also demonstrated by *S. officinalis* (30.66 mg/g DWP). Rosmarinic acid was also reported in *S. officinalis* as the main compound by Farhat, Chaouch-Hamada, Sotomayor, Landoulsi and Jordán (2014): its concentration was the highest (11,723 μg/g DWP) during the vegetative stage in Soliman. In another study, the content of rosmarinic acid in *S. officinalis* samples growing in different habitats was even higher, reaching up to 18.40 mg/g DW (Farhat, Landoulsi, Chaouch-Hamada, Sotomayor and Jordán, 2013). According to Cvetkovikj et al. (2013) who examined three *Salvia* spp. plants, the highest content of rosmarinic acid was present in *S. officinalis* (25.98 mg/g DWP) followed by *S. fruticosa* (10.72 mg/g DWP) and *S. pomifera* (6.74 mg/g DWP). The concentrations of rosmarinic acid in *S. sclarea* and *S. glutinosa* reported by Bandoniene, Murkovic, and Venskutonis (2005) were 41.1 mg/g DWP and 47.3 mg/g DWP, respectively, which is higher than the results obtained in our study.

The content of caffeic acid was also high in the ten analysed *Salvia* spp. plants. Its concentration varied from 0.51 to 3.72 mg/g DWE and from 171 to 1,142 μg/g DWP. The highest concentrations of caffeic acid were determined in the ethanolic extract of *S. nemorosa* followed by *S. forsskaolii*, *S. amplexicaulis*, *S. verticillata* and *S. austriaca*. The highest total concentration of this compound isolated by the all solvents was determined in *S. verticillata* (1.63 mg/g DWP) followed by *S. forsskaollii* (1.52 mg/g DWP) and *S. nemorosa* (1.47 mg/g DWP). Farhat, Chaouch-Hamada, Sotomayor, Landoulsi and Jordán (2014) measured the concentration of caffeic acid in methanolic extracts of *S. officinalis* isolated in the course of vegetative, flowering and fruiting phenological stages of the plant: it varied from 251 μg/g DWP to 352 μg/g DWP. The concentration of apigenin-7-*O*-β-D-glucuronide in various *Salvia* spp. extracts ranged from 0.80 to 37.43 mg/g DWE and from 217 to 6,789 μg/g DWP: the highest concentration of this constituent was found in the ethanolic extract of *S. sclarea* followed by the water extract of *S. sclarea*, the ethanolic extract of *S. pratensis* and the ethanolic extract of *S. forsskaolii*. The highest total concentration of apigenin-7-*O*-β-D-glucuronide isolated by CO_2, ethanol and water was also in *S. sclarea* (9.64 mg/g DWP). Walch, Tinzoh, Zimmermann, Stühlinger and Lachenmeier (2011) reported that the concentration of apigenin-glucuronide in *S. officinalis* tea infusions was from 8.6

mg/L to 41.1 mg/L. However, previously published results are difficult to compare due to different extraction methodologies and sample preparation techniques.

Carnosic acid concentration in various *Salvia* spp. extracts was from 0.97 to 24.24 mg/g DWE and from 193 to 8,294 μg/g DWP. The highest concentrations of carnosic acid were determined in the ethanolic extract of *S. officinalis* followed by the CO_2 extract of *S. officinalis*, the water extract of *S. nemorosa*, the water extract of *S. officinalis* and the water extract of *S. verticillata*. The highest total concentration of this compound isolated by CO_2, ethanol and water was also demonstrated by *S. officinalis* (9.15 mg/g DWP). Carnosol was detected only in the CO_2 and ethanolic extracts of *S. officinalis* (53.04 μg/g DWP and 1,155 μg/g DWP, respectively). The concentration of methyl carnosate was determined by using the commercial standard of carnosic acid: the highest concentrations of this compound were found in the CO_2 and ethanolic extracts of *S. officinalis*, 371 DWP and 987 μg/g DWP, respectively. Farhat, Chaouch-Hamada, Sotomayor, Landoulsi and Jordán (2014) studied variations in the content of phenolic diterpenes in *S. officinalis* depending on the plant collection site and the phenological stage. The highest content of carnosic acid (3,906 and 4,149 μg/g DWP), carnosol (8,405 and 7,726 μg/g DWP) and methyl carnosate (7,745 and 6,137 μg/g DWP) was observed during the flowering period of the plant. Phenolic diterpenes (1,056.90–1,148.42 μg/g DW) was reported as the most represented class of compounds in *S. verbenaca* (Farhat, Chaouch-Hamada, Sotomayor, Landoulsi and Jordan, 2015).

The highest concentrations of luteolin-7-*O*-β-D-glucuronide (in μg/g DWP) were determined in *S. officinalis* (ethanolic extracts: 1,479; water extracts: 4,364) followed by *S. forsskaolii*, *S. sclarea*, *S. nemorosa* and *S. verticillata*. Martins et al. (2015) reported the content of luteolin-7-*O*-glucuronide in aqueous (decoction) extract of *S. officinalis* at the level of 130 mg/g DWP. The highest concentrations of quercetin 3-glucuronide (in μg/g DWP) were observed in the water extracts of *S. officinalis* (1,270) followed by the water extracts of *S. forsskaolii* (649) and the ethanolic extracts of *S. sclarea* (297). The traces of 3',4',5,7-tetraxydroxy-3-methoxyflavone were determined in the ethanolic extracts of *S. amplexicaulis*, *S. austriaca*, *S. glutinosa*, *S. officinalis*, *S. pratensis*, *S. sclarea*, *S. stepposa* and *S. verticillata* and in the water extracts of *S. amplexicaulis*, *S. pratensis*, *S. sclarea* and *S. stepposa*. Ethyl gallate was determined in all the CO_2 extracts and in the ethanolic extracts of *S. glutinosa*, *S. officinalis* and *S. pratensis*. The traces of diosmetin were also determined in the ethanolic extracts of *S. glutinosa*, *S. officinalis* and *S. sclarea*, whereas rhamnetin was detected in the ethanolic extracts of *S. amplexicaulis*, *S. forsskaolii*, *S. glutinosa*, *S. sclarea*, *S. stepposa* and in the water extracts of *S. amplexicaulis*, *S. austriaca*, *S. glutinosa*, *S. nemorosa*, *S. pratensis*, *S. sclarea* and *S. stepposa*. To the best of our knowledge, quercetin 3-glucuronide, 3',4',5,7-tetraxydroxy-3-methoxyflavone, diosmetin and rhamnetin have never been reported previously in *Salvia* spp. plants.

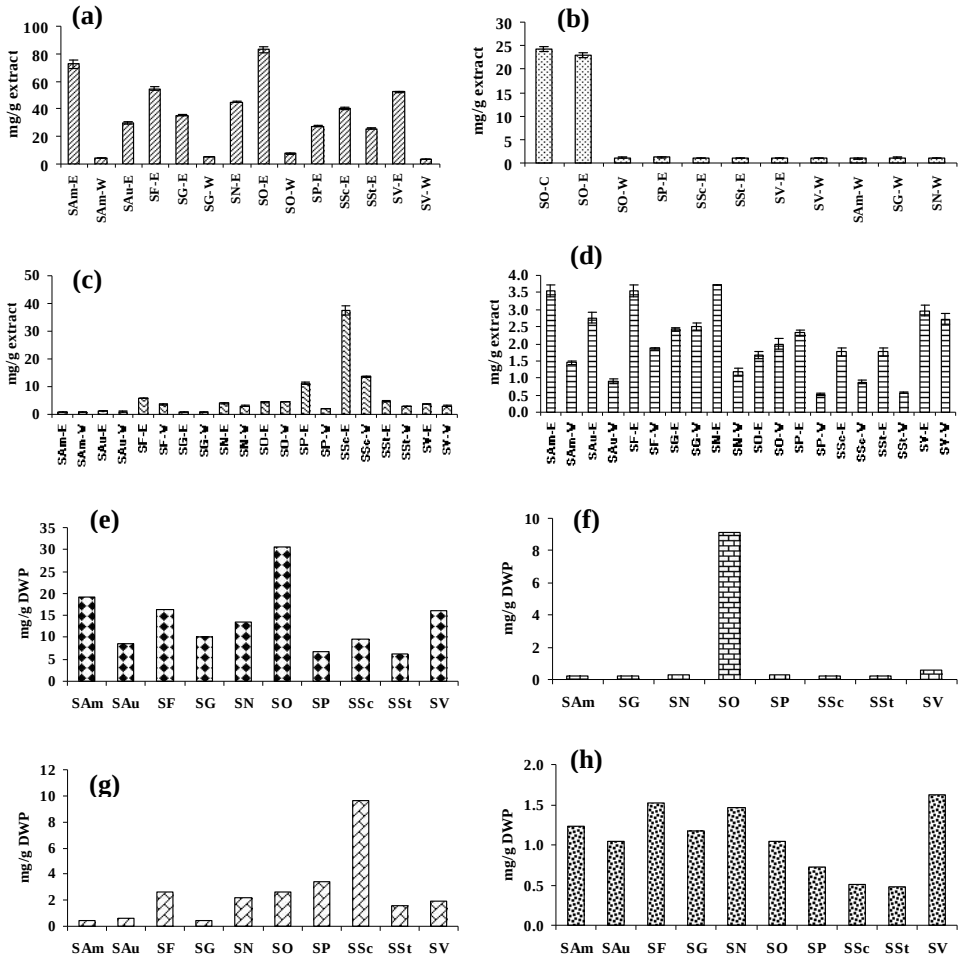


Fig. 4.6. Concentration of the main compounds in selected *Salvia* spp. plants: expressed as mg/g extract: (a) rosmarinic acid; (b) carnosic acid; (c) apigenin-7-*O*-β-D-glucuronide; (d) caffeic acid; isolated by the all solvents (CO_2, ethanol and water) and expressed in mg/g of dry weight plant (DWP): (e) rosmarinic acid; (f) carnosic acid; (g) apigenin-7-O-β-D-glucuronide; (h) caffeic acid

Table 4.5. Content of the main phytochemicals isolated from *Salvia* spp. plants by different solvents, in μg/g DWP

Samples	Compounds											
	Caffeic acid (LOD 1.76; LOQ 5.86)	Ethyl gallate (LOD 13.39; LOQ 44.65)	Quercetin 3-glucuronide (LOD 9.86; LOQ 32.88)	Luteolin-7-*O*-β-D-glucuronide (LOD 12.53; LOQ 41.76)	Apigenin-7-*O*-β-D-glucuronide (LOD 3.51; LOQ 11.70)	Rosmarinic acid (LOD 24.60; LOQ 81.99)	3',4',5,7-Tetrahydroxy-3-methoxyflavone (LOD 1.67; LOQ 5.56)	Diosmetin (LOD 4.64; LOQ 15.47)	Rhamnetin (LOD 2.24; LOQ 7.46)	Carnosol (LOD 6.60; LOQ 21.98)	Carnosic acid (LOD 6.77; LOQ 22.56)	Methyl carnosate (LOD 6.77; LOQ 22.56)
SAm-E	905±43^{e}	nd	tr.	tr.	217±15^{a}	18464±719^{g}	tr.	nd	tr.	nd	tr.	tr.
SAm-W	470±16^{d}	nd	tr.	nd	284±4^{a}	1460±20^{ab}	tr.	nd	tr.	nd	313±23^{a}	tr.
SAu-E	812±48^{cd}	nd	nd	tr.	367±5^{a}	8781±178^{c}	tr.	nd	nd	nd	tr.	tr.
SAu-W	370±28^{c}	nd	tr.	tr.	419±16^{b}	nd	nd	nd	tr.	nd	tr.	tr.
SF-E	1076±52^{f}	nd	tr.	1344±52^{c}	1733±15^{d}	16617±413^{f}	nd	nd	tr.	nd	tr.	tr.
SF-W	675±6^{e}	nd	649±5^{a}	2612±128^{c}	1356±74^{e}	tr.	nd	nd	nd	nd	tr.	tr.
SG-E	630±14^{b}	tr.	nd	nd	242±4^{a}	9225±111^{c}	tr.	tr.	tr.	nd	tr.	tr.
SG-W	776±31^{f}	nd	nd	nd	247±15^{a}	1524±18^{b}	nd	nd	tr.	nd	350±33^{ab}	tr.
SN-E	1142±3^{f}	nd	tr.	nd	1268±59^{c}	13837±212^{d}	nd	nd	nd	nd	tr.	tr.
SN-W	520±46^{d}	nd	tr.	717±26^{a}	1343±27^{e}	tr.	nd	nd	tr.	nd	464±6^{d}	tr.
SO-C	tr.	tr.	nd	nd	tr.	nd	nd	nd	nd	53.04±4.11	904±23	371±6
SO-E	605±34^{b}	tr.	nd	1479±39^{d}	1632±100^{d}	30017±734^{h}	tr.	tr.	nd	1155±29	8294±171^{b}	987±3
SO-W	754±63^{f}	nd	1270±89^{b}	4364±326^{e}	1698±9^{f}	2865±62^{c}	nd	nd	nd	nd	433±34^{cd}	tr.
SP-E	580±23^{b}	tr.	nd	tr.	2838±100^{e}	6836±129^{b}	tr.	nd	nd	nd	295±3^{a}	tr.
SP-W	218±14^{ab}	nd	tr.	tr.	906±1^{c}	nd	tr.	nd	tr.	nd	tr.	tr.

Table 4.5. Continued

Samples	Compounds											
	Caffeic acid (LOD 1.76; LOQ 5.86)	Ethyl gallate (LOD 13.39; LOQ 44.65)	Quercetin 3-glucuronide (LOD 9.86; LOQ 32.88)	Luteolin-7-*O*-β-D-glucuronide (LOD 12.53; LOQ 41.76)	Apigenin-7-*O*-β-D-glucuronide (LOD 3.51; LOQ 11.70)	Rosmarinic acid (LOD 24.60; LOQ 81.99)	3',4',5,7-Tetrahydroxy-3-methoxyflavone (LOD 1.67; LOQ 5.56)	Diosmetin (LOD 4.64; LOQ 15.47)	Rhamnetin (LOD 2.24; LOQ 7.46)	Carnosol (LOD 6.60; LOQ 21.98)	Carnosic acid (LOD 6.77; LOQ 22.56)	Methyl carnosate (LOD 6.77; LOQ 22.56)
SSc-E	319±24^{a}	nd	297±20	932±51^{a}	6789±296^{f}	7323±84^{b}	tr.	tr.	tr.	nd	195±14^{a}	tr.
SSc-W	266±14^{b}	nd	tr.	1281±55^{b}	4132±41^{g}	tr.	tr.	nd	tr.	nd	tr.	tr.
SSt-E	764±1^{c}	nd	nd	nd	912±42^{b}	5024±109^{a}	tr.	nd	tr.	nd	193±9^{a}	tr.
SSt-W	171±8^{a}	nd	nd	tr.	905±8^{c}	tr.	tr.	nd	tr.	nd	tr.	tr.
SV-E	879±42de	nd	nd	1210±3^{b}	1102±54^{bc}	15436±112^{e}	tr.	nd	nd	nd	294±7^{a}	tr.
SV-W	1104±61^{g}	nd	tr.	3826±53^{d}	1220±58^{d}	1383±32^{a}	nd	nd	nd	nd	402±6^{bc}	tr.

*Content of the main phytochemicals isolated from *Salvia* spp. plants by SFE-CO_2 was not determined at all or was only determined in trace and thus was not included in this table. Values represented as mean±standard deviation (n=3); a–h: the mean values followed by different letters are significantly different ($P<0.05$) between one type of solvent and a different *Salvia* spp plant; nd: not detected; tr.: trace.

4.3. Comparison of the Composition of Volatile Compounds in 10 *Salvia* spp. Plants Isolated by Using Various Methods

This section presents and discusses the results obtained in our study by using L-N and SFE-CO_2 extractions in the light of the previously published data on the selected *Salvia* spp. plants. However, it should be noted that such a comparison may be regarded as rather conditional because the majority of the previously performed studies reported the content of volatile compounds in GC area percentages (sometimes by using GC/MS total ion chromatogram data), i.e. these researches were conducted without using ISTD and without calculating the response factors for individual compounds. The shortcomings of such data handling as well as the recommendations for obtaining more reliable data on volatiles were prepared a few years ago by a group of experts (Cachet et al., 2016). Data handling in our study is based on the above mentioned recommendations, and the concentration of volatiles is expressed in mg per kg of DWP.

