„Dunărea de Jos" University of Galați

Doctoral School of Fundamental Sciences and Engineering

PHD THESIS

Separation and characterization of some organic compounds from Romanian varieties of aromatic plants

(PhD thesis summary)

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INTRODUCTION

Plants have been sources of food and medicine since ancient times [1]. In traditional medicine, plants are used both for their curative properties and the prevention of certain diseases [2,3]. When used for preventive purposes, i.e. to maintain general health, some medicinal plants can also be classified as functional foods and/or nutraceuticals. A good example is food flavoring which, in addition to its use as a source of flavor compounds for various food products, also has the role of helping to prevent various ailments such as metabolic, respiratory, or mental ones [4,5].

Over the centuries, empirical knowledge about the benefits of medicinal plants in alternative medicine has been passed down from generation to generation, and researchers around the world have shown an increasing interest in studying the chemical composition of plants and their therapeutic effects, with the aim of to scientifically argue these properties [6,7]. Plants synthesize a wide range of organic compounds that are classified as primary and secondary metabolites [8]. Primary metabolites are compounds that have essential roles associated with various physiological processes such as photosynthesis, respiration, growth, and development. Among them, we can mention carbohydrates, lipids, or amino acids [9].

Under the name of secondary metabolites are known phytochemical compounds that accumulate in varying concentrations in some plant species and that are not necessary for cell development and reproduction, but instead play an important role in plant defense mechanisms [10–13]. Many natural compounds belonging to the category of secondary metabolites are currently compounds of interest in various fields. In this context, numerous compounds have been separated and characterized for their applications in various branches of industry, such as the pharmaceutical, cosmetic, or food fields (medicines, antiseptics, disinfectants, insecticides, herbicides, dyes, flavoring agents or perfumes) [14, 15].

In recent years, the protective role of secondary metabolites in plant-based foods has been widely studied, becoming an increasingly important research area for human nutrition [16]. Studies show that these metabolites are not essential for short-term well-being, but there is well-argued evidence that low long-term intakes can have a favorable impact on the incidence of incurable, chronic diseases, including cardiovascular and type II diabetes, that occur in western populations with increasing frequency [4]. In conclusion, chemical compounds biosynthesized by plants have been and continue to be analyzed for their biologically-active properties and then separated, isolated, purified, or synthesized to be used for therapeutic purposes [17,18].

The doctoral thesis entitled: "***Separation and characterization of some organic compounds from Romanian varieties of aromatic plants***" includes six chapters. In the first chapter, the current state of the research is presented and includes the analysis of data from the specialized literature on the characterization of the studied aromatic plant species. In the following five chapters, the original personal contribution is presented regarding the chemical composition and some biological properties of the studied aromatic plant species, respectively *Ocimum basilicum*, *Ocimum citriodorum*, *Agastache foeniculum*, *Pimpinella anisum*, *Carum carvi,* and *Illicium verum*.

The general objective of the doctoral thesis "***Separation and characterization of some organic compounds from Romanian varieties of aromatic plants***" was the characterization of the chemical composition of six varieties of aromatic plants, cultivated in Romania, three species belonging to the Lamiaceae family: *Ocimum basilicum*, *Ocimum citriodorum,* and *Agastache foeniculum*, two plant species belonging to the Apiaceae family: *Pimpinella anisum* and *Carum carvi*, and one species belonging to the Schisandraceae family: *Illicium verum*. In the doctoral thesis, six varieties of aromatic plants were characterized by applying various methods of analysis depending on the nature of the samples obtained, and by following the current research directions in the field of chemistry of natural plant compounds. Thus, in the first stage, some phytochemical compounds from the analyzed species were identified, separated, and characterized, using various and modern methods of analysis such as spectrophotometric, chromatographic HPTLC, HPLC, GC, and spectral methods such as MS, IR, and NMR.

Another objective of this thesis consisted in finding new practical uses for the organic compounds identified in these plant species through preliminary *in vitro* and *in silico* studies and through studies of the biological activity of some of these compounds.

In the six chapters of the thesis, the original results obtained during the experiments are presented, in dependence with the data from the specialized literature.

The proposed **specific objectives** of the doctoral thesis were the following:

- Sampling plant material from the Buzău Vegetable Research - Development Station, Romania, and preparing the samples for analysis;
- Characterization of the plant material, the aromatic plants *Ocimum basilicum*, *Ocimum citriodorum*, *Agastache foeniculum*, *Pimpinella anisum*, *Carum carvi,* and *Illicium verum*, varieties cultivated in Romania, with the help of confocal laser scanning microscopy;
- Solvent extraction, by methods belonging to green chemistry, of natural organic compounds from the aromatic plant species *Ocimum basilicum*, *Ocimum citriodorum*, *Agastache foeniculum*, *Pimpinella anisum*, *Carum carvi,* and *Illicium verum*;
- Extraction of volatile oils from the aromatic plant species *Ocimum basilicum*, *Ocimum citriodorum*, *Agastache foeniculum*, *Pimpinella anisum*, *Carum carvi,* and *Illicium verum*;
- Qualitative and quantitative analysis of natural organic compounds extracted with organic solvents using high-performance liquid chromatography coupled with mass spectrometry (HPLC-DAD and UHPLC-MS);
- Characterization by NMR spectrometry of the essential oils obtained from the studied aromatic plant species, *Ocimum basilicum*, *Ocimum citriodorum*, *Agastache foeniculum*, *Pimpinella anisum*, *Carum carvi,* and *Illicium verum*;
- Identification and quantification of some classes of organic compounds, polyphenols, and flavonoids, from the studied plant species;
- Evaluation of the antioxidant potential of biologically active compounds from the species of aromatic plants studied, by microspectrophotometric methods of inhibition of free radicals, such as DPPH, and ABTS, and reducing metal ions such as FRAP;
- Evaluation of the antioxidant potential of the biologically active compounds from the aromatic plant species studied by electrochemical methods - cyclic voltammetry;
- Interaction of some isoflavonoids identified in the studied aromatic plant species with phosphatidylinositol 3-kinase alpha and xanthine oxidase through in silico studies.