Volatile constituents of *Salvia* spp. plants were extracted by using the L-N and SFE-CO_2 methods. However, it should be noted that supercritical CO_2 also dissolves lipophilic non-volatile constituents, such as waxes, chlorophylls, carotenoids and others which cannot be directly analysed and quantified by the GC; meanwhile, the principle of L-N is similar to that of hydrodistillation which is usually applied for isolating the EOs. The difference is that hydrodistilled volatile compounds in the L-N apparatus are simultaneously extracted into the organic solvents; therefore, this method is convenient when a limited amount of botanical material is available for the analysis and when the amount of volatiles in such a material is low (when difficulties of collecting in the typical Clevenger apparatus arise).

The composition of volatile constituents in the CO_2 and L-N extracts of 10 studied *Salvia* spp. plants is provided in Tables 4.6 and 4.7, respectively. Thus the total amount of volatiles extracted by SFE-CO_2 and L-N from 10 different *Salvia* spp. plants was in the range of 112–3,992 and 347–21,508 mg/kg, respectively. The differences between the methods were highly dependent on the plant species. The amount of volatiles extracted by L-N method from *S. amplexicaulis* was only 1.44 times higher than the result achieved with SFE-CO_2; whereas, in the case of *S. officinalis*, L-N gave a 5.66 times higher yield of volatile constituents than SFE-CO_2.

It may be observed that L-N was a considerably more efficient method for the extraction of mono and sesquiterpenes which are typical EO constituents of many aromatic herbs. The total amount of monoterpenes extracted by using the L-N method was from 8 (*S. glutinosa*) to 195 times (*S. sclarea*) higher than the values shown by the SFE-CO_2 method. Moreover, monoterpene hydrocarbons and oxygenated monoterpenes were found in the CO_2 extracts of only four and five studied *Salvia* spp. plants, respectively. In the extracts of other *Salvia* spp. plants, the compounds belonging to these groups were below the threshold of detection at the applied GC analysis parameters. Monoterpenes were detected and quantified in all the L-N extracts except for *S. pratensis* (they were not detected in the extract of this species). The total amount of sesquiterpenes extracted by L-N was from 3.4 (*S.*

nemorosa) to 29.8 times (*S. amplexicaulis*) higher than extracted by SFE-CO_2. Sesquiterpenes were detected and quantified in all the extracts except for sesquiterpene hydrocarbons in the CO_2 extract of *S. amplexicaulis*.

The compounds belonging to other classes of compounds were present in remarkably smaller amounts both in CO_2 and L-N extracts, except for diterpenes manool and sclareol which were abundant in *S. officinalis* and *S. sclarea*, respectively. However, the main fragrant chemical compound of *S. sclarea*, sclareol, was comparatively more effectively isolated when employing SFE-CO_2 than manool: L-N delivered a 1.76 and 4 times higher yields of sclareol and manool, respectively, than SFE-CO_2. The ungrouped constituents ('others') were also better extracted by L-N.

Alkanes are the only class of compounds which were better extracted by SFE-CO_2 than by L-N; the total amount of alkanes extracted by using the SFE-CO_2 method was from 3.5 (*S. sclarea*) to 40.8 times (*S. officinalis*) higher than by L-N. This group consists mainly of longer-chain hydrocarbons ($C \geq 16$), such as hexadecane, heptacosane, octacosane and others.

As it was already indicated, remarkably higher amounts of volatile compounds were extracted from various *Salvia* spp. plants when employing L-N (Table 4.7) rather than SFE-CO_2 (Table 4.6). Most likely, volatile constituents may be partially lost with the flow of CO_2 during the collection of extracts after depressurising the extraction system. It should be noted that, in many previously performed researches, the extracts isolated from the EO-bearing plants by SFE-CO_2 have been collected in some organic solvent, e.g. hexane. In addition, the composition of volatile compounds in the majority of the previously studied CO_2 extracts of aromatic herbs was expressed in the GC area percentage (Reverchon and De Marco, 2006), which is not reliable for evaluating the efficiency of the method for isolating volatiles. In our study, one of the objectives was to avoid the use of hazardous organic solvents which should be necessarily removed from the final product. Such an approach complies with the principles of green process development for extracting lipophilic plant constituents, including EO constituents. However, the obtained results demonstrate that in terms of the yields of volatile constituents, SFE-CO_2 should be supplemented with fraction separators or a specially designed trap for the volatiles, e.g. cooled at low temperature loops.

Among other reasons which may be considered for explaining the differences between the applied extraction methods are the risks of the formation of thermal artefacts during hydrodistillation (boiling in water) and the possibilities of hydrolysis of glycosidically bound EO compounds which afterwards may be released into the hydrodistilled volatile fraction. However, the formation of such artefacts is accepted as a result of the traditional process (Sgorbini et al., 2015); whereas, what regards glycosidically bound compounds, in a recent study by Sgorbini et al. (2015), it was concluded that glycosidically bound forms which are not recovered during hydrodistillation may be of considerable strength. Consequently, it is rather unlikely that these two reasons may play an important role in the remarkable differences between L-N and SFE-CO_2 methods.

S. officinalis yielded the highest amount of volatile compounds when employing both SFE-CO_2 and L-N methods. The qualitative and quantitative composition of the main *S. officinalis* constituents was completely different compared with the other investigated species. For instance, the most abundant in *S. officinalis* L-N extracts α-thujone and 1,8-cineole were not detected in any of the other researched *Salvia* spp. plant, whereas β-thujone, camphor and manool were found in trace amounts only in one or two other species. In the case of CO_2 extracts, the above mentioned as well as many other typical EO compounds were found solely in *S. officinalis* (see the footnote of Table 4.6).

In general, regarding the dominating volatiles in *S. officinalis*, our results are in agreement with those obtained by Santos-Gomes and Fernandes-Ferreira (2003), Avato, Fortunato, Ruta and D'Elia (2005) and Pinto, Salgueiro, Cavaleiro, Palmeira and Gonçalves (2007) who reported oxygenated monoterpenes as the most common compounds in *S. officinalis* EO. Lima et al. (2004) reported *cis*-thujone (17.4%), α-humulene (13.3%), 1,8-cineole (12.7%), *β*-caryophyllene (8.5%) and borneol (8.3%) as the quantitatively main EO constituents in *S. officinalis* cultivated in Portugal. These findings are also in agreement with the previously reported data by Marino, Bersani and Comi (2001), except for manool which was not detected in their study, while in some *Salvia* plants analysed in our study this compound was present in reasonable amounts. Thus the amount of manool isolated from *S. officinalis* by employing SFE-CO_2 and L-N was 689 mg/kg and 2,751 mg/kg, respectively; from *S. stepposa* and *S. sclarea* when employing L-N the yields were 96 mg/kg and 44 mg/kg, respectively. Previously, manool was reported in relatively large percentage rates in *S. officinalis* originating from Cuba (15%) (Pino, Estarrón and Fuentes, 1997). The amount of sesquiterpenes in *S. officinalis* analysed in our study was also high both in SFE-CO_2 and L-N extracts, 1,101 and 3,773 mg/kg, respectively.

SFE-CO_2 extracts of *S. officinalis* were previously studied by Mićić, Lepojević, Jotanoviae, Tadić and Pejović (2011) and Occhipinti, Capuzzo, Arceusz and Maffei (2014); however, in these studies, the content of volatiles was expressed in the GC area percent without using ISTD. Thus in the article by Mićić, Lepojević, Jotanoviae, Tadić and Pejović (2011), depending on the CO_2 pressure which varied from 8 to 30 MPa, the major components were α-thujone (16–27%), camphor (16–24%), and γ-elemene (7.5–16%), whereas in the latter article (by Occhipinti, Capuzzo, Arceusz and Maffei, 2014), viridiflorol (22%), camphor (16%) and borneol (8.4%) were the dominating components in the volatile fraction of *S. officinalis* extracted at 25 MPa. As it was already mentioned, it is rather difficult to compare the results obtained by using SFE-CO_2 in our study with the previously published ones mainly due to the essential differences in the analysis procedure and data handling. It is obvious that the content of volatile compounds in SFE-CO_2 extracts expressed in the GC area percentage may provide rather preliminary information which does not reliably evaluate the process in terms of the effectiveness of the isolation of volatiles from DWP. Supercritical CO_2, particularly at higher pressures, may extract large amounts of non-volatile compounds, such as waxes, chlorophylls, fixed oils; therefore, GC analysis of such extracts may give

somewhat erroneous results regarding the amount of isolated volatile constituents due to their dilution with high boiling point molecules which may not elute from the chromatographic column. Therefore, the results were obtained by using the internationally recognised practice for the quantification of volatiles. Cachet et al. (2016) provides more comprehensive information both on the composition of volatiles in *Salvia* spp. plants and on the method of their isolation.

Many other factors such as the geographical origin, the environmental conditions and the genotype may also be responsible for high intraspecific variability within the EOs of *Salvia* spp. (Miguel, 2010). These factors may exert a considerable influence on the plant's biosynthetic pathways and, consequently, on the produced amounts of the secondary metabolites, including volatile compounds. However, the reports on the EO's of the numerous *Salvia* spp. plants are very scarce. For instance, only one article was available on the EO composition of *S. pratensis*: Anačkov (2009) reported that sesquiterpenes were predominant compounds in this species (55%), whereas monoterpenes (1.4%) were present in remarkably lower concentrations. *E*-Caryophyllene and caryophyllene oxide were the most abundant constituents in *S. pratensis* analysed in our study; however, these sesquiterpenes are typical EO compounds in many aromatic plants; they were also present in the other studied *Salvia* spp. plants. Askun, Baser, Tumen and Kurkcuoglu (2010) reported that *S. verticillata* collected from different geographical regions accumulated different main constituents, β-pinene (21.4%) and 1,8-cineole (16.1%). In our study the major components of *S. verticillata* L-N extracts were spathulenol (90 mg/kg; the second highest amount after *S. forsskaolii*), followed by germacrene D (56 mg/kg; the highest amount compared to other studied *Salvia* spp. plants) and germacra-4(15),5,10(14)-trien-1-α-ol (37 mg/kg), while in SFE-CO_2 extracts, the dominating constituents were squalene (44 mg/kg) followed by germacrene D (36 mg/kg), phytol (28 mg/kg) and vitamin E (24 mg/kg). Velickovic, Ristic and Velickovic (2003) investigated the chemical composition of the EOs obtained from the flower, the leaf and the stem of *S. glutinosa* originating from the Southeast region of Serbia and, in agreement with our findings, they determined the same main constituents: caryophyllene oxide (28.9% in the leaf), humulene epoxide II (13.8% in the leaf), β-caryophyllene (9.0% in the flower) and α-humulene (5.9% in the flower). Veličković et al. (2012) reported the EO composition of *S. austriaca* and *S. amplexicaulis* from Serbia: spathulenol (17%), hexahydrofarnesyl acetone (14%), isobornyl acetate (13%), palmitic acid (14%), *trans*-phytol (7.4%) and caryophyllene oxide (2.5%) were the main constituents in *S. austriaca*, whereas germacrene D (21%), caryophyllene oxide (15%), β-caryophyllene (9.2%), α-cadinol (6.7%), germacra-4(15),5,10(14)-trien-1α-ol (5.4%) and phytol (5.1%) were dominant in *S. amplexicaulis*. 1,8-cineole, α- and β-thujone and camphor, which are characteristic constituents of several *Salvia* spp. plants, were not present in the EOs of *S. austriaca* and *S. amplexicaulis*.

The chemical composition of *S. sclarea* EO has been also reported. In our study, the main components in L-N extracts of *S. sclarea* were sclareol (4,241 mg/kg), linalyl acetate (411 mg/kg), linalool (296 mg/kg), caryophyllene oxide (272 mg/kg) and (5E,9E)-farnesyl acetone (234 mg/kg). Sclareol was also the major

component in the SFE-CO_2 extract (2,411 mg/kg); however, other volatile compounds of *S. sclarea* which were found in reasonable amounts in L-N extracts in SFE-CO_2 extracts were present in remarkably lower amounts. It was found that *S. sclarea* EO accumulates a high amount of linalool (25%), linalyl acetate (21%), geranyl acetate (6.3%), (E)-β-ocimene (5.7%), and caryophyllene oxide (5.3%) (Askun, Baser, Tumen and Kurkcuoglu, 2010). However, Carrubba, la Torre, Piccaglia and Marotti (2002) reported germacrene D as the main compound (68–69%) in the *S. sclarea* leaves, while linalool (26–29%) and linalyl acetate (35–53%) were major constituents in the inflorescences. Different distribution of the main constituents in EOs isolated from various anatomical parts of *S. sclarea* was determined by Farakaš, Hollá, Tekel, Mellen and Štefánia (2005): linalool (19%), sclareol (16%), and linalyl acetate (14%) were most abundant in EO distilled from inflorescences, whereas gemacrene D (29%), bicyclogermacrene (13%), spathulenol (10%) and β-caryophyllene (6.2%) and were the main constituents in the EO of the leaf.

To the best of our knowledge, the chemical composition of volatiles in *S. forsskaolii* and *S. stepposa* has not been reported previously. Our study shows that *E*-caryophyllene, spathulenol and caryophyllene oxide are the major volatile constituents in *S. forsskaolii*, while caryophyllene oxide is dominating in *S. stepposa.*

Table 4.6. Compounds* isolated from *Salvia* spp. plant material by SFE-CO_2, in mg/kg

Component	KI[th]	KI[♦]	Samples									
			SAm-C	SAu-C	SF-C	SG-C	SN-C	SO-C	SP-C	SSc-C	SSt-C	SV-C
α-Thujene	930	921	0.56	-	-	-	-	6.61	-	-	0.65	-
Sabinene	975	968	0.71	-	-	-	-	9.23	-	-	0.59	-
Thymol	1290	1286	-	13.9	-	-	-	-	-	-	-	-
α-Copaene	1376	1374	-	-	-	-	-	10.0	-	4.94	-	-
Tetradecane	1400	1396	0.59	6.04	-	-	0.95	0.77	-	2.00	0.95	0.73
(E)-Caryophyllene	1419	1413	6.14	-	14.7	5.16	5.92	111	14.0	12.3	0.78	5.74
Coumarin	1439[♠]	1437	-	-	-	5.78	0.66	-	-	-	-	-
α-Humulene	1454	1448	-	-	7.04	5.61	-	395	-	-	0.94	2.48
Germacrene D	1480	1476	3.66	-	9.32	-	-	2.18	0.61	9.55	-	35.7
δ-Cadinene	1523	1515	-	-	4.48	-	-	35.6	0.72	-	-	-
Spathulenol	1578	1574	-	-	11.8	-	-	3.37	-	1.52	4.80	13.1
Caryophyllene oxide	1583	1581	3.85	-	2.75	6.10	71.1	10.2	3.84	14.2	24.7	2.46
Hexadecane	1600	1595	2.94	46.0	-	-	-	7.90	1.66	1.69	-	0.84
Humulene epoxide II	1608	1605	-	-	-	4.54	2.90	39.4	-	-	1.03	2.01
allo-Aromadendrene epoxide	1641	1648	0.62	8.04	1.45	-	-	-	-	-	-	0.89
(Z)-Coniferyl alcohol	1667	1657	-	-	0.63	-	-	-	-	-	-	5.28
14-hydroxy-(Z)-Caryophyllene	1667	1668	-	-	-	-	11.2	1.96	-	-	4.22	0.56
Fluorensadiol	1869	1857	-	-	-	-	-	5.41	2.20	1.86	-	4.99
Phytol	1943	1943	9.15	7.25	9.12	8.29	7.30	41.6	20.0	-	6.72	28.1
Linoleic acid	2133	2135	-	-	-	1.57	-	252	-	-	-	-
Linolenic acid	2134[♣]	2140	-	-	-	-	4.63	17.0	-	-	-	-
(E)-phytol acetate	2218	2216	0.71	0.71	8.11	11.8	3.17	-	0.81	-	2.93	-
Sclareol	2223	2227	-	-	-	-	-	-	-	2411	-	-
Labd-7,13-dien-15-ol	2292	2287	-	-	-	-	-	15.4	-	7.82	-	-
(Z)-9-Octadecenamide	2397[♣]	2408	15.9	8.00	27.5	-	18.5	285	-	-	-	1.23
Glycerol 2-palmitate	2488[♣]	2509	0.68	5.30	-	-	4.03	21.4	4.60	0.86	-	1.11
Hexacosane	2600	2590	2.96	24.1	21.3	16.9	11.4	67.7	14.1	6.63	12.9	14.3
Unidentified alkane		2653	10.5	35.5	7.39	-	15.6	-	14.9	5.77	12.6	10.1
Unidentified alkane		2828	27.2	-	54.5	5.17	-	-	40.0	-	-	3.24
Squalene	2847[♣]	2841	10.6	24.3	-	14.7	32.2	61.5	26.0	10.9	20.1	44.2

Table 4.6. Continued

Component	KI[th]	KI[♦]	Samples									
			SAm-C	SAu-C	SF-C	SG-C	SN-C	SO-C	SP-C	SSc-C	SSt-C	SV-C
Unidentified alkane		2943	5.43	3.47	5.54	1.51	-	-	-	32.7	-	4.39
Triacontane	3000	3006	5.00	5.71	8.01	-	8.69	12.0	4.96	1.29	8.95	7.02
Unidentified alkane		3123	-	22.9	-	0.90	54.6	36.5	29.5	14.4	62.2	-
Vitamin E	3112[♣]	3126	7.92	18.5	28.4	3.40	-	40.2	-	-	-	23.9
β-Sitosterol	3200	3164	-	5.14	4.65	3.18	4.17	18.0	5.69	2.23	-	-
Monoterpene hydrocarbons			**1.27**	**-**	**-**	**-**	**-**	**110**	**-**	**0.73**	**1.25**	**-**
Oxygenated monoterpenes			**-**	**14.7**	**-**	**3.78**	**-**	**1067**	**-**	**3.99**	**-**	**0.88**
TOTAL MONOTERPENES			**1.27**	**14.7**	**-**	**3.78**	**-**	**1177**	**-**	**4.73**	**1.25**	**0.88**
Sesquiterpene hydrocarbons			**9.80**	**-**	**42.5**	**12.7**	**5.92**	**659**	**18.5**	**28.2**	**1.72**	**46.2**
Oxygenated sesquiterpenes			**5.77**	**8.04**	**20.0**	**12.2**	**88.3**	**443**	**6.04**	**21.4**	**34.8**	**27.6**
TOTAL SESQUITERPENES			**15.6**	**8.04**	**62.5**	**24.9**	**94.3**	**1101**	**24.5**	**49.6**	**36.5**	**73.8**
Diterpenes			**9.15**	**7.25**	**9.12**	**8.29**	**7.30**	**853**	**20.0**	**2419**	**6.72**	**28.1**
Triterpenes			**10.5**	**24.3**	**-**	**14.7**	**32.2**	**61.5**	**26.0**	**10.9**	**20.1**	**44.2**
Alkanes			**56.5**	**144**	**97.4**	**25.2**	**92.3**	**127**	**106**	**64.5**	**97.6**	**40.7**
Others			**25.9**	**43.4**	**74.3**	**33.3**	**39.5**	**673**	**11.9**	**6.07**	**5.13**	**38.2**
Total			**119**	**241**	**243**	**110**	**265**	**3992**	**189**	**2555**	**167**	**226**

*The following compounds were determined only in *S. officinalis* extract and are not included in table (mg/kg): α-pinene (17.8), camphene (12.2), β-pinene (15.8), δ-3-carene (9.73), limonene (12.1), 1,8-cineole (92.9), γ-terpinene (12.4), linalool (15.4), α-thujone (351), β-thujone (85.4), camphor (210), borneol (279), terpinen-4-ol (7.38), α-terpineol (5.78), myrtenol (7.05), neo-iso-3-thujyl acetate (21.0), α-Cubebene (8.90), aromadendrene (16.3), geranyl acetone (6.52), γ-muurolene (23.2), γ-amorphene (17.0), α-muurolene (11.8), viridiflorol (357), (E)-sesquilavandulol epoxide (18.2), manool (689), (E)-totarol (19.1), (E)-ferruginol (55.1), labd-(13E)-8,15-diol (31.3).

** These volatile compounds which were determined in amounts lower than 5.00 mg/kg are not included in table. "-": not detected; [♦] *Kováts* retention indices calculated against C_7–C_{30} n-alkanes on nonpolar Elite-5 column; [th] *Kováts* retention indices on nonpolar DB-5 column reported in scholarly literature (Adams, 2009); [♠] *Kováts* retention indices from the database http://www.flavornet.org/; [♣] *Kováts* retention indices from the database http://webbook.nist.go

Table 4.7. Compounds isolated from various *Salvia* spp. plant material by simultaneous distillation-solvent extraction in a Likens-Nickerson apparatus, in mg/kg

Component	KI[th]	KI[♦]	Samples									
			SAm	SAu	SF	SG	SN	SO	SP	SSc	SSt	SV
α-Pinene	939	939	-	-	8.75	-	-	326	-	-	1.35	4.75
Camphene	954	943	-	-	-	-	-	218	-	-	-	-
Sabinene	975	971	2.95	-	3.60	4.20	-	21.6	-	-	3.35	-
1-Octen-3-one	977	972	-	-	-	4.20	-	173	-	-	-	-
1-Octen-3-ol	979	973	8.68	-	-	4.15	41.9	25.1	44.1	-	25.2	12.6
β-Pinene	979	979	-	-	2.24	-	-	35.8	-	-	-	5.40
p-Cymene	1024	1020	1.29	1.56	-	-	5.58	67.4	-	6.09	10.5	1.62
1,8-Cineole	1031	1025	-	-	-	-	-	1394	-	-	-	-
Benzyl alcohol	1031	1031	8.81	11.2	3.46	3.24	4.33	462	6.53	6.58	5.05	1.56
γ-Terpinene	1059	1052	7.35	-	-	-	-	43.7	-	-	4.82	-
Linalool	1096	1093	-	-	1.28	1.44	-	864	-	296	10.3	5.50
α-Thujone	1102	1104	-	-	-	-	-	3391	-	-	-	-
β-Thujone	1114	1115	-	-	-		-	2041	-	-	1.54	-
Camphor	1146	1146	-	-	-	-	-	2690	-	3.67	2.60	-
δ-Terpineol	1166	1166	-	-	-	-	-	38.5	-	-	-	-
Borneol	1169	1169	-	-	-	-	-	2458	-	-	-	3.90
Terpinen-4-ol	1177	1170	11.5	-	-	-	4.77	98.8	-	-	8.85	-
α-Terpineol	1188	1183	1.27	-	4.04	-	1.80	48.8	-	105	1.19	2.16
2,3-Dihydrobenzofuran	1224[♣]	1216	-	-	-	-	25.3	-	-	-	-	-
Linalyl acetate	1257	1247	-	-	-	-	-	-	-	411	-	-
(3Z)-Hexenyl valerate	1281	1279	-	-	-	-	-	179	-	-	-	-
neoiso-3-Thujyl acetate	1283	1281	-	33.5	1.20	-	-	-	-	-	-	-
Thymol	1290	1286	-	124	2.25	1.14	1.17	3.44	-	-	1.19	-
Carvacrol	1299	1294	1.61	8.35	2.56	12.5	1.65	16.4	2.03	56.1	1.76	-
p-Vinylguaiacol	1309	1306	14.4	2.14	1.74	-	52.5	5.46	11.2	3.19	7.58	1.81
4'-Methoxy-acetophenone	1350	1340	22.3	-	-	-	-	-	-	-	-	-
α-Ylangene	1375	1363	1.18	-	-	-	-	24.9	-	11.3	-	2.56
α-Copaene	1376	1377	10.7	3.77	3.95	-	-	64.7	-	84.4	2.47	3.48
(E)-Caryophyllene	1419	1413	151	2.77	50.5	33.2	12.0	588	291	107	4.24	28.0
Aromadendrene	1441	1441	-	-	3.79	-	-	65.9	1.26	2.83	-	1.23
(Z)-β-Farnesene	1442	1442	12.5	-	1.69	-	-	-	30.5	-	-	2.47
α-Humulene	1454	1447	6.94	1.25	23.4	30.2	2.25	2058	11.6	-	1.64	11.6

Table 4.7. Continued

Component	KI[th]	KI[♦]	Samples									
			SAm	SAu	SF	SG	SN	SO	SP	SSc	SSt	SV
Germacrene D	1480	1477	15.6	2.28	15.6	5.48	3.62	7.52	5.62	34.6	-	56.2
Germacrene A	1509	1509	-	-	-	1.13	-	21.9	-	16.4	-	-
(E)-Calamenene	1522	1514	4.94	2.23	5.78	1.20	-	57.4	-	-	-	-
α-Muurolene	1523[♠]	1505	8.60	-	4.71	-	-	31.3	-	-	-	-
δ-Cadinene	1523	1520	14.5	5.45	22.0	1.42	-	159	-	14.6	2.07	5.37
Spathulenol	1578	1571	1.79	14.8	100	2.25	-	20.3	40.0	72.6	27.9	89.7
Caryophyllene oxide	1583	1579	147	3.04	45.4	56.5	220	60.4	200	272	120	24.1
Humulene epoxide II	1608	1604	15.9	-	23.7	49.3	9.47	290	8.36	34.5	5.54	21.8
Caryophylla-4(12),8(13)-dien5-β-ol	1640	1641	3.53	-	1.84	1.63	16.6	182	4.73	12.0	7.79	-
α-Cadinol	1654	1650	-	-	-	-	-	16.4	-	26.5	-	-
14-Hydroxy-(Z)-caryophyllene	1667	1666	19.0	-	4.71	8.08	52.5	-	21.0	43.1	23.7	2.08
Germacra-4(15),5,10(14)-trien-1-α-ol	1686	1682	14.9	1.15	10.0	1.96	2.91	3.10	1.45	24.7	1.96	36.9
(5E,9E)-Farnesyl acetone	1913	1899	5.45	7.28	7.32	7.88	3.81	9.44	10.2	234	-	8.03
Phytol	1943	1943	37.4	32.7	9.97	34.0	14.1	129	72.8	42.2	11.8	13.7
Manool	2057	2060		-	-	-	-	2751	-	43.5	95.5	-
Sclareol	2223	2223	-	-	-	-	-	-	-	4241	-	-
Monoterpene hydrocarbons			**20.5**	**1.56**	**19.8**	**4.20**	**11.6**	**764**	**-**	**24.1**	**27.4**	**17.9**
Oxygenated monoterpenes			**22.7**	**138**	**14.2**	**26.6**	**18.3**	**13133**	**7.05**	**891**	**56.8**	**13.3**
TOTAL MONOTERPENES			**43.2**	**140**	**33.9**	**30.8**	**29.9**	**13898**	**7.05**	**915**	**84.3**	**31.3**
Sesquiterpene hydrocarbons			**254**	**17.7**	**161**	**92.6**	**19.0**	**3193**	**353**	**315**	**14.3**	**145**
Oxygenated sesquiterpenes			**211**	**19.0**	**193**	**142**	**302**	**579**	**277**	**504**	**186**	**175**
TOTAL SESQUITERPENES			**464**	**36.8**	**354**	**235**	**321**	**3773**	**631**	**819**	**201**	**319**
Diterpenes			**37.4**	**34.9**	**13.4**	**37.2**	**14.1**	**2880**	**72.8**	**4326**	**109**	**15.1**
Triterpenes			**3.84**	**6.11**	**9.59**	**5.39**	**5.39**	**-**	**6.95**	**3.55**	**3.23**	**5.12**
Alkanes			**13.3**	**19.4**	**6.19**	**5.66**	**7.32**	**16.5**	**24.0**	**18.2**	**5.37**	**10.1**
Others			**137**	**110**	**87.1**	**78.5**	**210**	**941**	**153**	**350**	**123**	**83.0**
Total			**699**	**347**	**504**	**392**	**588**	**21508**	**895**	**6433**	**526**	**464**

* These volatile compounds which were determined in amounts lower than 20.0 mg/kg are not included in table. "-" not detected; [♦] *Kováts* retention indices calculated against C_7–C_{30} n-alkanes on nonpolar Elite-5 column; [th] *Kováts* retention indices on nonpolar DB-5 column reported in scholarly literature (Adams, 2009); [♠] *Kováts* retention indices from the database http://www.flavornet.org/; [♣] *Kováts* retention indices from the database http://webbook.nist.go

4.4. Preliminary Evaluation of the Potential of Studied *Salvia* species in the Development of Ingredients for Functional Foods and Nutraceuticals

Aromatic plants and spices accumulate various phytochemicals which are an important source of functional ingredients because they possess a wide range of beneficial health effects (Lopresti, 2017). The addition of bioactive compounds to foods is one of the main ways to develop functional foods which can be further tested as candidates for health claims (Viuda-Martos, Ruiz-Navajas, Fernández-López and Pérez-Álvarez, 2011). Therefore, nowadays, the search and valorisation of new plant sources which would be rich in bioactive compounds has become an important issue both for scientists as well as food manufacturers.

Salvia extracts contain a large variety of bioactive compounds including flavonoids and phenolic acids which are potential sources of natural antioxidants and are widely used commercially at present in various types of food, beverage and cosmetic applications either as astringents or as flavouring agents (Lopresti, 2017). These natural antioxidants avoid the toxicity problems which may arise from the use of synthetic antioxidants such as butylated hydroxy anisole (BHA), butylated hydroxy toluene (BHT) and propyl gallate.

Rosmarinic acid was found to be the dominating compound (up to 30 mg/g DWP) in various *Salvia* spp. plants, particularly in their ethanolic extracts. The highest concentration of this compound was determined in ethanolic *S. officinalis* extracts followed by *S. amplexicaulis*, *S. forsskaolii* and *S. verticillata*. The antioxidant capacity of rosmarinic acid was shown to be more than 3 times higher than that of trolox; therefore, it inhibits xanthine oxidase and can be expected to scavenge the surplus free radicals in the body. In addition, rosmarinic acid reduces Mo (VI) to Mo (V) and therefore may prevent the production of free radicals caused by the polyvalent metal catalysts of oxidation (Petersen and Simmonds, 2003). The content of caffeic acid was also high in the 10 analysed *Salvia* spp plants. Its concentration varied from 0.51 to 3.72 mg/g DWE and from 0.17 to 1.14 mg/g DWP. The highest concentrations of caffeic acid were determined also in the ethanolic extract of *S. nemorosa* followed by *S. forsskaolii*, *S. amplexicaulis*, and *S. verticillata*. The role of caffeic acid in the prevention of cardiovascular diseases and certain types of cancers is well documented (Chang et al., 2010); therefore such constituents can also serve as functional food and nutraceutical ingredients. Various *Salvia* spp. plants are also a good source of apigenin-7-O-β-D-glucuronide. Its concentration in different *Salvia* spp. extracts was found to range from 0.80 to 37.43 mg/g DWE and from 0.22 to 6.79 mg/g DWP: the highest concentration of this constituent was found in the ethanolic extract of *S. sclarea* followed by the water extract of *S. sclarea*, the ethanolic extract of *S. pratensis* and the ethanolic extract of *S. forsskaolii*. Apigenin-7-O-β-D-glucuronide possesses multiple pharmacological activities, including antioxidant, anti-complement, and aldose reductase inhibitory activities (Viuda-Martos, Ruiz-Navajas, Fernández-López, and Pérez-Álvarez, 2011).

In conclusion, the ethanolic extracts of *S. officinalis*, *S. sclarea*, *S. amplexicaulis*, *S. verticillata*, *S. nemorosa*, *S. forsskaolii* and *S. pratensis* are good

sources of phytochemicals for the valorisation of *Salvia* spp. plants as raw materials for the isolation of functional ingredients for human nutrition and nutraceuticals.

Conclusions

1. From 10 different *Salvia* spp. plants, three fractions per plant were isolated by consecutive high pressure extraction with CO_2, ethanol (96%) and water. The yields obtained with water (30.2–43.7%) were higher compared to the extracts isolated with ethanol (18.1–36.2%) and CO_2 (1.8–5.2%).

2. The antioxidant potential of 10 *Salvia* spp. extracts was evaluated by using TPC, $ABTS^{+\bullet}$ and ORAC assays. Ethanol extracts possessed significantly higher antioxidant capacity and the total content of phenolics than the extracts obtained with CO_2 or water. The antioxidant power of the plant material extraction residues evaluated by the QUENCHER method was also comparatively high, particularly after SFE-CO_2; it suggests that a considerable amount of antioxidatively active compounds remain in the plant material after extraction. A strong correlation (r^2=0.812–0.942) between TPC and antioxidant activity measured by $ABTS^{\bullet+}$ and ORAC was also observed.