Keywords: **phytochemical compounds, aromatic plants, plant extracts, essential oils, bioactive compounds, structural analysis, chromatography, *in silico* studies.**

PART I. CURRENT STATE OF RESEARCH

Chapter 1. Characterization of the studied aromatic plant species – Analysis of data from the specialized literature

1.1. Morphological characterization of the studied species

Lamiaceae family

Description of *Ocimum* species

a. *Ocimum basilicum* L. is a native Asian species of the Lamiaceae family. The plant can reach up to 65 cm in height, with light green, silky leaves, 1.5–5 cm long and 1–3 cm wide [64]. The taxonomic classification of the species *Ocimum basilicum* includes the kingdom *Plantae*, division *Magnoliophyta*, from the class *Magnoliopsida*, order *Lamiales* [65].

Due to its phenolic and aromatic compounds, basil has demonstrated antioxidant activities, demonstrated by free radical inactivation capacity [76,77], and antimicrobial [78–81]. The main phenolic components present in basil are phenolic acids and flavonol glycosides [80,82–86]. The presence of caffeic acid derivatives has been reported in basil [82,83,85–87], but complete phenolic profiles of basil have not been reported. Among the caffeic acid derivatives from basil, chicoric and caftaric acids are the major phenolic acids [88] that exhibit antioxidant activities [89].

b. *Ocimum citriodorum* or lemon basil, is the second basil species analyzed in this study and is known to be a cultivar obtained by cross-pollination from two chemotypes of sweet basil: *Ocimum basilicum* and *Ocimum americanum* [64,91,92] (Figure 1.3).

Lemon basil is a strongly aromatic annual plant with a lemon scent due to the presence of citral in the form of its geometric isomers, neral, and geranial [93]. Due to its lemon scent and flavor, it can be used as a source of citrus flavor and as an alternative to lemon and other lemon-scented botanicals.

Description of *Agastache foeniculum (Lophantus anisatus)* species

The genus Agastache is part of the Lamiaceae family and includes 22 species of perennial ornamental and medicinal plants [107].

Agastache foeniculum has fragrant leaves and purple flowers, being widely used for ornamental purposes due to its appearance and pleasant aromas similar to anise, but also for flavoring sweets and other foods [108]. This plant is used in the form of teas that are especially preferred by Native Americans for therapeutic purposes, such as relief of various symptoms of cold, fever, cough, heart disease, inflammation, and pain [109].

A herbaceous and aromatic perennial plant, it is native to North America and has been referred to in other studies as anise hyssop, *Lophantus anisatus*, giant blue hyssop, flagrant giant hyssop, or giant lavender hyssop [110–113].

The taxonomic classification of the species *Agastache foeniculum* includes the kingdom *Plantae* division *Magnoliophyta*, class *Magnoliopsida* and order *Lamiales* [112].

The phytochemical profile of these plants includes non-volatile metabolites belonging to several classes such as flavones and glycosylated flavones (a rare dimeric malonylflavone - agastachin, agastachoside, acacetin, apigenin, tilianin, myricetin, luteolin) [108,122], phenolic compounds (rosmarinic acid, caffeic acid) [123], lignans (agastenol, agastinol) [124], terpenoids including triterpenoids (betulin, betulinic acid, maslinic acid, oleanolic acid, β-amyrin, ursolic acid, corosolic acid, α-amyrin), diterpenes (agastaquinone, agastol) and sterols [108,111,125]. Volatile metabolites have been reported in its chemical composition, such as estragole, pulegone, eugenol, methyleugenol, menthone, isomenthone, spathulenol [111–113,126,127]. According to literature data, the yield of essential oil obtained from *Agastache foeniculum* (dried plant) varies from 0.02 to 3% (mL/g) [94,113,117,118,128–130].

Apiaceae* family *(Umbelifere)

The species *Pimpinella anisum* and *Carum carvi* are part of this family of Apiaceae and whose chemical compositions, natural compounds from these species as well as some of their biological properties have been studied in this thesis.

a. Description of *Pimpinella anisum* (anise) species

Pimpinella anisum is an annual plant known by the popular name of anise, has a height of about 30-60 cm, with small white inflorescences, and small, oval seeds, characterized by a sweet aromatic smell and aroma (Figure 1.6) [134].

Taxonomic classification of the species *Pimpinella anisum*: kingdom: Plantae; subkingdom: *Tracheobionta*; superdivision: *Spermatophyta*; division: *Magnoliophyta*; class: *Magnoliopsida*; subclass: *Rosidae*; order: *Apiales*; family: *Apiaceae*; genus: *Pimpinella*; species: *Anisum*; scientific name: *Pimpinella anisum* [135].

The species *Pimpinella anisum* L has been known since ancient times in traditional Asian medicine for its disinfectant, antifungal, antimicrobial, antiviral, antioxidant, anticoagulant, and anti-inflammatory properties [136–140].

b. Description of *Carum carvi* species (caraway)

Caraway (*Carum carvi*) is a biennial plant, and in cultivated forms, it is also an annual plant, native to Europe and western Asia (Figure 1.8).

Taxonomic classification of *Carum carvi* species: domain: Eukaryotae; kingdom: Plantae; subkingdom: Tracheobionta; superdivision: Spermatophyta; division: Magnoliophyta; class: Magnoliopsida; subclass: Rosidae; order: Apiales; family: Apiaceae; genus: Carum; species: Carvi L. [145].

The plant belongs to the Apiaceae family and originates from the temperate areas of Western Asia and is currently cultivated mainly in Poland, Hungary, and Morocco being a medicinal and spice plant known since ancient times [148].

***Schisandraceae* family (*Illiciaceae*)**

Description of *Illicium verum* species (star anise)

Illicium verum is also known as star anise or Chinese star anise and is a member of the Schisandraceae family. The taxonomic classification of the *Illicium verum* species is kingdom: Plantae; division: Magnoliophyta; class: Magnoliopsida; order: Austrobaileyales; family: Schisandraceae (Illiciaceae); genus: Illicium; species: Verum [135].

Illicium verum is an evergreen, well-branched tree or shrub that is around 8–15 m tall [180]. It was included in the family Illiciaceae (Badianaceae) in the older systematic classification. In the APG IV system (2016), *Illicium verum* is classified in the genus Illicium belonging to the Schisandraceae family [181,182]. The bark of *I. verum* plants is white to light gray. The leaves are light green, lanceolate, and alternate, measure 6 to 12 cm long, and are located at the ends of the branches [135,183,184]. The flowers are solitary, bisexual, yellowish-white or greenish, and 1–7 cm in diameter. They grow either alone or arranged in clusters [183]. The fruit is star-shaped and has 6-10 capsule-like follicles with a small brown seed inside each. The seeds are ovoid with a glossy and smooth surface. Each part of the fruit carries an aromatic odor [183,184].

1.2.2. The therapeutic importance of the studied species

Pharmacological properties

The Lamiaceae family comprises important species containing active phytochemical constituents and exhibiting a wide range of pharmacological and biological activities. The plants from this family studied are rich sources of compounds with antioxidant properties, also recognized for their antibacterial, antitumor, anthelmintic, hypolipidemic, antihepatotoxic, and antidiabetic properties [70,254,255].

The pharmacological properties of the studied species are due to some chemical compounds that produce an important physiological action on the human body. *In vivo* research on these studied species has demonstrated numerous biological properties, such as cardioprotective, hepatoprotective, antidiabetic, antioxidant, anti-inflammation, and anticancer [255].

The flavonoids present in basil have attracted interest due to the anti-inflammatory, analgesic, antitumor, antimicrobial, antioxidant, and immunostimulating activities of extracts from this plant [23].

Part II PERSONAL CONTRIBUTIONS

Chapter 2. Preparation of plant material and separation of biologically active compounds

2.3. Sampling and characterization of plant material

The basil varieties analyzed were grown and harvested at the Vegetable Research - Development Station (SCDL) Buzău, Romania. The two new varieties of basil studied in this thesis were registered as Romanian varieties by SCDL Buzău and named "Aromat de Buzău" (*Ocimum basilicum*) and "Macedon" (*Ocimum citriodorum*). For the analysis of the plant material, the plants were cultivated by sowing in March 2019, planted in the open field in April 2019, and the plants with inflorescence were harvested at maturity at the beginning of August 2019 (Figure 2.1).

a)

b)

Figure 2.1. Cultures of *O. basilicum* „Aromat de Buzau" (a) and *O. citriodorum* „Macedon" (b)

A new species of Lophantus, *Agastache foeniculum* variety "Aromat de Buzău", was adapted to the climatic conditions in Romania and also cultivated at SCDL Buzău. For the analysis of the plant material, the plants were grown by sowing in March 2020 and the mature flowering plants were harvested at the beginning of August 2020 (Figure 2.2).

Figure 2.2. Cultures of *Lophantus* (*Agastache foeniculum* „Aromat de Buzău”)

Whole aerial parts of Lamiaceae plants (stems, leaves, flowers, seeds) were dried at room temperature, ground using an electric grinder (Heinner grinder, Grinder Optim150, 150W, 50/60 Hz, China), and subjected to assisted extractions by ultrasound and by hydrodistillation.

Two plant species from the Apiaceae family: aniseed (*Pimpinella anisum*) and caraway (*Carum carvi*) were analyzed to determine whether they potentiate the biologically active effect of new plant varieties from the Lamiaceae family. The seeds were obtained in August 2019 from SCDL Buzău (Figure 2.3).

(a) (b) (c)

Figure 2.3. Seeds of (a) anise (*Pimpinella anisum*), (b) caraway (*Carum carvi*), and (c) star anise (*Illicium verum*)

2.4. Extraction and separation of biologically active compounds from the studied species

The extraction of the biologically active compounds from the plant materials in this study was carried out by the following extraction methods:

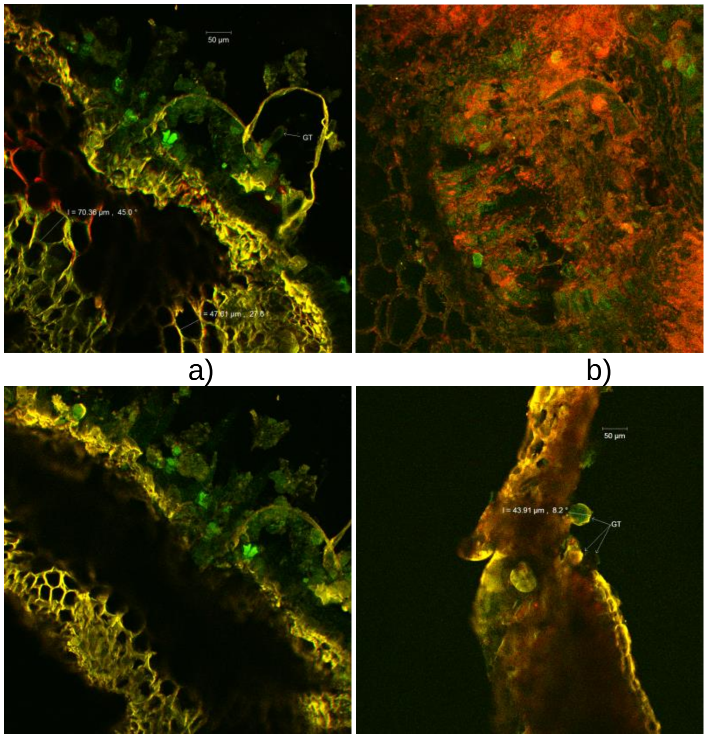
a) *simple ultrasound-assisted solvent extraction*

b) *sequential extraction with solvents assisted by ultrasound* (ESUS)

c) *extraction by hydrodistillation (HD)*

2.5. Results and Discussion

The two varieties of basil selected for this study, *Ocimum basilicum* L. "Aromat de Buzău" and *Ocimum citriodorum* "Macedon", stand out for the specific aromas of all plant organs, mentholated for the first variety and lemony for the second.