3. Rosmarinic, caffeic and carnosic acids and apigenin-7-*O*-β-D-glucuronide were identified as the main compounds in the selected *Salvia* spp. extracts. In addition, ethyl gallate, 3',4',5,7-tetrahydroxy-3-methoxyflavone, hyperoside and isorhamnetin-glucoside were also identified in the selected *Salvia* spp. extracts for the first time.

4. Rosmarinic acid was found to be the dominating compound (up to 30,017 μg/g DWP) in various *Salvia* spp. plants, particularly in their ethanolic extracts. Apigenin-7-*O*-β-D-glucuronide (up to 6,798 μg/g DWP), caffeic (up to 1,142 μg/g DWP) and carnosic (up to 8,294 μg/g DWP) acids were quantitatively important phytochemicals in the majority other *Salvia* spp. plants. Rosmarinic, caffeic and carnosic acids were determined to be the most active radical scavengers in the investigated extracts when employing the on-line HPLC-UV-$DPPH^{\bullet}$ method. In total, 15 compounds were identified in *Salvia* spp. extracts by using commercial standards, 11 of them were quantified, whereas some other compounds were identified tentatively based on the obtained MS fragments and a comparison with data provided in scholarly literature.

5. The total yield of tocopherol isomers in the studied *Salvia* spp. plants varied from 2,360 to 10,071 μg/g DWE and from 52.64 to 221 μg/g DWP. The highest total amount of the extracted tocopherols from dry plant material was determined in *S. nemorosa* (221 μg/g DWP) followed by *S. forsskaolii* (193 μg/g DWP) and *S. verticillata* (187 μg/g DWP). α-Tocopherol was found to be the dominating vitamin E isomer in 10 different *Salvia* species; its concentration in the extracts and plant material were from 2242 to 8473 μg/g DWE and from 50.01 to 179 μg/g DWP, respectively. The concentrations of γ- and δ-tocopherols were remarkably lower. β isomer was not detected in any of the extracts.

6. A comparison of simultaneous distillation/extraction in a *Likens-Nickerson* (L-N) apparatus and supercritical fluid extraction with carbon dioxide (SFE-CO_2) methods for the isolation of volatile compounds revealed that the amount of volatiles isolated from 1 kg of dried plant material by SFE-CO_2 method was remarkably

lower comparing to the L-N method, most likely, due to the losses of volatiles with the exhaust from the system CO_2 after depressurising the extraction equipment.

7. Experimental studies revealed that ethanolic extracts of *S. officinalis*, *S. sclarea*, *S. amplexicaulis*, *S. verticillata*, *S. nemorosa*, *S. forsskaolii* and *S. pratensis* accumulate various valuable phytochemicals such as rosmarinic acid and tocopherols. Based on the existing knowledge on the health benefits of these constituents, it is assumed that these investigated *Salvia* spp. plants are promising plants which could be cultivated for the development of valuable ingredients for functional foods and nutraceuticals.

References

1. ABDILLE, Md.,H., SINGH, R.P., JAYAPRAKASHA, G.K. and JENA, B.S. Antioxidant Activity of the Extracts from *Killenia Indica* Fruits. *Food Chemistry.* 2005, 90(4), 891–896. ISSN 0021-8561.

2. ADAMS, R.P. *Identification of Essential Oils Components by Gas Chromatography/Mass Spectrometry*. 4th ed. Allured Business Media, Carol Stream, IL. 2007, 698. ISBN 978-1932633214.

3. AGGARWAL, B.B. and SUNG, B. Pharmacological Basis for the Role of Curcumin in Chronic Diseases: an Age-Old Spice with Modern Targets. *Trends in Pharmacological Sciences.* 2009, 30(2), 85–94. ISSN 0165-6147.

4. AHMADI, L. and MIRZA, M. Essential Oil of *Salvia Multicaulis* Vahl from Iran. *Journal of Essential Oil Research.* 1999, 11(3), 289–290. ISSN 1041-2905.

5. ALI, B., AL-WABEL, N.A., SHAMS, S., AHAMAD, A., KHAN, S.A. and ANWAR, F. Essential Oils Used in Aromatherapy: a Systemic Review. *Asian Pacific Journal of Tropical Biomedicine.* 2015, 5(8), 601–611. ISSN 2221-1691.

6. ALLAHGHADRI, T., RASOOLI, I., OWLIA, P., NADOOSHAN, M.J., GHAZANFARI, T., TAGHIZADEH, M. and ASTANEH, S.D.A. Antimicrobial Property, Antioxidant Capacity, and Cytotoxicity of Essential Oil from Cumin Produced in Iran. *Journal of Food Science.* 2010, 75(2), H54–H61. ISSN 0022-1147.

7. AL-ASHEH, S., ALLAWZI, M., AL-OTOOM, A., ALLABOUN, H. and AL-ZOUBI, A. Supercritical Fluid Extraction of Useful Compounds from Sage. *Natural Science.* 2012, 4(8), 544–551. ISSN 2334-2943.

8. AL-REZA, S.M., RAHMAN, A., PARVIN, T., RAHMAN, M.M. and RAHMAN, M.S. Chemical Composition and Antibacterial Activities of Essential Oil and Organic Extracts of *Curcuma Aromatica* Salisb. *Journal of Food Safety.* 2011, 31(4), 433–438. ISSN 1745-4565.

9. AMAROWICZ, R., PEGG, R.B., RAHIMI-MOGHADDAM, P., BARL, B. and WEIL, J.A. Free-Radical Scavenging Capacity and Antioxidant Activity of Selected Plant Species from the Canadian Prairies. *Food Chemistry.* 2004, 84(4), 551–562. ISSN 0021-8561.

10. ANAČKOV, G., BOŽIN, B., ZORIĆ, L., VUKOV, D., MIMICA-DUKIĆ, N., MERKULOV, L., IGIĆ, R., JOVANOVIĆ, M. and BOŽA, P. Chemical Composition of Essential Oil and Leaf Anatomy of *Salvia Bertolonii* Vis. and *Salvia Pratensis* L. (Sect. *Plethiosphace*, Lamiaceae). *Molecules.* 2009, 14(1), 1–9. ISSN 1420-3049.

11. ANDREASEN, A.F., KROON, P.A., WILLIAMSON, G. and GARCIA-CONESA, M.T. Esterase Activity Able to Hydrolyze Dietary Antioxidant Hydroxycinnamates Is Distributed along the Intestine of Mammals. *Journal of Agricultural and Food Chemistry.* 2001, 49(11), 5679–5684. ISSN 0021-8561.

12. ASKUN, T., BASER, K.H.C., TUMEN, G. and KURKCUOGLU M. Characterization of Essential Oils of Some *Salvia* Species and Their Antimycobacterial Activities. *Turkish Journal of Biology.* 2010, 34, 89–95. ISSN 1300-0152.

13. ATOUI, A.K., MANSOURI, A., BOSKOU, G. and KEFALAS, P. Tea and Herbal Infusions: Their Antioxidant Activity and Phenolic Profile. *Food Chemistry*. 2005, 89(1), 27–36. ISSN 0021-8561.

14. AVATO, P., FORTUNATO, I.M., RUTA, C. and D'ELIA, R. (2005). Glandular Hairs and Essential Oils in Micropropagated Plants of *Salvia Officinalis* L. *Plant Science*. 2005, 169(1), 29–36. ISSN 0168-9452.

15. BAKKALI, F., AVERBECK, S., AVERBECK, D. and IDAOMAR, M. Biological Effects of Essential Oils – a Review. *Food and Chemical Toxicology*. 2008, 46(2), 446–475. ISSN 0278-6915.

16. BANDONIENE, D., MURKOVIC, M. and VENSKUTONIS, P.R. Determination of Rosmarinic Acid in Sage and Borage Leaves by High-Performance Liquid Chromatography with Different Detection Methods. *Journal of Chromatographic Science*. 2005, 43(7), 372–376. ISSN 0021-9665.

17. BANDONIENĖ, D., VENSKUTONIS, P.R., GRUZDIENĖ, D. and MURKOVIC, M. Antioxidative Activity of Sage (*Salvia Officinalis* L.), Savory (*Satureja Hortensis* L.) and Borage (*Borago Officinalis* L.) Extracts in Rapeseed Oil. *European Journal of Lipid Science and Technology*. 2002, 104(5), 286–292. ISSN 1438-7697.

18. BARANAUSKIENĖ, R., VENSKUTONIS, P.R., VIŠKELIS, P., and DAMBRAUSKIENĖ, E. Influence of Nitrogen Fertilizers on the Yield and Composition of Thyme (*Thymus Vulgaris*). *Journal of Agricultural and Food Chemistry*. 2003, 51(26), 7751–7758. ISSN 0021-8561.

19. BARICEVIC, D. and BARTOL, T. The Biological/Pharmacological Activity of the *Salvia* Genus. In: Kintzios, S.E. (ed.). *The Genus Salvia*. Amsterdam: Harwood Academic Publishers., 2000, 143–184. ISBN 978-8123922577.

20. BARTÁK, P., FRNKOVÁ, P. and ČÁP, L. Determination of Phenols Using Simultaneous Steam Distillation-Extraction. *Journal of Chromatography A*. 2000, 867(1–2), 281–287. ISSN 0021-9673.

21. BENCHAAR, C., CALSAMIGLIA, S., CHAVES, A.V., FRASER, G.R., COLOMBATTO, D, MCALLISTER, T.A. and BEAUCHEMIN, K.A. A Review of Plant-Derived Essential Oils in Ruminant Nutrition and Production. *Animal Feed Science and Technology*. 2008, 145(1–4), 209–228. ISSN 0377-8401.

22. BETTAIEB, I., BOURGOU, S., WANNES, W.A., HAMROUNI, I., LIMAM, F. and MARZOUK, B. Essential Oils, Phenolics, and Antioxidant Activities of Different Parts of Cumin (*Cuminum Cyminum* L.). *Journal of Agricultural and Food Chemistry*. 2010, 58(19), 10410–10418. ISSN 0021-8561.

23. BHATT, A. and PATEL, V. Antioxidant Activity of Garlic Using Conventional Extraction and *in Vitro* Gastrointestinal Digestion. *Free Radicals and Antioxidants*. 2013, 3(1), 30–34. ISSN 0975-3575.

24. BIESALSKI, H.K., DRAGSTED, L.O., ELMADFA, I., GROSSKLAUS, R., MÜLLER, M., SCHRENK, D., WALTER, P. and WEBER, P. Bioactive Compounds: Definition and Assessment of Activity. *Nutrition*. 2009, 25(11–12), 1202–1205. ISSN 0899-9007.

25. BIGLARI, F., ALKARKHI, A.F.M. and EASA, A.M. Antioxidant Activity and Phenolic Content of Various Date Palm (*Phoenix Dactylifera*) Fruits from Iran. *Food Chemistry*. 2008, 107(4), 1636–1641. ISSN 0021-8561.

26. BISIO, A., ROMUSSI, G., CIARALLO, G. and DE TOMMASI, N. Flavonoide und Triterpenoide aus *Salvia Blepharophylla* Brandegee ex. *Epling Pharmazie*. 1997, 52, 330–331. ISSN 0031-7144.

27. BLANCH, G.P., REGLERO, G. and HERRAIZ, M. Rapid Extraction of Wine Aroma Compounds Using a New Simultaneous Distillation-Solvent Extraction Device. *Food Chemistry*. 1996, 56(4), 439–444. ISSN 0021-8561.

28. BLANCO TIRADO, C., STASHENKO, E.E., COMBARIZA, M.Y. and MARTINEZ, J.R. Comparative Study of Colombian Citrus Oils by High-Resolution Gas Chromatography and Gas Chromatography-Mass Spectrometry. *Journal of Chromatography A*. 1995, 697(1–2), 501–513. ISSN 0021-9673.

29. BURDOCK, G.A. and CARABIN, I.G. Safety Assessment of Coriander (*Coriandrum Sativum* L.) Essential Oil as a Food Ingredient. *Food and Chemical Toxicology*. 2009, 47(1), 22–34. ISSN 0278-6915.

30. BURT, S. Essential Oils: Their Antibacterial Properties and Potential Applications in Foods – a Review. *International Journal of Food Microbiology*. 2004, 94(3), 223–253. ISSN 0168-1605.

31. CACHET, T., BREVARD, H., CHAINTREAU, A., DEMYTTENAERE, J., FRENCH, L., GASSENMEIER, K., JOULAIN, D., KOENIG, T., LEIJS, H., LIDDLE, P., LOESING, G., MARCHANT, M., MERLE, Ph., SAITO, K., SCHIPPA, C., SEKIYA, F. and SMITH, T. IOFI Recommended Practice for the Use of Predicted Relative-Response Factors for the Rapid Quantification of Volatile Flavouring Compounds by GC-FID. *Flavour and Fragrance Journal*. 2016, 31(3), 191–194. ISSN 0882-5734.

32. CALLISTE, C.A., TROUILLAS, P., ALLAIS, D.P., SIMON, A. and DUROUX, J.L. Free Radical Scavenging Activities Measured by Electron Spin Resonance Spectroscopy and B16 Cell Antiproliferative Behaviors of Seven Plants. *Journal of Agricultural and Food Chemistry*. 2001, 49(7), 3321–3327. ISSN 0021-8561.

33. CAPECKA, E., MARECZEK, A. and LEJA, M. Antioxidant Activity of Fresh and Dry Herbs of Some *Lamiaceae* Species. *Food Chemistry*. 2005, 93(2), 223–226. ISSN 0021-8561.

34. CARAZZONE, C., MASCHERPA, D., GAZZANI, G. and PAPETTI, A. (2013). Identification of Phenolic Constituents in Red Chicory Salads (*Cichorium Intybus*) by High Performance Liquid Chromatography with Diode Array Detection and Electrospray Ionisation Tandem Mass Spectrometry. *Food Chemistry*. 2013, 138(2–3), 1062–1071. ISSN 0021-8561.

35. CARRUBBA, A., LA TORRE, R., PICCAGLIA, R. and MAROTTI, M. Characterization of an Italian Biotype of Clary Sage (*Salvia Sclarea* L.) Grown in a Semi-Arid Mediterranean Environment. *Flavour and Fragrance Journal*. 2002, 17(3), 191–194. ISSN 0882-5734.

36. CHAILLOU, L.L. and NAZARENO, M.A. New Method to Determine Antioxidant Activity of Polyphenols. *Journal of Agricultural and Food Chemistry*. 2006, 54(22), 8397–8402. ISSN 0021-8561.

37. CHAINTREAU, A. Simultaneous Distillation-Extraction: from Birth to Maturity – Review. *Flavour and Fragrance Journal*. 2001, 16(2), 136–148. ISSN 0882-5734.

38. CHALCHAT, J.C., MICHET, A. and PASQUIER, B. Study of Clones of *Salvia Officinalis* L. Yields and Chemical Composition of Essential Oil. *Flavour and Fragrance Journal*. 1998, 13(1), 68–70. ISSN 0882-5734.

39. CHANG, W.C., HSIEH, C.H., HSIAO, M.W., LIN, W.C., HUNG, Y.C. and YE, J.C. Caffeic Acid Induces Apoptosis in Human Cervical Cancer Cells through the Mitochondrial Pathway. *The Taiwanese Journal of Obstetrics and Gynecology*. 2010, 49(4), 19–424. ISSN 1028-4559.

40. CHAO, S.C., YOUNG, G. and OBERG, C.J. Screening for Inhibitory Activity of Essential Oils on Selected Bacteria, Fungi and Virus. *Journal of Essential Oil Research.* 2000, 12(5), 639–649. ISSN 1041-2905.

41. CHEN, S.W., MIN, L., LI, W.J., KONG, W.X., LI, J.F. and ZHANG, Y.J. The Effects of Angelica Essential Oil in Three Murine Tests of Anxiety. *Pharmacology, Biochemistry and Behavior.* 2007, 79(2), 377–382. ISSN 0091-305.

42. CHIZZOLA, R. Composition and Variability of the Essential Oil of *Salvia Nemorosa* (Lamiaceae) from the Vienna Area of Austria. *Natural Product Communications.* 2012, 7(12), 1671–1672. ISSN 1934-578X.

43. CHIZZOLA, R., MICHITSCH, H. and FRANZ, C. Antioxidative Properties of Thymus Vulgaris Leaves: Comparison of Different Extracts and Essential Oil Chemotypes. *Journal of Agricultural and Food Chemistry.* 2008, 56(16), 6897–6904.