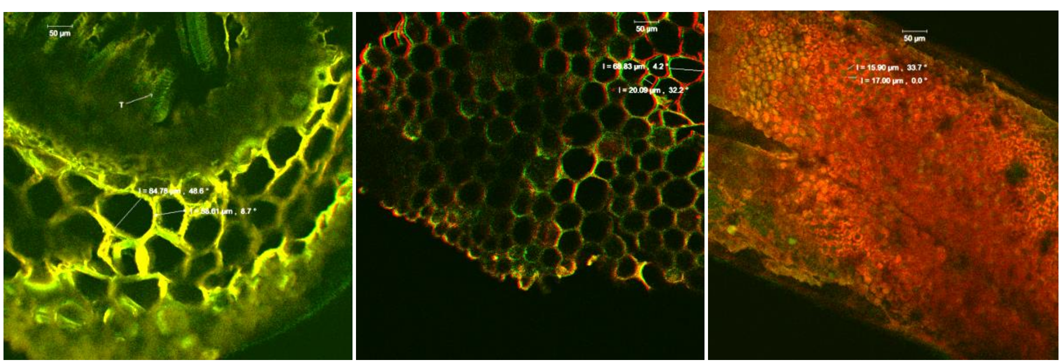


a) b)

c) d)

Figure 2.7. Cross-section image through (a) stem of *O. basilicum* and (b) *O. citriodorum,* respectively through leaves (c) of *O. basilicum and* (d) *O. citriodorum* observed using CLSM

Sections through stems and leaves of the mature plant of the new variety *A. foeniculum* AdB indicated plant cells with normal, healthy morphology and structures as observed by confocal laser scanning microscopy (Figure 2.8).



a) b) c)

Figure 2.8. Images of (a, b) stem and (c) leaf cross-sections of *A. foeniculum* observed using CLSM

2.6. Partial conclusions

- The samples analyzed from the aromatic plants *Ocimum basilicum*, *Ocimum citriodorum,* and *Agastache foeniculum*, family Lamiaceae were cultivated and harvested at the Vegetable Research - Development Station (SCDL) Buzău, Romania.

- The samples analyzed from the fruits of *Pimpinella anisum* and *Carum carvi*, Apiaceae family, were also purchased from the Vegetable Research - Development Station (SCDL) Buzău, Romania.
- For the extraction with methanol and ethanol organic solvents of biologically active natural organic compounds from the studied aromatic plants, such as *Ocimum basilicum*, *Ocimum citriodorum,* and *Agastache foeniculum*, the ultrasound extraction method was used.
- To obtain the essential oils from all six species of aromatic plants, hydro-distillation with a Clevenger-type apparatus was used as an extraction method.
- Yields of 1.04 ± 0.76% of volatile oils obtained by hydrodistillation, from the variety *O. basilicum* "Aromat de Buzău" and 0.92 ± 0.48% of volatile oil from the variety *O. citriodorum* "Macedon" were obtained ".
- By simple ultrasound-assisted solvent extraction (EUS) from *Ocimum basilicum* with methanol, 3.49 ± 0.64%, respectively 4.95 ± 0.47% of *Ocimum citriodorum*, total extractable components and with ethanol were obtained 4.08 ± 0.82% of *Ocimum citriodorum*, respectively 3.11 ± 0.51% of *Ocimum basilicum*.
- Simple solvent extraction assisted by ultrasound with methanol yielded 11.06 ± 0.95% total extractable components from *A. foeniculum* respectively 7.21 ± 0.69% with ethanol.
- A yield of 1.86 ± 0.64% volatile oil of *A. foeniculum* was obtained by hydrodistillation.
- Through hydrodistillation, a yield of 1.85 ± 0.73% volatile oil was obtained for *Pimpinella anisum*, 3.04 ± 0.86% for *Illicium verum,* and 2.6 ± 0.68% for *Carum carvi* L.

Chapter 3. Chromatographic and spectral analysis of natural organic compounds separated from the studied aromatic plants

3.2. Analysis of natural organic compounds by the HPTLC chromatographic technique

3.2.3. Results and Discussion

In this study, TLC was used as a separation and identification method for various compounds in alcoholic extracts and volatile oils. Mobile phases were selected after multiple optimization attempts for good, clear migration and easy identification of organic compounds. Based on the values of the retention factors (R_f) of the pure standard compounds, obtained under the same conditions as the Rf values for the analyzed samples, rutin was identified (R_f=0.15) in the methanolic extracts from both analyzed basil species but also in the ethanolic extract of *O. citriodorum* (Figure 3.1). Rutin is part of the class of flavonoids, a class of molecules important due to their diverse therapeutic properties. Rutin, also known as vitamin P or rutoside, chemically is a glycoside containing the flavonol aglycone quercetin together with the disaccharide rutinose. In numerous studies, rutin has demonstrated several pharmacological activities such as antioxidant, cardioprotective, cytoprotective, vasoprotective, neuroprotective, and anticancer activities [341]. Therefore, it is a therapeutically important component identified in the analyzed alcoholic extracts of basil.

Quercetin (Rf=0.82) could not be identified in the alcoholic extracts of basil by CSS, but daidzein was identified in the four analyzed methanolic and ethanolic extracts of *O. basilicum* and *O. citriodorum*.

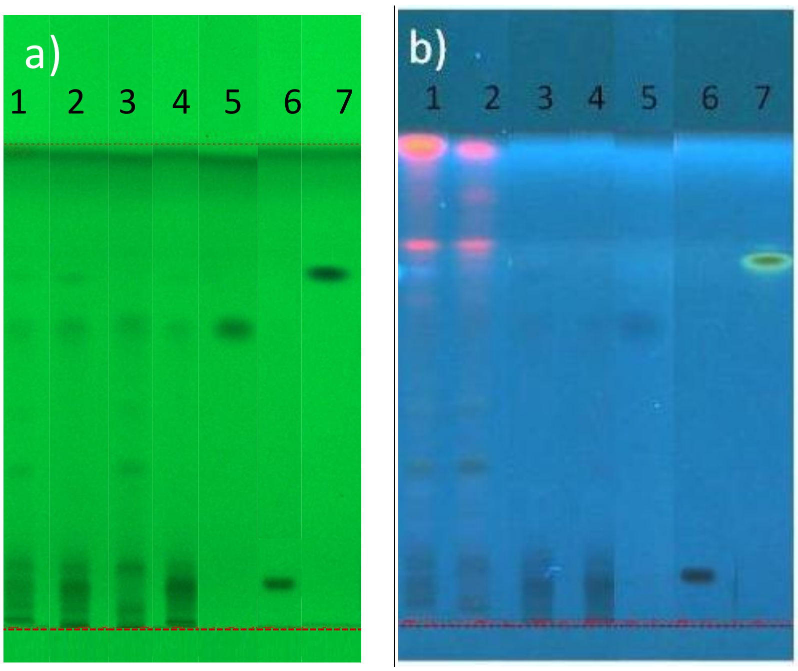


Figure 3.1. HPTLC chromatogram of (1) methanolic and (2) ethanolic extracts of *O. basilicum*, (3) methanolic and (4) ethanolic extracts of *O. citriodorum*, (5) daidzein, (6) rutin, and (7) quercetin, at (a) 254 nm and (b) 366 nm

In the analysis of the alcoholic extracts of A. foeniculum, caffeic acid (Rf=0.80), rutin (Rf=0.15), and quercetin (Rf=0.82) could be identified (Figure 3.2).

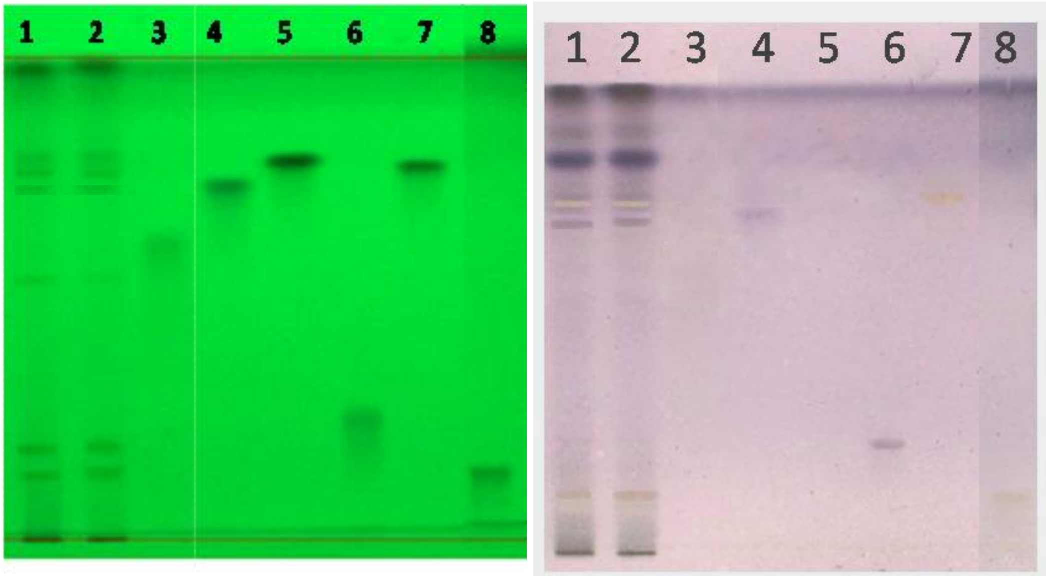


Figure 3.2. HPTLC chromatogram of (1) methanolic and (2) ethanolic extracts of A.foeniculum, and standards (3) gallic acid, (4) caffeic acid, (5) vanillic acid, (6) chlorogenic acid, (7) quercetin, (8) routine, before development at λ=254 nm and after chemical development with anisaldehyde-sulfuric acid under white light

Through TLC analysis of volatile oils, anethole was identified in *I. verum* and *P. anisum* oils, estragole in *A. foeniculum* and *O. basilicum* oils, linalool in *O. basilicum* and *O. citriodorum* basil oils, eugenol in *O. basilicum* oil and carvone in *C. carvi* oil (Figure 3.3).

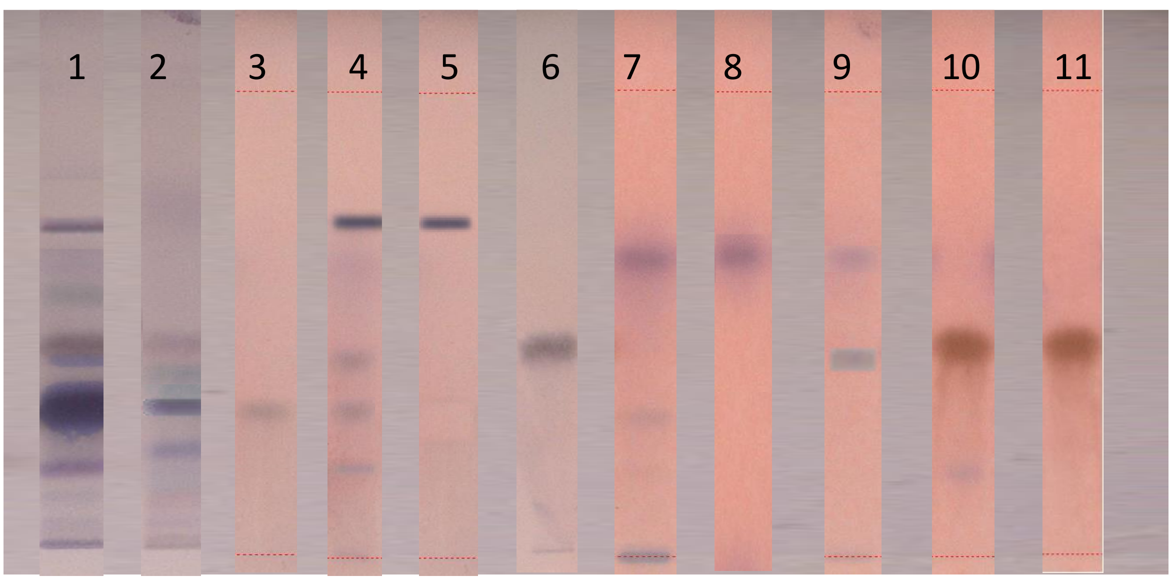


Figure 3.3. HPTLC chromatogram of analyzed oils and standard compounds: (1) O.basilicum essential oil, (2) *O. citriodorum* essential oil, (3) linalool, (4) *A. foeniculum* essential oil, (5) estragole, (6) eugenol, (7) *I. verum* essential oil, (8) trans-anethole, (9) *P. anisum* essential oil, (10) *C. carvi* essential oil, and (11) carvone, after chemical revelation with anisaldehyde-sulphuric acid under white light