44. CHOI, S.W., LEE, S.K., KIM, E.O., OH, J.H., YOON, K.S., PARRIS, N., HICKS, K.B. and MOREAU, R.A. Antioxidant and Antiomelanogenic Activities of Polyamine Conjugates from Corn Bran and Related Hydroxycinnamic Acids. *Journal of Agricultural and Food Chemistry.* 2007, 55(10), 3920–3925. ISSN 0021-8561.

45. CHUN, S.S., VATTEM, D.A., LIN, Y.T. and SHETTY, K. Phenolic Antioxidants from Clonal Oregano (*Origanum Vulgare*) with Antimicrobial Activity against *Helicobacter Pylori*. *Process Biochemistry.* 2005, 40(2), 809–816. ISSN 1359-5113.

46. CLIFFORD, M.N. Chlorogenic Acids and Other Cinnamates: Nature, Occurrence, Dietary Burden, Absorption and Metabolism. *Journal of Agricultural and Food Chemistry.* 2000, 80(7), 1033–1043. ISSN 0021-8561.

47. COISIN, M., NECULA, R., GRIGORAŞ, V., GILLE, E., ROSENHECH, E. and ZAMFIRACHE, M.M. Phytochemical Evaluation of some *Salvia* Species from Romanian Flora. *Scientific Annals of Alexandru Ioan Cuza University of Iasi. New Series, Section 2. Vegetal Biology.* 2012, 58(1), 35–44. ISSN 1223-6578.

48. COSIO, M., BURATTI, S., MANNINO, S. and BENEDETTI, S. Use of an Electrochemical Method to Evaluate the Antioxidant Activity of Herb Extracts from the Labiateae Family. *Food Chemistry.* 2006, 97(4), 725–731. ISSN 0021-8561.

49. CUVELIER, M.E., BERSET, C. and RICHARD, H. Antioxidant Constituents in Sage (*Salvia Officinalis*). *Journal of Agricultural and Food Chemistry.* 1994, 42(3), 665–669. ISSN 0021-8561.

50. CVETKOVIKJ, I., STEFKOV, G., ACEVSKA, J., STANOEVA, J.P., KARAPANDZOVA, M., STEFOVA, M., DIMITROVSKA, A. and KULEVANOVA, S. Polyphenolic Characterization and Chromatographic Methods for Fast Assessment of Culinary *Salvia* Species from South East Europe. *Journal of Chromatography A.* 2013, 1282(22), 38–45. ISSN 0021-9673.

51. DAPKEVICIUS, A., van BEEK, T.A., LELYVELD, G.P., van VELDHUIZEN, A., de GROOT, A., LINSSEN, J.P.H. and VENSKUTONIS, R. Isolation and Structure Elucidation of Radical Scavengers from *Thymus Vulgaris* Leaves. *Journal of Natural Products.* 2002, 65(6), 892–896. ISSN 0163-3864.

52. DAI, J. and MUMPER, R.J. Plant Phenolics: Extraction, Analysis and Their Antioxidant and Anticancer Properties. A Review. *Molecules.* 2010, 15(10), 7313–7352. ISSN 1420-3049.

53. DASTMALCHI, K., DORMAN, H.J.D., OINONEN, P.P., DARWIS, Y., LAAKSO, I. and HILTUNEN, R. Chemical Composition and *in Vitro* Antioxidative Activity

of a Lemon Balm (*Melissa Officinalis* L.) Extract. *LWT – Food Science and Technology*. 2008, 41(3), 391–400. ISSN 0023-6438.

54. DAUKŠAS, E., VENSKUTONIS, P.R., POVILAITYTĖ, V., and SIVIK, B. Rapid Screening of Antioxidant Activity of Sage (*Salvia Officinalis* L.) Extracts Obtained by Supercritical Carbon Dioxide at Different Extraction Conditions. *Food/Nahrung*. 2001, 45(5), 338–341. ISSN 0027-769X.

55. DELAMARE, A.P.L., MOSCHEN-PISTORELLO, I.T., ARTICO, L., ATTI-SERAFINI, L. and ECHEVERRIGARAY, S. Antibacterial Activity of the Essential Oils of *Salvia Officinalis* L. and *Salvia Triloba* L. Cultivated in South Brazil. *Food Chemistry*. 2007, 100(2), 603–608. ISSN 0021-8561.

56. DEMO, A., PETRAKIS, C., KEFALAS, P. and BOSKOU, D. Nutrient Antioxidants in Some Herbs and Mediterranean Plant Leaves. *Food Research International*. 1998, 31(5), 351–354. ISSN 0963-9969.

57. DEVI, M.P., CHAKRABARTY, S., GHOSH, S.K. and BHOWMICK, N. Essential Oil: Its Economic Aspect, Extraction, Importance, Uses, Hazards and Quality. In: Sharangi, A.B., Datta, S. (eds.). *Value Addition of Horticultural Crops: Recent Trends and Future Directions*. Springer: Dordrecht, 2015, 15, 269. ISBN 978-81-322-2261-3.

58. DIXON, R.A., ACHNINE, L., KOTA, P., LIU, C.J., REDDY, M.S.S. and WANG, L. The Phenylpropanoid Pathway and Plant Defence – a Genomics Perspective. *Molecular Plant Pathology*. 2002, 3(5), 371–390. ISSN 0885-5765.

59. DŽAMIĆ, A., SOKOVIĆ, M., RISTIĆ, M., GRUJIĆ-JOVANOVIĆ, S., VUKOJEVIĆ, J. and MARIN, P.D. Chemical Composition and Antifungal Activity of *Salvia Sclarea* (Lamiaceae) Essential Oil. *Archives of Biological Sciences*. 2008, 60(2), 233–237. ISSN 0354-4664.

60. EIKANI, M.H., GOLMOHAMMAD, F., ROWSHANZAMIR, S. and MIRZA, M. Recovery of Water-Soluble Constituents of Rose Oil Using Simultaneous Distillation-Extraction. *Flavour and Fragrance Journal*. 2005, 20(6), 555–558. ISSN 0882-5734.

61. EISENREICH, W., BACHER, A., ARIGONI, D. and ROHDICH, F. Biosynthesis of Isoprenoids via the Non-Mevalonate Pathway. *Cellular and Molecular Life Sciences*. 2004, 61(12), 1401–1426. ISSN 1420-682X.

62. EMBUSCADO, M.E. Spices and Herbs: Natural Sources of Antioxidants – a Mini Review. *Journal of Functional Foods*. 2015, 18(Part B), 811–819. ISSN 1756-4646.

63. EMMONS, C.L., PETERSON, D.M. and PAUL, G.L. Antioxidant Capacity of Oat (*Avena Sativa* L.) Extracts. 2. In Vitro Antioxidant Activity and Contents of Phenolic and Tocol Antioxidants. *Journal of Agricultural and Food Chemistry*. 1999, 47(12), 4894–4898. ISSN 0021-8561.

64. ERDOĞAN, S.S., KARIK, Ü., HÜSNÜ, K. and BAŞER, K.H.C. The Determination of Antioxidant Activity of some Sage Populations in the Marmara Region. *Turkish Journal of Agricultural and Natural Sciences*. 2014, 2, 1877–1882. ISSN 2148-3647.

65. FARAKAŠ, P., HOLLÁ, M., TEKEL, J., MELLEN, S. and ŠTEFÁNIA, V. Composition of the Essential Oils from the Flowers and Leaves of *Salvia Sclarea* (Lamiaceae) Cultivated in the Slovak Republic. *Journal of Essential Oil Research*. 2005, 17(2), 141–144. ISSN 1041-2905.

66. FARHAT, B.M., CHAOUCH-HAMADA, R., SOTOMAYOR, J.A., LANDOULSI, A. and JORDAN, M.J. Antioxidant Properties and Evaluation of

hytochemical Composition of *Salvia Verbenaca* L. Extracts at Different Developmental Stages. *Plant Foods for Human Nutrition.* 2015, 70(1), 15–20. ISSN 0921-9668.

67. FARHAT, B.M., CHAOUCH-HAMADA, R., SOTOMAYOR, J.A., LANDOULSI, A. and JORDÁN, M.J. Antioxidant Potential of *Salvia Officinalis* L. Residues as Affected by the Harvesting Time. *Industrial Crops and Products.* 2014, 54, 78–85. ISSN 0926-6690.

68. FARHAT, M.B., JORDÁN, M.J., CHAOUECH-HAMADA, R., LANDOULSI, A. and SOTOMAYOR, J.A. Variations in Essential Oil, Phenolic Compounds and Antioxidant Activity of Tunisian Cultivated *Salvia Officinalis* L. *Journal of Agricultural and Food Chemistry*. 2009, 57(21), 10349–10356. ISSN 0021-8561.

69. FARHAT, M., LANDOULSI, A., CHAOUCH-HAMADA, R., SOTOMAYOR, J. A. and JORDÁN, M. J. Phytochemical Composition and in Vitro Antioxidant Activity of By-Products of *Salvia Verbenaca* L. Growing Wild in Different Habitats. *Industrial Crops and Products.* 2013, 49, 373–379. ISSN 0926-6690.

70. FISHER, K. and PHILLIPS, C. Potential Antimicrobial Uses of Essential Oils in Food: Is Citrus the Answer? *Trends in Food Science and Technology*. 2008, 19(3), 156–164. ISSN 0924-2244.

71. FRATERNALE, D., GIAMPERI, L., BUCCHINI, A., RICCI, D., EPIFANO, F., GENOVESE, S. and CURINI, M. Composition and Antifungal Activity of Essential Oil of *Salvia Sclarea* from Italy. *Chemistry of Natural Compounds.* 2005, 41(5), 604–606. ISSN 0009-3130.

72. GAŠIĆ, U.M., NATIĆ, M.M., MIŠIĆ, D.M., LUŠIĆ, D.V., MILOJKOVIĆ-OPSENICA, D.M., TEŠIĆ, Ž.Lj. and LUŠIĆ, D. Chemical Markers for the Authentication of Unifloral *Salvia Officinalis* L. Honey. *Journal of Food Composition and Analysis.* 2015, 44, 128–138. ISSN 0889-1575.

73. GAZZANI, G., PAPETTI, A., MASSOLINI, G. and DAGLIA, M. Anti- and Prooxidant Activity of Water Soluble Components of Some Common Diet Vegetables and the Effect of Thermal Treatment. *Journal of Agricultural and Food Chemistry*. 1998, 46(10), 4118–4122. ISSN 0021-8561.

74. GIÃO, M.S., PEREIRA, C.L., FONSECA, S.C., PINTADO, M.E. and MALCATA, F.H. Effect of Particle Size upon the Extent of Extraction of Antioxidant Power from the Plants *Agrimonia Eupatoria, Salvia* sp. and *Satureja Montana*. *Food Chemistry*. 2009, 117(3), 412–416. ISSN 0021-8561.

75. GÖKMEN, V., SERPEN, A. and FOGLIANO, V. Direct Measurement of the Total Antioxidant Capacity of Foods: the 'QUENCHER' Approach. *Trends in Food Science and Technology*. 2009, 20, 278–288. ISSN 0924-2244.

76. GRUNOVAITĖ, L., PUKALSKIENĖ, M., PUKALSKAS, A. and VENSKUTONIS, P.R. Fractionation of Black Chokeberry Pomace into Functional Ingredients Using High Pressure Extraction Methods and Evaluation of Their Antioxidant Capacity and Chemical Composition. *Journal of Functional Foods.* 2016, 24, 85–96. ISSN 1756-4646.

77. GRUSZKA, J. and KRUK, J. RP-LC for Determination of Plastochromanol, Tocotrienols and Tocopherols in Plant Oils. *Chromatographia.* 2007, 66(11–12), 909–913. ISSN 0009-5893.

78. GRYSZCZYNSKA, A., OPALA, B., LOWICKI, Z., DREGER, M., GORSKA-PAUKSZTA, M., SZULC, M., KAMINSKA, E., LITWIN, E., STRUZIK, P., DYR, W., WYSZOGRODZKA, E. and MIKOLAJCZAK, P.L. Bioactive Compounds Determination in

the Callus and Hydroalcoholic Extracts from *Salvia Miltiorrhiza* and *Salvia Przewalskii* – Preliminary Study on Their Anti-Alcoholic Activity Effects. *Phytochemistry Letters.* 2015, 11, 399–403. ISSN 1874-3900.

79. HALLIWELL, B. and GUTTERIDGE, J.M. The Definition and Measurement of Antioxidants in Biological Systems. *Free Radical Biology and Medicine.* 1995, 18(1), 125–126. ISSN 0891-5849.

80. HE, X.J and LIU R.H. Cranberry Phytochemicals: Isolation, Structure Elucidation, and Their Antiproliferative and Antioxidant Activities. *Journal of Agricultural and Food Chemistry.* 2006, 54(19), 7069–7074. ISSN 0021-8561.

81. HERRERO, M., CIFUENTES, A. and IBAÑEZ, E. Sub- and Supercritical Fluid Extraction of Functional Ingredients from Different Natural Sources: Plants, Food-By-Products, Algae and Microalgae. A Review. *Food Chemistry.* 2006, 98(1), 136–148. ISSN 0021-8561.

82. HERRERO, M., PLAZA, M., CIFUENTES, A. and IBÁÑEZ, E. Green Processes for the Extraction of Bioactives from Rosemary: Chemical and Functional Characterization via Ultra-Performance Liquid Chromatography-Tandem Mass Spectrometry and In-Vitro Assays. *Journal of Chromatography A.* 2010, 1217(16), 2512–2520. ISSN 0021-9673.

83. HOLASOVA, M., FIEDLEROVA, V., SMRCINOVA, H., ORSAK, M., LACHMAN, J., and VAVREINOVA, S. Buckwheat – the Source of Antioxidant Activity in Functional Foods. *Food Research International.* 2002, 35(2–3), 207–211. ISSN 0963-9969.

84. HOSSAIN, M. B., BARRY-RYAN, C., MARTIN-DIANA, A.B. and BRUNTON, N.P. Optimisation of Accelerated Solvent Extraction of Antioxidant Compounds from Rosemary (*Rosmarinus Officinalis* L.), Marjoram (*Origanum Majorana* L.) and Oregano (*Origanum Vulgare* L.) Using Response Surface Methodology. *Food Chemistry.* 2011, 126(1), 339–346. ISSN 0308-8146.

85. HOSSAIN, M.B., RAI, D.K., BRUNTON, N.P., MARTIN-DIANA, A.B. and BARRY-RYAN, C. Characterization of Phenolic Composition in Lamiaceae Spices by LC-ESI-MS/MS. *Journal of Agricultural and Food Chemistry.* 2010, 58(19), 10576–10581. ISSN 0021-8561.

86. HUANG, S.W. and FRANKEL, E.N. Antioxidant Activity of Tea Catechins in Different Lipid Systems. *Journal of Agricultural and Food Chemistry.* 1997, 45(8), 3033–3038. ISSN 0021-8561.

87. HUANG, D., OU, B. and PRIOR, R.L. The Chemistry behind Antioxidant Capacity Assays. *Journal of Agricultural and Food Chemistry.* 2005, 53(6), 1841–1856. ISSN 0021-8561.

88. JÄPELT, R.B. and JAKOBSEN, J. Analysis of Vitamin K_1 in Fruits and Vegetables Using Accelerated Solvent Extraction and Liquid Chromatography Tandem Mass Spectrometry with Atmospheric Pressure Chemical Ionization. *Food Chemistry.* 2016, 192, 402–408. ISSN 0308-8146.

89. JAVANMARDI, J., KHALIGHI, A., KASHI, A., BAIS, H.P. and VIVANCO, J.M. Chemical Characterization of Basil (*Ocimum Basilicum* L.) Found in Local Accessions and Used in Traditional Medicines in Iran. *Journal of Agricultural and Food Chemistry.* 2002, 50(21), 5878–5883. ISSN 0021-8561.

90. JAYASINGNE, C., GOTOH, N., AOKI, T. and WADA, S. Phenolic Composition and Antioxidant Activity of Sweet Basil (Ocimum Basilicum L.). *Journal of Agricultural and Food Chemistry.* 2003, 51(15), 4442–4449. ISSN 0021-8561.

91. JUSTESEN, U. and KNUTHSEN, P. Composition of Flavonoids in Fresh Herbs and Calculation of Flavonoid Intake by Use of Herbs in Traditional Danish Dishes. *Food Chemistry*. 2001, 73(2), 245–250. ISSN 0021-8561.