3.2.4. Partial conclusions

- The TLC profile of extracts and volatile oils showed that they are mixtures of compounds with different Rf values. In the aromatic plants studied, the identified compounds were varied and secreted in different concentrations depending on the species, the plant parts used for extractions, and the extraction methods.
- By analyzing the alcoholic extracts of *A. foeniculum*, the polyphenols caffeic acid, rutin, and quercetin were identified.
- Through the CSS analysis of volatile oils, anethole was identified in *I. verum* and *P. anisum* oils, estragole in *A. foeniculum* and *O. basilicum* oils, linalool in *O. basilicum* and *O. citriodorum* oils, eugenol in *O. basilicum* oil and carvone in *C. carvi* oil.
- Therefore, the presence of important compounds with beneficial properties for human health was confirmed in the analyzed samples. Some compounds were further identified and quantified by high-performance liquid chromatography and gas chromatography.

3.3. Analysis of natural organic compounds by the GS-MS technique

3.3.3. Results and Discussion

The analyzed essential oils were complex mixtures of natural organic compounds, composed predominantly of terpene hydrocarbons (limonene, pinene, myrcene, p-cymene, α-phellandrene) and terpenoids such as monoterpene alcohols (borneol), monocyclic ketones (carvone) and aromatic phenols (carvacrol, thymol, eugenol). The diverse nature of these compounds, coupled with interspecies and intraspecies variation, results in a wide range of possible applications of extracted compounds from aromatic plants.

The essential oils in this study were extracted by hydrodistillation and the separation and identification of the organic compounds was carried out using a gas chromatograph coupled with a mass spectrometer. The chromatograms recorded by GC-MS for the essential oil of *Ocimum basilicum* "Aromat de Buzău" presented 16 compounds whose structure was identified (Figure 3.18). The essential oil of *Ocimum citriodorum* "Macedon lemon" presented 15 compounds whose structure was identified (Figure 3.19). The essential oil of *A. foeniculum* "Aromat de Buzău" presented 7 compounds whose structure was also identified (Figure 3.20). In the essential oil of caraway (*Carum carvi*) 2 compounds were identified (Figure 3.21), in the essential oil of star anise (*Illicium verum*) 16 compounds were highlighted (Figure 3.22), and 5 compounds were identified in the essential oil of *Pimpinella anisum* (Figure 3.23).

Estragole (methyl-chavicol/p-allylanisol), a compound widely used in the perfume industry, is the majority compound present in the essential oil of *Agastache foeniculum* (94.89%±1.02%), also identified in the essential oils of *O. basilicum* (12.81%±0.44%), *O. citriodorum* (7.14%±0.08%), *Illicium verum* (0.69%±0.04%) and *P.anisum* (0.33%± 0.02%). Estragole has the molecular formula $C_{10}H_{12}O$, having the base peak with mass m/z=148. The mass spectrum of estragole (Figure 3.6) shows a high abundance of the molecular ion peak, which could be explained by the chemical stability of this compound. The fragmentation of the molecular ion of estragole can follow three fragmentation paths proposed based on the fragments resulting from the mass spectrum and the fragmentation rules from the specialized literature [358,362]: a) the formation of ionic fragments with mass m/z=133 which is due to the formation of the ion $[M-CH_3]^{+\bullet}$ by the loss of the methyl radical from the molecular ion, b) the formation of ionic fragments with mass m/z=121, $[M-C_2H_4]^{+\bullet}$, due to the loss of the C_2H_4 radical from the molecular ion and c) the formation of ionic fragments with mass m/z=117, $[M-OCH_3]^{+\bullet}$, which is due to the loss of the methoxy group from the molecular ion (Figure 3.7). Next, the fragment with mass m/z=117 can lose the $C_2H_4^{\bullet}$ radical with the formation of the fragment with mass m/z=105, $[M-OCH_3-C_2H_4]^{+}$, which can also be obtained from the ionic fragment $[M-C_2H_4]^{+\bullet}$ by the loss of a methyl radical. The fragment with mass m/z=117 can lose the radical $CH_2=CH^{\bullet}$ to form the ion with mass m/z=91 [M-

OCH_3-CH_2=$CH^{\bullet}]^+$ which by tropyl fragmentation and loss of $CH_2^{\bullet}$ leads to the ion with mass m/z=77.

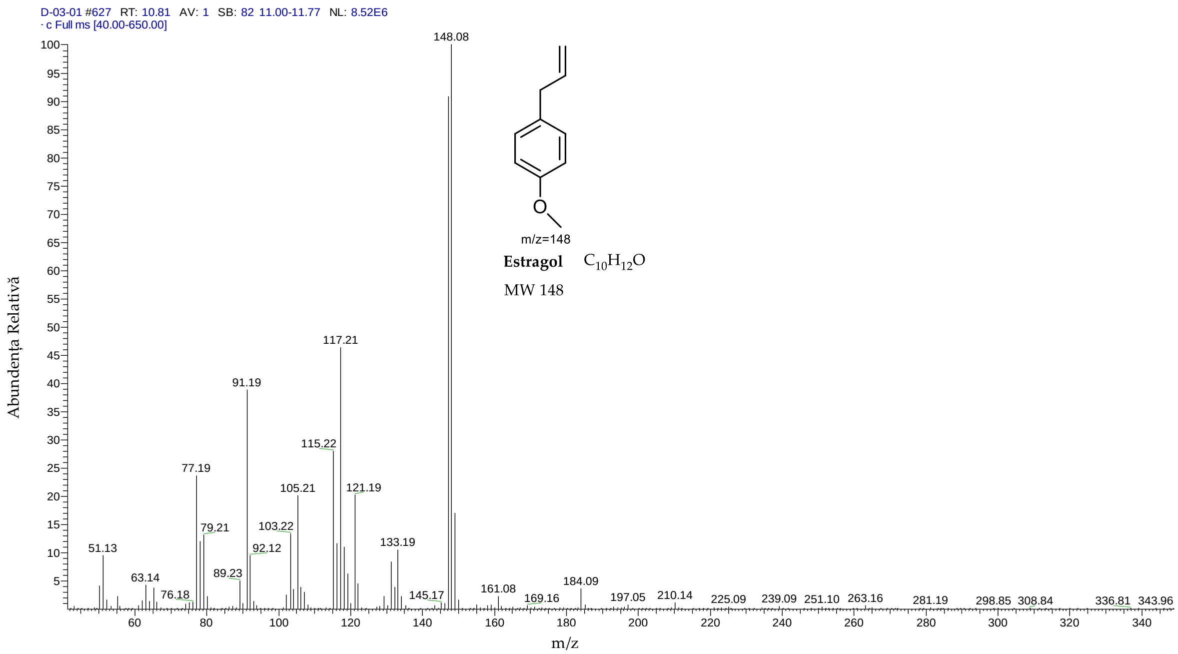


Figure 3.6. MS spectrum and fragmentation of methyl-chavicol (estragole)

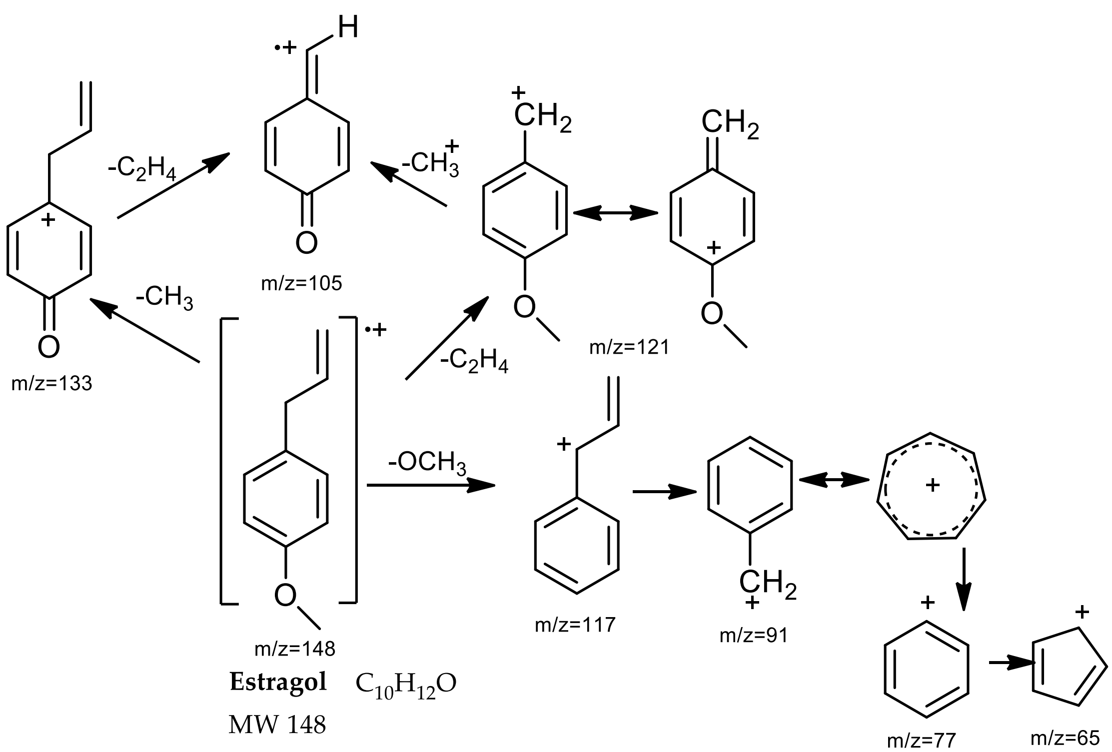


Figure 3.7. Proposed fragmentation mechanism for methyl-chavicol (estragole)

The main component detected by GC-MS analysis in the essential oil of *Agastache foeniculum* also called " Aromat de Buzău " was estragole (1-allyl-4-methoxybenzene), an allylbenzene analog, colorless liquid, with an aromatic odor of anise and sweet taste.