92. JUSTESEN, U., KNUTHSEN, P. and LETH, T. Quantitative Analysis of Flavanols, Flavones, and Flavanones in Fruits, Vegetables and Beverages by High Performance Liquid Chromatography with Photodiode Array and Mass Spectrometric Detection. *Journal of Chromatography A*. 1988, 799(1–2), 101–110. ISSN 0021-9673.

93. KAHKONEN, M.P., HOPIA, A.I., VUORELA, H.J., RAUHA, J.P., PIHLAJA, K., KUJALA, T.S and HEINONEN, M. Antioxidant Activity of Plant Extracts Containing Phenolic Compounds. *Journal of Agricultural and Food Chemistry*. 1999, 47(10), 3954–3962. ISSN 0021-8561.

94. KAMATH, S.D., ARUNKUMAR, D., AVINASH, N.G. and SAMSHUDDIN, S. Determination of Total Phenolic Content and Total Antioxidant Activity in Locally Consumed Food Stuffs in Moodbidri, Karnataka, India. *Advances in Applied Science Research*. 2015, 6(6), 99–102. ISSN 0976-8610.

95. KANATT, S.R., CHANDER, R., RADHAKRISHNA, P. and SHARMA, A. Potato Peel Extract – a Natural Antioxidant for Retarding Lipid Peroxidation in Radiation Processed Lamb Meat. *Journal of Agricultural and Food Chemistry*. 2005, 53(5), 1499–1504. ISSN 0021-8561.

96. KAUFMANN, B. and CHRISTEN, P. Recent Extraction Techniques for Natural Products: Microwave-Assisted Extraction and Pressurised Solvent Extraction. *Phytochemical Analysis*.2002, 13(2), 105–113. ISSN 0958-0344.

97. KEMZŪRAITĖ, A., VENSKUTONIS, P.R., BARANAUSKIENĖ, R. and NAVIKIENĖ, D. Optimization of Supercritical CO_2 Extraction of Different Anatomical Parts of Lovage (*Levisticum Officinale* Koch.) Using Response Surface Methodology and Evaluation of Extracts Composition. *The Journal of Supercritical Fluids*. 2014, 87, 93–103. ISSN 0896-8446.

98. KHODDAMI, A., WILKES, M.A. and ROBERTS, T.H. Techniques for Analysis of Plant Phenolic Compounds. *Molecules*. 2013, 18(2), 2328–2375. ISSN 1420-3049.

99. KINTZIOS, S.E. Sage: The Genus *Salvia*. In: Kintzios, S.E. (ed.). *Medicinal and Aromatic Plants*. The Netherlands: Harwood Academic Publishers, 2000, 14, 296. ISBN 90-5823-005-8.

100. KONTOGIANNI, V.G., TOMIC, G., NIKOLIC, I., NERANTZAKI, A.A., SAYYAD, N., STOSIC-GRUJICIC, S. and TZAKOS, A.G. Phytochemical Profile of *Rosmarinus Officinalis* and *Salvia Officinalis* Extracts and Correlation to Their Antioxidant and Anti-Proliferative Activity. *Food Chemistry*. 2013, 136(1), 27–36. ISSN 0021-8561.

101. KOUL, O., WALIA, S. and DHALIWAL, G.S. Essential Oils as Green Pesticides: Potential and Constraints. *Biopesticides International*. 2008, 4(1), 63–84. ISSN 0973-483X.

102. KRYŽEVIČIŪTĖ, N., KRAUJALIS, P. and VENSKUTONIS, P.R. Optimization of High Pressure Extraction Processes for the Separation of Raspberry Pomace into Lipophilic and Hydrophilic Fractions. *Journal of Supercritical Fluids*. 2016, 108, 61–68. ISSN 0896-8446.

103. KULIŠIĆ, T., DRAGOVIĆ-UZELAC, V. and MILOŠ, M. Antioxidant Activity of Aqueous Tea Infusions Prepared from Oregano, Thyme and Wild Thyme. *Food Technology and Biotechnology*. 2006, 44(4), 485–492. ISSN 1330-9862.

104. KUŹMA, Ł, KALEMBA, D., RÓŻALSKI, M., RÓŻALSKA, B., WIĘCKOWSKA-SZAKIEL, M., KRAJEWSKA, U. and WYSOKIŃSKA, H. Chemical Composition and Biological Activities of Essential Oil From *Salvia Sclarea* Plants Regenerated *in Vitro*. *Molecules*. 2009, 14(4): 1438–1447. ISSN 1420-3049.

105. KRISTINOVÁ, V., MOZURAITYTE, R., STORRØ, I. and RUSTAD, T. Antioxidant Activity of Phenolic Acids in Lipid Oxidation Catalyzed by Different Prooxidants. *Journal of Agricultural and Food Chemistry*. 2009, 57(21), 10377–10385. ISSN 0021-8561.

106. LANG, Q. and WAI, C.M. Supercritical Fluid Extraction in Herbal and Natural Product Studies – a Practical Review. *Talanta*. 2001, 53(4), 771–782. ISSN 0039-9140.

107. LAWLESS, J. *The Illustrated Encyclopedia of Essential Oils*. Shaftesbury, Dorset: Element, 1995, 83. ISBN 1852307218.

108. LAWRENCE, B.M. Commercial Production of Non-Citrus Essential Oils in North America. *Perfumer and Flavorist*. 1979, 3(6), 21–33. ISSN 0361-8587.

109. LEAHY, M., SPERONI, J. and STARR, M. Latest Developments in Cranberry Health Research. *Pharmaceutical Biology*. 2002, 40, 50–54. ISSN 1388-0209.

110. LEE, J. Caffeic Acid Derivatives in Dried Lamiaceae and *Echinacea Purpurea* Products. *Journal of Functional Foods*. 2010, 2(2), 158–162. ISSN 1756-4646.

111. LEE, J. and SCAGEL, C.F. Chicoric Acid Found in Basil (*Ocimum Basilicum* L.) Leaves. *Food Chemistry*. 2009, 115(2), 650–656. ISSN 0021-8561.

112. LI, M., WANG, F., HUANG, Y., DU, F., ZHONG, C., OLALEYE, O.E., LI, C. Systemic Exposure to and Disposition of Catechols Derived from *Salvia Miltiorrhiza* Roots (Danshen) after Intravenous Dosing DanHong Injection in Human Subjects, Rats, and Dogs. *Drug Metabolism and Disposition*. 2015, 43(5), 679–690. ISSN 0090-9556.

113. LIMA, C.F., CARVALHO, F., FERNANDES, E., BASTOS, M.L., SANTOS-GOMES, P.C., FERNANDES-FERREIRA, M. and PEREIRA-WILSO, C. Evaluation of Toxic/Protective Effects of the Essential Oil of *Salvia Officinalis* on Freshly Isolated Rat Hepatocytes. *Toxicology in Vitro*. 2004, 18(4), 457–465. ISSN 0887-2333.

114. LIU, A.H., LIN, Y.H., YANG, M., GUO, H., GUAN, S.H., SUN, J.H. and GUO, D.A. Development of the Fingerprints for the Quality of the Roots of *Salvia Miltiorrhiza* and Its Related Preparations by HPLC-DAD and LC–MSn. *Journal of Chromatography B*. 2007, 846(1–2), 32–41. ISSN 1570-0232.

115. LOBO, V., PATIL, A., PHATAK, A. and CHANDRA, N. Free Radicals, Antioxidants and Functional Foods: Impact on Human Health. *Pharmacognosy Reviews*. 2010, 4(8), 118–126. ISSN 0973-7847.

116. LOPRESTI, A.L. *Salvia* (Sage): a Review of Its Potential Cognitive-Enhancing and Protective Effects. *Drugs in R&D*. 2017, 17(1), 53–64. ISSN 1174-5886.

117. LU, Y.R. and FOO, L.Y. (1999). Rosmarinic Acid Derivatives from *Salvia Officinalis*. *Phytochemistry*. 1999, 51(1), 91–94. ISSN 0031-9422.

118. LU, Y.R. and FOO, L.Y. (2001). Antioxidant Activities of Polyphenols from Sage (*Salvia Officinalis*). *Food Chemistry*. 75(2), 197–202. ISSN 0021-8561.

119. LU, Y.R. and FOO, L.Y. (2002). Polyphenolics of *Salvia* – a Review. *Phytochemistry*. 2002, 59(2), 117–140. ISSN 0031-9422.

120. LUQUE DE CASTRO, M.D. and JIMÉNEZ-CARMONA, M.M. Where Is Supercritical Fluid Extraction Going? *Trends in Analytical Chemistry*. 2000, 19(4), 223–228. ISSN 0165-9936.

121. LYONS, M., YU, C., TOMA, R.B., CHO, S.Y., REIBOLDT, W., LEE, J. and VAN BREEMEN, R.B. Resveratrol in Raw and Baked Blueberries and Bilberries. *Journal of Agricultural and Food Chemistry.* 2003, 51(20), 5867–5870. ISSN 0021-8561.

122. MA, Q., SU, X., YANG, J., ZHANG, L., LIU, C. and WEI, R. Chemistry and Pharmacology of *Salvia Plebeia* R. Brown (Lamiaceae). *Journal of Chemical and Pharmaceutical Research.* 2014, 6(10), 777–783. ISSN 0975-7384.

123. MACDONALD-WICKS, L.K., WOOD, L.G. and GARG, M.L. Methodology for the Determination of Biological Antioxidant Capacity in Vitro: a Review. *Journal of the Science of Food and Agriculture.* 2006, 86(13), 2046–2056. ISSN 0022-5142.

124. MADSEN, H.L. and BERTELSEN, G. Spices as Antioxidants. *Trends in Food Science and Technology.* 1995, 6(8), 271–277. ISSN 0924-2244.

125. MAESTRI, D.M., NEPOTE, V., LAMARQUE, A.L. and ZYGADLO, J.A. Natural Products as Antioxidants. *Phytochemistry: Advances in Research.* 2006, 105–135. ISBN 81-308-0034-9.

126. MANOSROI, J., DHUMTANOM, P. and MANOSROI, A. Anti-Proliferative Activity of Essential Oil Extracted from Thai Medicinal Plants on KB and P388 Cell Lines. *Cancer Letters.* 2006, 235(1), 114–120. ISSN 0304-3835.

127. MARINO, M., BERSANI, C. and COMI, G. Impedance Measurements to Study the Antimicrobial Activity of Essential Oils from Lamiaceae and Compositae. *International Journal of Food Microbiology.* 2001, 67(3), 187–195. ISSN 0168-1605.

128. MARTINS, N., BARROS, L., SANTOS-BUELGA, C., HENRIQUES, M., SILVA, S. and FERREIRA, I.C.F.R. Evaluation of Bioactive Properties and Phenolic Compounds in Different Extracts Prepared from *Salvia Officinalis* L. *Food Chemistry*. 2015, 170, 378–385. ISSN 0021-8561.

129. MARTINS, A.P., SALGUEIRO, L.R., VILA, R., TOMI, F., CAÑIGUERAL, S., CASANOVA, J., DA CUNHA, A.P. and ADZET, T. Composition of the Essential Oils of *Ocimum Canum, O. Gratissimum* and *O. Minimum. Planta Medica.* 1999, 65(2), 187–189. ISSN 0032-0943.

130. MATA, A.T., PROENÇE, C., FERREIRA, A.R., SERRALHEIRO, M.L.M., NOGUEIRA, J.M.F. and ARAÚJO, M.E.M. Antioxidant and Antiacetylcholinestarase Activities of Five Plants Used as Portuguese Food Spices. *Food Chemistry.* 2007, 103(3), 778–786. ISSN 0021-8561.

131. MIĆIĆ, V., LEPOJEVIĆ, Z., JOTANOVIAE, M., TADIĆ, G. and PEJOVIĆ, B. Supercritical Extraction of *Salvia Officinalis* L. *Journal of Applied Sciences.* 2011, 11(21), 3630–3634. ISSN 1678-7757.

132. MIGUEL, M.G. Antioxidant Activity of Medicinal and Aromatic Plants. A Review. *Flavour and Fragrance Journal.* 2010, 25(5), 291–312. ISSN 0882-5734.

133. MIKALAUSKAS, G. Screening, Isolation and Evaluation of Antioxidative Compounds from *Geranium Macrorrhizum*, *Potentilla Fruticosa* and *Rhaponticum Carthamoides*. Holland, Wageningen. Thesis Wageningen University, 2006. pp. 1–155. ISBN 90-8504-487-1.

134. MILLER, H.E., RIGELHOF, F., MARQUART, L., PRAKASH, A. and KANTER, M. Antioxidant Content of Whole Grain Breakfast Cereals, Fruits and Vegetables. *Journal of the American College of Nutrition.* 2000, 19(3), 312S–319S. ISSN 1541-1087.

135. MINH TU, N.T., THANH, L.X., UNE, A., UKEDA, H. and SAWAMURA, M. (2002). Volatile Constituents of Vietnamese Pummelo, Orange, Tangerine and Lime Peel Oils. *Flavour and Fragrance Journal.* 2002, 17(3), 169–174. ISSN 0882-5734.

136. MIRJALILI, M.H., SALEHI, P., SONBOLI, A. and VALA, M.M. Essential Oil Variation of *Salvia Officinalis* Aerial Parts during Its Phenological Cycle. *Chemistry of Natural Compounds.* 2006, 42(1), 19–23. ISSN 0009-3130.

137. MIRODDI, M., NAVARRA, M., QUATTROPANI, M.C., CALAPAI, F., GANGEMI, S. and CALAPAI, G. Systematic Review of Clinical Trials Assessing Pharmacological Properties of *Salvia* Species of Memory, Cognitive Impairment and Alzheimer's Disease. *CNS Neuroscience and Therapeutics.* 2014, 20(6), 485–495. ISSN 1755-5930.

138. MIURA, K., KIKUZAKI, H. and NAKATANI, N. Apianane Terpenoids from *Salvia Officinalis. Phytochemistry*. 2001, 58(8), 1171–1175. ISSN 0031-9422.

139. MOON, J.K. and SHIBAMOTO, T. Antioxidant Assays for Plant and Food Components. *Journal of Agricultural and Food Chemistry.* 2009, 57(5), 1655–1666. ISSN 0021-8561.

140. MUNNÉ-BOSCH, S. and FALK, J. New Insights into the Function of Tocopherols in Plants. *Journal of Plant Biology*. 2004, 218(3), 323–326. ISSN 0032-0935.

141. MURBACH TELES, B.F., ANDRADE, M.T., BARBOSA, L.N., PROBST, I.S., and FERNANDES JÚNIOR, A. Antimicrobial Activity of Essential Oils. *Journal of Essential Oil Research.* 2014, 26(1), 34–40. ISSN 1041-2905.

142. NAGHIBI, F., MOSADDEGH, M., MOHAMMADI MOTAMED, S. and GHORBANI, A. Labiatae Family in Folk Medicine in Iran: from Ethnobotany to Pharmacology. *Iranian Journal of Pharmaceutical Research.* 2005, 4(2), 63–79. ISSN 1735-0328.

143. NAMBIAR, V.S., DANIEL, M. and GUIN, P. Characterization of Polyphenols from Coriander Leaves (*Coriandrum Sativum*), Red Amaranthus (*A. Paniculatus*) and Green Amaranthus (*A. Frumentaceus*) Using Paper Chromatography and Their Health Implications. *Journal of Herbal Medicine and Toxicology.* 2010, 4(1), 173–177. ISSN 0973-4643.

144. NIKI, E. Assessment of Antioxidant Capacity in Vitro and in Vivo. *Free Radical Biology and Medicine.* 2010, 49(4), 503–515. ISSN 0891-5849.

145. NINFALI, P., MEA, G., GIORGINI, S., ROCCHI, M. and BACCHIOCCA, M. Antioxidant Capacity of Vegetables, Spices and Dressings Relevant to Nutrition. *British Journal of Nutrition.* 2005, 93(2). 257–266. ISSN 0007-1145.

146. OCCHIPINTI, A., CAPUZZO, A., ARCEUSZ, A. and MAFFEI, M.E Comparative Analysis of α- and β-Thujone in the Essential Oil and Supercritical CO_2 Extract of Sage (*Salvia Officinalis* L.). *Journal of Essential Oil Research.* 2014, 26(2), 85–90. ISSN 1041-2905.

147. ÖZGEN, U., MAVI, A., TERZI, Z., YILDIRIM, A, COŞKUN, M. and HOUGHTON, P.J. Antioxidant Properties of some Medicinal Lamiaceae (Labiatae) Species. *Pharmaceutical Biology.* 2006, 44(2), 107–112. ISSN 1388-0209.

148. PAN, M.H., LAI, C.S., and HO, C.T. Anti-Inflammatory Activity of Natural Dietary Flavonoids. *Food and Function.* 2010, 1(1), 15–31. ISSN 2042-6496.

149. PANDEY, A.K., SINGH, P. and TRIPATHI, N.N. Chemistry and Bioactivities of Essential Oils of Some Ocimum Species: an Overview. *Asian Pacific Journal of Tropical Biomedicine.* 2014, 4(9), 682–694. ISSN 2221-1691.

150. PAREDES-LÓPEZ, O., CERVANTES-CEJA, M.L., VIGNA-PÉREZ, M. and HERNÁNDEZ-PÉREZ, T. Berries: Improving Human Health and Healthy Aging, and Promoting Quality Life – a Review. *Plant Foods for Human Nutrition.* 2010, 65(3), 299–308. ISSN 0921-9668.

151. PASTORIZA, S., DELGADO-ANDRADE, C., HARO, A. and RUFIÁN-HENARES, J.A. A Physiologic Approach to Test the Global Antioxidant Response of Foods. The GAR Method. *Journal of Agricultural and Food Chemistry*. 2011, 129(4), 1926–1932. ISSN 0021-8561.

152. PERRY, N.B., ANDERSON, R.E., BRENNAN, N.J., DOUGLAS, M.H., HEANEY, A.J., MCGIMPSEY, J.A. and SMALLFIELD, B.M. Essential Oils from Dalmatian Sage (*Salvia Officinalis* L.): Variations among Individuals, Plant Parts, Seasons, and Sites. *Journal of Agricultural and Food Chemistry.* 1999, 47(5): 2048–2054. ISSN 0021-8561.

153. PETERSON, D.M., EMMONS, C.L. and HIBBS, A.H. Phenolic Antioxidants and Antioxidant Activity in Pearling Fractions of Oat Groats. *Journal of Cereal Science.* 2001, 33(1), 97–103. ISSN 0733-5210.

154. PETERSEN, M. and SIMMONDS, M.S.J. Rosmarinic Acid. *Phytochemistry.* 2003, 62(2), 121–125. ISSN 0031-9422.

155. PHAM-HUY, L.A., HE, H. PHAM-HUY, C. Free Radicals, Antioxidants in Disease and Health. *Journal of Biomedical Science.* 2008, 4(2), 89–96. ISSN 1550-9702.

156. PHIPPS, S., SHARAF, M.H.M. and BUTTERWECK, V. Assessing Antioxidant Activity in Botanicals and Other Dietary Supplements. *Pharmacopeial Forum.* 2007, 33(4), 1–6. ISSN 0363-4655.

157. PINO, J.A., ESTARRÓN, M. and FUENTES, V. Essential Oil of Sage (*Salvia Officinalis* L.) Grown in Cuba. *Journal of Essential Oil Research.* 1997, 9(2), 221–222. ISSN 1041-2905.

158. PINTO, E., SALGUEIRO, L.R., CAVALEIRO, C., PALMEIRA, A. and GONÇALVES, M.J. *In Vitro* Susceptibility of Some Species of Yeasts and Filamentous Fungi to Essential Oils of *Salvia Officinalis*. *Industrial Crops and Products.* 2007, 26(2), 135–141. ISSN 0926-6690.

159. PITAROKILI, D., COULADIS, M., PETSIKOS-PANAYOTAROU, N. and TZAKOU, O. Composition and Antifungal Activity on Soil-Borne Pathogens of the Essential Oil of *Salvia Sclarea* from Greece. *Journal of Agricultural and Food Chemistry*. 2002, 50(23): 6688–6691. ISSN 0021-8561.

160. PORRES-MARTÍNEZ, M., GONZÁLEZ-BURGOS, E., ACCAME, M.E.C. and GÓMEZ-SERRANILLOS, M.P. Phytochemical Composition, Antioxidant and Cytoprotective Activities of Essential Oil of *Salvia Lavandulifolia* Vahl. *Food Research International.* 2013, 54(1), 523–531. ISSN 0963-9969.

161. POVILAITIS, D., ŠULNIŪTĖ, V., VENSKUTONIS, P.R. and KRAUJALIENĖ, V. Antioxidant Properties of Wheat and Rye Bran Extracts Obtained by Pressurized Liquid Extraction with Different Solvents. *Journal of Cereal Science*. 2015, 62, 117–123. ISSN 0733-5210.

162. PRASAD, S. and TYAGI, A.K. Ginger and Its Constituents: Role in Prevention and Treatment of Gastrointestinal Cancer. *Gastroenterology Research and Practice*. 2015, 2015 (article ID: 142979), 11. ISSN 1687-6121.

163. PRICE, S.F., BREEN, P.J., VALLADAO, M. and WATSON, B.T. Cluster Sun Exposure and Quercetin in Pinot Noir Grapes and Wine. *American Journal of Enology and Viticulture*. 1995, 46(2), 187–194. ISSN 0002-9254.

164. PRIOR, R.L. and CAO, G. Antioxidant Phytochemicals in Fruits and Vegetables: Diet and Health Implications. *Horticulture Science*. 2000, 35(4). 588–592. ISSN 0003-1062.

165. PRIOR, R.L., HOANG, H., GU, L., WU, X., BACCHIOCCA, M., HOWARD, L., HAMPSCH-WOODILL, M., HUANG, D., OU, B. and JACOB, R. Assays for Hydrophilic and Lipophilic Antioxidant Capacity (Oxygen Radical Absorbance Capacity (ORAC(FL))) of Plasma and Other Biological and Food Samples. *Journal of Agricultural and Food Chemistry*. 2003, 51(11), 3273–3279. ISSN 0021-8561.

166. PRIOR, R.L., WU, W. and SCHAICH, K. Standardized Methods for the Determination of Antioxidant Capacity and Phenolics in Foods and Dietary Supplements. *Journal of Agricultural and Food Chemistry.* 2005, 53(10), 4290–4302. ISSN 0021-8561.

167. PRONYK, C. and MAZZA, G. Design and Scale-up of Pressurized Fluid Extractors for Food and Bioproducts. *Journal of Food Engineering.* 2009, 95(2), 215–226. ISSN 0260-8774.

168. PUKALSKAS, A. Isolation, Identification and Activity of Natural Antioxidants from Sweet Grass *(Hierochloe Odorata)*, Costmary *(Chrysanthemum Balsamita)* and Horehound *(Marrubium Vulgare)*, Cultivated in Lithuania. Holland, Wageningen. Thesis Wageningen University, 2008. pp. 1–137. ISBN 978-90-8504-982-1.

169. RAAL, A., ORAV, A. and ARAK, E. Composition of the Essential Oil of *Salvia Officinalis* L. from Various European Countries. *Natural Product Research.* 2007, 21(5): 406–411. ISSN 1478-6419.

170. RAMASUBRAMANIA RAJA, R. Medicinally Potential Plants of Labiatae (Lamiaceae) Family: an Overview. *Research Journal of Medicinal Plants.* 2012, 6(3), 203–213. ISSN 1996-0875.

171. RAUT, P., BHOSLE, D., JANGHEL, A., DEO, S., VERMA, C., KUMAR, S.S., AGRAWAL, M., AMIT, N., SHARMA, M., GIRI, T., TRIPATHI, D.K., AJAZ, A. and ALEXANDER, A. Emerging Pressurized Liquid Extraction (PLE) Techniques as an Innovative Green Technologies for the Effective Extraction of the Active Phytopharmaceuticals. *Research Journal of Pharmacy and Technology*. 2015, 8(6), 801–812. ISSN 0974-3618.

172. RE, R., PELLEGRINI, N., PROTEGGENTE, A., PANNALA, A., YANG, M. and RICE-EVANS, C.A. Antioxidant Activity Applying an Improved ABTS Radical Cation Decolorization Assay. *Free Radical Biology and Medicine.* 1999, 26(9–10), 1231–1237. ISSN 0891-5849.

173. REVERCHON, E. and DE MARCO, I. Supercritical Fluid Extraction and Fractionation of Natural Matter. *Journal of Supercritical Fluids.* 2006, 38(2), 146–166. ISSN 0896-8446.

174. RICHTER, B.E., JONES, B.E., EZZELL, J.L. and PORTER, N.L. Accelerated Solvent Extraction: a Technique for Sample Preparation. *Analytical Chemistry*. 1996, 68(6), 1033–1039. ISSN 0003-2700.

175. RIMANDO, M.A., KALT, W., MAGEE, B.J., DEWEY, J. and BALLINGTON, R.J. Resveratrol, Pterostilbene and Piceatannol in Vaccinium Berries. *Journal of Agricultural and Food Chemistry.* 2004, 52(15), 4713–4719. ISSN 0021-8561.

176. ROGINSKY, V. and LISSI, E.A. Review of Methods to Determine Chain-Breaking Antioxidant Activity in Food. *Food Chemistry.* 2005, 92(2), 235–254. ISSN 0021-8561.

177. RYYNÄNEN, M., LAMPI, A.M., SALO-VÄÄNÄNEN, P., OLLILAINEN, V. and PIIRONEN, V. A Small-Scale Sample Preparation Method with HPLC Analysis for Determination of Tocopherols and Tocotrienols in Cereals. *Journal of Food Composition and Analysis.* 2004, 17(6), 749–765. ISSN 0889-1575.

178. SÁ, C.M., RAMOS, A.A., AZEVEDO, M.F., LIMA, C.F., FERNANDES-FERREIRA, M. and PEREIRA-WILSON, C. Sage Tea Drinking Improves Lipid Profile and Antioxidant Defences in Humans. *International Journal of Molecular Sciences.* 2009, 10(9), 3937–3950. ISSN 1661-6596.

179. SAID-AL AHL, H., HUSSEIN, M.S, GENDY, A.S.H.,and TKACHENKO, K.G. Quality of Sage (*Salvia Officinalis* L.) Essential Oil Grown in Egypt. *Journal of Plant Science and Ecology.* 2015, 1(4), 119–123. ISSN 1752-9921.

180. SAITO, S.T., GOSMANN, G., SAFFI, J., PRESSER, M., RICHTER, M.F. and BERGOLD, A.M. Characterization of the Constituents and Antioxidant Activity of Brazilian Green Tea (*Camellia Sinensis* Var. Assamica IAC-259 Cultivar) Extracts. *Journal of Agricultural and Food Chemistry.* 2007, 55(23), 9409–9414. ISSN 0021-8561.

181. SANGWAN, N.S., FAROOQI, A.H.A., SHABIH, F. and SANGWAN, R.S. Regulation of Essential Oil Production in Plants. *Plant Growth Regulation.* 2001, 34(1), 3–21. ISSN 0167-6903.

182. SANTOS-GOMES, P.C. and FERNANDES-FERREIRA, M. Essential Oils Produced by in Vitro Shoots of Sage (*Salvia Officinalis* L.). *Journal of Agricultural and Food Chemistry.* 2003, 51(8), 2260–2266. ISSN 0021-8561.

183. SAWAI, Y. and SAKATA, K. NMR Analytical Approach to Clarify the Antioxidative Molecular Mechanism of Catechins Using 1,1-diphenyl-2-picrylhydrazyl. *Journal of Agricultural and Food Chemistry.* 1998, 46(1), 111–114. ISSN 0021-8561.

184. SCHANTZ, M.M. Pressurized Liquid Extraction in Environmental Analysis. *Analytical and Bioanalytical Chemistry.* 2006, 386(4), 1043–1047. ISSN 1618-2642.

185. SCHMIDERER, C., GRASSI, P., NOVAK, J., WEBER, M. and FRANZ, C. Diversity of Essential Oil Glands of Clary Sage (*Salvia Sclarea* L., Lamiaceae). *Journal of Plant Biology.* 2008, 10(4), 433–440. ISSN 1226-9239.

186. SCHMIDT, B., RIBNICKY, D.M., POULEV, A., LOGENDRA, S., CEFALU, W.T. and RASKIN, I.A Natural History of Botanical Therapeutics. *Metabolism.* 2008, 57(1), S3–S9. ISSN 0026-0495.

187. SCHUHMACHER, A., REICHLING, J. and SCHNITZLER, P. Virucidal Effect of Peppermint Oil on the Enveloped Viruses Herpes Simplex Virus Type 1 and Type 2 *in Vitro*. *Phytomedicine.* 2003, 10(6-7), 504–510. ISSN 0944-7113.

188. SEJALI, S.N.F. and ANUAR, M.S. Effect of Drying Methods on Phenolic Contents of Neem (*Azadirachta Indica)* Leaf Powder. *Journal of Herbs, Spices & Medicinal Plants*. 2011, 17(2), 119–131. ISSN 1049-6475.

189. SERPEN, A., CAPUANO, E., FOGLIANO, V. and GÖKMEN, V. A New Procedure to Measure the Antioxidant Activity of Insoluble Food Components. *Journal of Agricultural and Food Chemistry.* 2007, 55(19), 7676–7681. ISSN 0021-8561.

190. SERPEN, A., GÖKMEN, V. and FOGLIANO, V. Solvent Effects on Total Antioxidant Capacity of Foods Measured by Direct QUENCHER Procedure. *Journal of Food Composition and Analysis.* 2012, 26(1–2), 52–57. ISSN 0889-1575.

191. SGORBINI, B., CAGLIERO, C., PAGANI, A., SGANZERLA, M., BOGGIA, L., BICCHI, C. and RUBIOLO, P. Determination of Free and Glucosidically-Bound Volatiles in Plants. Two Case Studies: *L*-Menthol in Peppermint (*Mentha x Piperita* L.) and Eugenol in Clove (*Syzygium Aromaticum* (L.) Merr. & L.M.Perry). *Phytochemistry.* 2015, 117, 296–305. ISSN 0031-942.

192. SHAN, B., CAI, Y., SUN, M. and CORKE, H. Antioxidant Capacity of 26 Spices Extracts and Characterization of Their Phenolic Constituents. *Journal of Agricultural and Food Chemistry.* 2005, 53(20), 7749–7759. ISSN 0021-8561.

193. SHRIVASTAVA, A. and GUPTA, V.B. (2011). Methods for the Determination of Limit of Detection and Limit of Quantitation of the Analytical Methods. *Chronicles of Young Scientists.* 2011, 2(1), 21–25. ISSN 2229-5186.

194. SINGLETON, V.L. and ROSSI, J.A. Jr. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *American Journal of Enology and Viticulture.* 1965, 16, 144–158. ISSN 0002-9254.

195. SMOLSKAITĖ, L., VENSKUTONIS, P.R. and TALOU, T. Comprehensive Evaluation of Antioxidant and Antimicrobial Properties of Different Mushroom Species. *LWT – Food Science and Technology.* 2015, 60(1), 462–471. ISSN 0023-6438.

196. SOKOVIĆ, M. and VAN GRIENSVEN, L. J. L. D. Antimicrobial Activity of Essential Oils and Their Components against the Three Major Pathogens of the Cultivated Button Mushroom, *Agaricus Bisporus*. *European Journal of Plant Pathology.* 2006, 116(3), 211–224. ISSN 0929-1873.

197. STASHENKO, E.E., JARAMILLO, B.E. and MARTÍNEZ, J.R. Analysis of Volatile Secondary Metabolites from Colombian *Xylopia Aromatica* (Lamarck) by Different Extraction and Headspace Methods and Gas Chromatography. *Journal of Chromatography A.* 2004, 1025(1), 105–113. ISSN 0021-9673.

198. SU, X., DUAN, J., JIANG, Y., DUAN, X. and CHEN, F. Polyphenolic Profile and Antioxidant Activities of Oolong Tea Infusion under Various Steeping Conditions. *Molecular Sciences.* 2007, 8(12), 1196–1205. ISSN 1422-0067.

199. SVILAAS, A., SAKHI, A.K., ANDERSEN, L.F., SVILAAS, T., STRÖM, E.C., JACOBS, D.R. Jr., OSE, L. and BLOMHOFF, R. Intakes of Antioxidants in Coffee, Wine, and Vegetables Are Correlated with Plasma Carotenoids in Humans. *Journal of Nutrition.* 2004, 134(3), 562–567. ISSN 0022-3166.

200. SVOBODA, K.P. and GREENAWAY, R.I. Lemon Scented Plants. *International Journal of Aromatherapy.* 2003, 13(1), 23–32. ISSN 0962-4562.

201. SZETO, Y.T., TOMLINSON, B. and BENZIE, I.F.F. Total Antioxidant and Ascorbic Acid Content of Fresh Fruits and Vegetables: Implications for Dietary Planning and Food Preservation. *British Journal of Nutrition.* 2002, 87(1), 55–59. ISSN 0007-1145.

202. TEIXEIRA, S., MENDES, A., ALVES, A. and SANTOS, L. Simultaneous Distillation-Extraction of High-Value Volatile Compounds from *Cistus Ladanifer* L. *Analytica Chimica Acta.* 2007, 584(2), 439–446. ISSN 0003-2670.

203. TEPE, B., SOKMEN, M., AKPULAT, H.A. and SOKMEN, A. Screening of the Antioxidant Potentials of Six *Salvia* Species from Turkey. *Food Chemistry*. 2006, 95(2), 200–204. ISSN 0021-8561.

204. TIKEKAR, R.V., LUDESCHER, R.D. and KARWE, M.V. Processing Stability of Squalene in Amaranth and Antioxidant Potential of Amaranth Extract. *Journal of Agricultural and Food Chemistry.* 2008, 56(22), 10675–10678. ISSN 0021-8561.

205. TOPÇU, G., TAN, N., ULUBELEN, A., SUN, D. and WATSON, W.H. Terpenoids and Flavonoids from the Aerial Parts of *Salvia Candidissima*. *Phytochemistry.* 1995, 40(2), 501–504. ISSN 0031-9422.

206. TOPÇU, G. and ULUBELEN, A. Structure Elucidation of Organic Compounds from Natural Sources Using 1D and 2D NMR Techniques. *Journal of Molecular Structure.* 2007, 834–836(27), 57–73. ISSN 0022-2860.

207. TSUDA, T., OSAWA, T., OHSHIMA, K. and KAWAKISHI, S. Antioxidative Pigments Isolated from the Seeds of *Phaseolus Vulgaris* L. *Journal of Agricultural and Food Chemistry.* 1994, 42(2), 248–251. ISSN 0021-8561.

208. UPADHYAY, R., and MISHRA, H.N. Antioxidant Activity Measurement of Oleoresin from Rosemary and Sage. *Industrial Crops and Products.* 2014, 61, 453–459. ISSN 0926-6690.

209. VELICKOVIC, D., RISTIC, M. and VELICKOVIC, A. Chemical Composition of the Essential Oils Obtained from the Flower, Leaf and Stem of *Salvia Aethiopis* L. and *Salvia Glutinosa* L. Originating from the Southeast Region of Serbia. *Journal of Essential Oil Research.* 2003, 15(5), 346–349. ISSN 1041-2905.

210. VELIČKOVIĆ, D.T., RISTIĆ, M.S., MILOSAVLJEVIĆ, N.P., KARABEGOVIĆ, I.T., STOJIČEVIĆ, S.S. and LAZIĆ, M.L. Chemical Composition of the Essential Oils of *Salvia Austriaca* Jacq. and *Salvia Amplexicaulis* Lam. from Serbia. *Agro FOOD Industry Hi Tech.* 2012, 23(3), 8–10. ISSN 1722-6996.

211. VENNERIA, E., FANASCA, S., MONASTRA, G., FINOTTI, E., AMBRA, R., AZZINI, E., DURAZZO, A., FODDAI, M.S. and MAIANI, G. Assessment of the Nutritional Values of Genetically Modified Wheat, Corn, and Tomato Crops. *Journal of Agricultural and Food Chemistry.* 2008, 56(19), 9206–9214. ISSN 0021-8561.

212. VIROT, M., TOMAO, V., GINIES, C., VISINONI, F. and CHEMAT, F. Green Procedure with a Green Solvent for Fats and Oils' Determination: Microwave-Integrated Soxhlet Using Limonene Followed by Microwave Clevenger Distillation. *Journal of Chromatography A.* 2008, 1196–1197, 147–152. ISSN 0021-9673.

213. VIUDA-MARTOS, M., RUIZ-NAVAJAS, Y., FERNÁNDEZ-LÓPEZ, J. and PÉREZ-ÁLVAREZ, J.A. Spices as Functional Foods. *Critical Reviews in Food Science and Nutrition.* 2011, 51(1), 13–28. ISSN 1040-8398.

214. WALCH, S.G., TINZOH, L.N., ZIMMERMANN, B.F., STÜHLINGER, W. and LACHENMEIER, D.W. Antioxidant Capacity and Polyphenolic Composition as Quality Indicators for Aqueous Infusions of *Salvia Officinalis* L. (Sage Tea). *Pharmacology.* 2011, 2, 1–6. ISSN 0031-7012.

215. WANG, J.S., ZHAO, M.M., ZHAO, Q.Z. and JIANG, Y.M. Antioxidant Properties of Papain Hydrolysates of Wheat Gluten in Different Oxidation Systems. *Food Chemistry*. 2007, 101(4), 1658–1663. ISSN 0021-8561.

216. WANG, Y., CATANA, F., YANG, Y., RODERICK, R. and VAN BREEMEN, R.B. An LC-MS Method for Analyzing Total Resveratrol in Grape Juice, Cranberry Juice and Wine. *Journal of Agricultural and Food Chemistry.* 2002, 50(3), 431–435. ISSN 0021-8561.

217. WHITE, P.J. and XING, Y. Antioxidants from Cereals and Legumes. In: SHAHIDI, F. (ed.). *Natural Antioxidants: Chemistry, Health Effects, and Applications*. Champaign IL: AOCC press, 1997, 25–63. ISBN 0-935315-77-2.

218. WHITTLESEY, J. *The Plant Lover's Guide to Salvias.* Portland: Timber Press, 2014. ISBN 978-1-60469-419-2.

219. WOJDYŁO, A, OSZMIÁNSKI, J. and CZEMERYS, R. Antioxidant Activity and Phenolic Compounds in 32 Selected Herbs. *Food Chemistry*. 2007, 105(3), 940–949. ISSN 0308-8146.

220. WU, Y.B., NI, Z.Y., SHI, Q.W., DONG, M., KIYOTA, H., GU, Y.C. and CONG, B. Constituents from *Salvia* Species and Their Biological Activities. *Chemical Reviews.* 2012, 112(11), 5967–6026. ISSN 0009-2665.

221. YADAV, S. and MUKUNDAN, U. In Vitro Antioxidant Properties of *Salvia Coccinea* Buc'hoz ex etl. and *Salvia Officinalis* L. *Indian Journal of Fundamental and Applied Life Sciences.* 2011, 1(3), 232–238. ISSN 2231-6345.

222. YAN, X., MURPHY, B.T., HAMMOND, G.B., VINSON, J.A. and NETO, C.C. Antioxidant Activities and Antitumor Screening of Extracts from Cranberry Fruit (Vaccinium Macrocarpon). *Journal of Agricultural and Food Chemistry*. 2002, 50(21), 5844–5849. ISSN 0021-8561.

223. YANISHLIEVA, N.V. and MARINOVA, E.M. Stabilisation of Edible Oils with Natural Antioxidants. *European Journal of Lipid Science and Technology*. 2001, 103(11), 752–767. ISSN 1438-7697.

224. YAO, L.H., JIANG, Y.M., SHI, J., TOMÁS-BARBERÁN, F.A., DATTA, N., SINGANUSONG R. and CHEN S.S. Flavonoids in Food and Their Health Benefits. *Plant Foods for Human Nutrition.* 2004, 59(3), 113–122. ISSN 0921-9668.

225. ZGÓRKA, G. and GŁOWNIAK, K. Variation of Free Phenolic Acids in Medicinal Plants Belonging to the *Lamiaceae* Family. *Journal of Pharmaceutical and Biomedical Analysis.* 2001, 26(1), 79–87. ISSN 0731-7085.

226. ZHANG, D. and HAMAUZU, Y. Phenolics, Ascorbic Acid, Carotenoids and Antioxidant Activity of Broccoli and Their Changes during Conventional and Microwave Cooking. *Food Chemistry.* 2004, 88(4), 503–509. ISSN 0308-8146.

227. ZHANG, Y., YANG, L., ZU, Y., CHEN, X., WANG, F. and LIU, F. Oxidative Stability of Sunflower Oil Supplemented with Carnosic Acid Compared with Synthetic Antioxidants during Accelerated Storage. *Food Chemistry*. 2010, 118(3), 656–662. ISSN 0021-8561.

228. ZIMMERMANN, B.F., WALCH, S.G., TINZOH, L.N., STÜHLINGER, W. and LACHENMEIER, D.W. Rapid UHPLC Determination of Polyphenols in Aqueous Infusions of *Salvia Officinalis* L. (Sage Tea). *Journal of Chromatography B.* 2011, 879(24), 2459–2464. ISSN 1570-0232.

229. ZULUETA, A., ESTEVE, M.J. and FRÍGOLA, A. ORAC and TEAC Assays Comparison to Measure the Antioxidant Capacity of Food Products. *Food Chemistry*. 2009, 114(1), 310–316. ISSN 0308-8146.

230. ZUPKÓ, I., HOHMANN, J., RÉDEI, D., FALKAY, G., JANICSÁK, G. and MÁTHÉ, I. (2001). Antioxidant Activity of Leaves of *Salvia* Species Inenzyme-Dependent and Enzyme-Independent Systems of Lipid Peroxidation and Their Phenolic Constituents. *Planta Medica.* 2001, 67(4), 366–368. ISSN 0032-0943.

231. ZUZARTE, L. and SALGUEIRO, M.L. Essential Oils Chemistry. In: DE SOUSA, D.P. (ed.). *Bioactive Essential Oils and Cancer*. Switzerland: Springer International Publishing. 2015, Chapter 2, 19–61. ISBN 978-3-319-19143-0.

List of Publications

Publications on the Theme of the Dissertation Corresponding to the List of the *Clarivate Analytics* (formerly *Thomson Reuters*) *Web of Science* Database

1. **Šulniūtė, Vaida**; Baranauskienė, Renata; Ragažinskienė, Ona; Venskutonis, Petras Rimantas. Comparison of Composition of Volatile Compounds in Ten *Salvia* Species Isolated by Different Methods // *Flavour and Fragrance Journal*. Chichester: John Wiley & Sons Ltd, 2017, vol. 32, no. 4, p. 254-264. ISSN: 0882-5734. (I.F. 1.693 (2015)).

2. **Šulniūtė, Vaida**; Pukalskas, Audrius; Venskutonis, Petras Rimantas. Phytochemical Composition of Fractions Isolated from Ten *Salvia* Species by Supercritical Carbon Dioxide and Pressurized Liquid Extraction Methods // *Food Chemistry*. Oxford: Elsevier, 2017, vol. 224, p. 37–47. ISSN: 0308-8146. (I.F. 4.052 (2015)).

3. **Šulniūtė, Vaida**; Ragažinskienė, Ona; Venskutonis, Petras Rimantas. Comprehensive Evaluation of Antioxidant Potential of 10 *Salvia* Species Using High Pressure Methods for the Isolation of Lipophilic and Hydrophilic Plant Fractions // *Plant Foods for Human Nutrition*. Dordrecht: Springer, 2016, vol. 71, no. 1, p. 64–71. ISSN: 0921-9668. (I.F. 2.276 (2015)).

Other Publications Corresponding to the List of the *Clarivate Analytics* (formerly *Thomson Reuters*) *Web of Science* Database

1. Kitrytė, Vaida; Povilaitis, Darius; Kraujalienė, Vaida; **Šulniūtė, Vaida**; Pukalskas, Audrius; Venskutonis, Petras Rimantas. Fractionation of Sea Buckthorn Pomace and Seeds into Valuable Components by Using High Pressure and Enzyme-Assisted Extraction Methods // *LWT-Food Science and Technology*. (I.F. 2.711 (2015)) (in press).

2. Basegmez, Hatice Imge Oktay; Povilaitis, Darius; Kitrytė, Vaida; Kraujalienė, Vaida; **Šulniūtė, Vaida**; Alasalvar, Cesarettin; Venskutonis, Petras Rimantas. Biorefining of Blackcurrant Pomace into High Value Functional Ingredients Using Supercritical CO_2, Pressurized Liquid and Enzyme Assisted Extractions // *The Journal of Supercritical Fluids*. Amsterdam: Elsevier, 2017, vol. 124, p. 10-19. ISSN: 0896-8446 (print). (I.F. 2.579 (2015)).

3. **Šulniūtė, Vaida**; Jaime, Isabel; Rovira, Jordi; Venskutonis, Petras Rimantas. Rye and Wheat Bran Extracts Isolated with Pressurized Solvents Increase Oxidative Stability and Antioxidant Potential of Beef Meat Hamburgers // *Journal of Food Science*. Hoboken, NJ: Wiley-Blackwell, 2016, vol. 81, no. 2, p. H519–H527. ISSN: 0022-1147. (I.F. 1.649 (2015)).

4. Povilaitis, Darius; **Šulniūtė, Vaida**; Venskutonis, Petras Rimantas; Kraujalienė, Vaida. Antioxidant Properties of Wheat and Rye Bran Extracts Obtained by Pressurized Liquid Extraction with Different Solvents // *Journal of Cereal Science*. London: Academic Press-Elsevier Science, 2015, vol. 62, p. 117–123. ISSN 0733-5210. (I.F. 2.402 (2015)).

List of Presentations on the Theme of the Dissertation in International Scientific Conferences

1. **Sulniute, Vaida**; Pukalskas, Audrius; Venskutonis, Petras Rimantas. Phytochemical Composition and Antioxidants from Ten Different *Salvia* spp. Isolated by High Pressure Extraction Methods // 3rd International Conference on Food and Biosystems Engineering (FaBE2017), June 1–4, 2017, Rhodes Island, Greece. Book of Abstracts/ Thessaly: Technological Educational Institute of Thessaly, 2017, p. 138.

2. **Sulniute, Vaida**; Venskutonis, Petras Rimantas; Pukalskas, Audrius; Baranauskiene, Renata. Antioxidant Activities and Phytochemical Composition of Products Isolated by High Pressure Extraction Methods from *Salvia* spp. // 11th Baltic Conference on Food Science and Technology "Food Science and Technology in a Changing World" (FoodBalt-2017), April 27–28, 2017, Jelgava, Latvia. Abstract Book/ Latvia University of Agriculture, Faculty of Food Technology. Riga: Drukātava, 2017, p. 29. ISSN 2501-0190.

3. **Šulniūtė, Vaida**; Venskutonis, Petras Rimantas; Baranauskienė, Renata. Essential Oils and Antioxidants from Ten *Salvia* spp. Isolated by High Pressure Extraction Methods // 47th International Symposium on Essential Oils (ISEO2016), September 11–14, 2016, Nice, France. Program and Book of Abstracts/ Université de Nice-Sophia Antipolis**.** Nice: Centre de Production Numérique Universitaire (CPNU), 2016, p. 141. *(I received the IFEAT Young Scientist Fellowship award which covered registration fees reimbursement).*

4. Venskutonis, Petras Rimantas; **Sulniute, Vaida**. Comprehensive Evaluation of Antioxidant Properties of Different *Salvia* Species as Sources of Nutraceutical Ingredients // Institute of Food Technologists Annual Meeting and Food Expo "Where Science Feeds Innovation" (IFT-2015), July 11–14, 2015, McCormick Place South, Chicago, IL USA. Book of abstracts/ Chicago (Illinois): Institute of Food Technologists, 2015, p. 122. ISSN 2470-1416.

5. **Šulniūtė, Vaida**; Venskutonis, Petras Rimantas. Screening of Antioxidant Properties of Ten *Salvia* Species by the *in Vitro* Assays // 10th Baltic Conference on Food Science and Technology "Future Food: Innovations, Science and Technology" (FoodBalt-2015), May 21–22, 2015, Kaunas, Lithuania. Abstract Book/ Department of Food Science and Technology. Kaunas: Kaunas University of Technology, 2015, p. 51. e-ISBN 978-609-02-1138-0. (*I received the nomination for the best poster presentation).*

SL344. 2017-06-23, 12,25 leidyb. apsk. l. Tiražas 12 egz. Užsakymas 205.
Išleido Kauno technologijos universitetas, K. Donelaičio g. 73, 44249 Kaunas
Spausdino leidyklos „Technologija" spaustuvė, Studentų g. 54, 51424 Kaunas