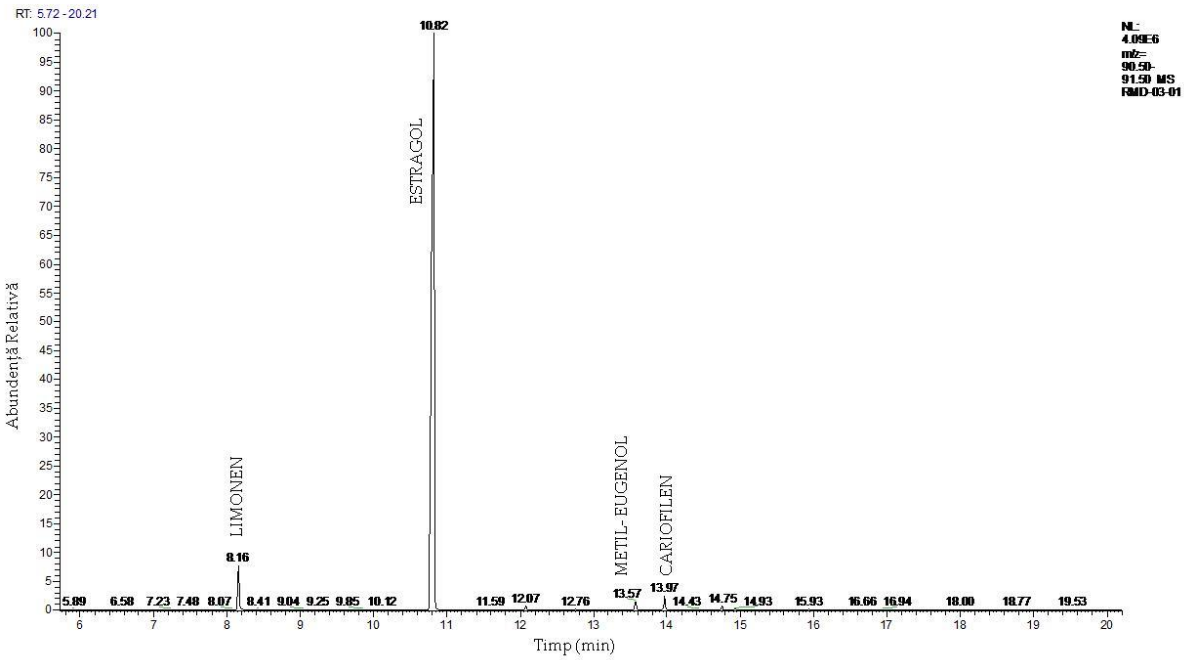


Figure 3.20. GC–MS chromatogram of the essential oil of *A. foeniculum* „Aromat de Buzău"

3.3.4. Partial conclusions

- The chemical composition of the extracts obtained by hydrodistillation from species of aromatic plants from the Lamiaceae family: *Ocimum basilicum*, *Ocimum citriodorum,* and *Agastache foeniculum*, cultivated in Romania, was analyzed for the first time.
- This experimental study confirms the presence of the major compounds linalool, methyl chavicol (estragole), eugenol, methyl-eugenol, d-germacrene, 1,8-cineole, β-caryophyllene in accordance with the data from the specialized literature, at this stage of the research analyzing -at the same time, the chemical composition of two species of plants from the Apiaceae family, *Pimpinella anisum* and *Carum carvi*, but also of a species from the Schisandraceae family, namely *Illicium verum*, in which the presence of the majority compounds trans-anethole, ɣ-himachalene, carvone, limonene, and estragole
- The main component detected by GC-MS analysis in the essential oil of *Ocimum basilicum* "Aromat de Buzau" (34.87 ± 1.05%) was the acyclic terpenoid linalool (3,7-dimethylocta-1,6-dien-3- ol).
- The main component detected by GC-MS analysis in the essential oil of *Agastache foeniculum* also called "Buzău Aromat" was estragole (1-allyl-4-methoxybenzene), an allylbenzene analog, colorless liquid, with an aromatic smell of anise and sweet taste.
- The essential oil of caraway (*Carum carvi*) analyzed was rich in carvone (68.49 ± 1.41%), a monocyclic ketone responsible for the aroma of caraway, and limonene (29.01 ± 1.61%), a cyclic monoterpene.
- The main component identified in the analyzed essential oils of star anise (*Illicium verum*) and anise (*Pimpinella anisum*) by GC-MS analysis was trans-anethole.

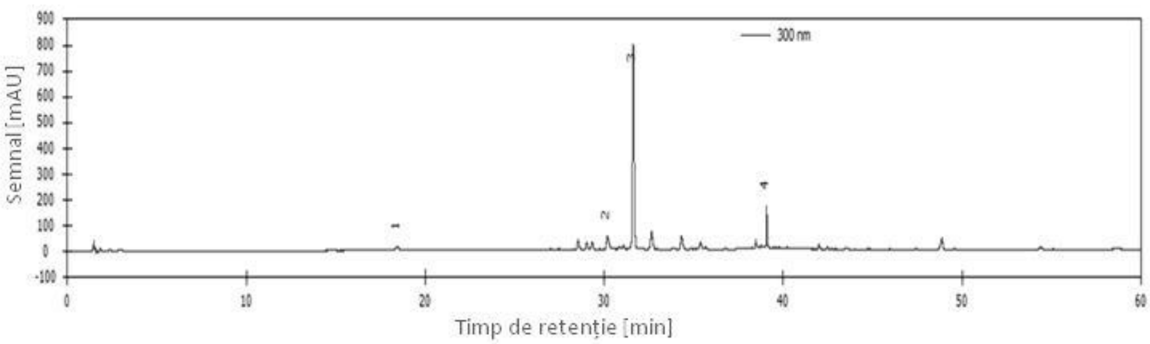
3.4. Analysis of Natural Organic Compounds by High-Performance Liquid Chromatography (HPLC-DAD)

3.4.1. Main objectives

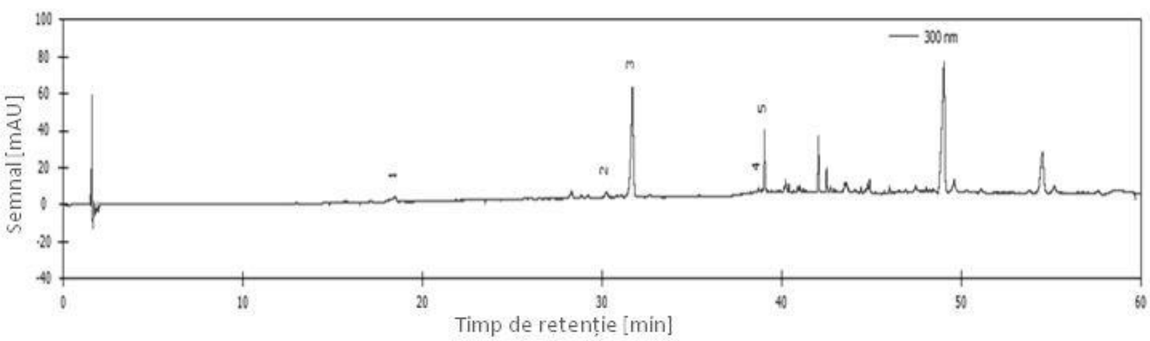
One of the main objectives of the thesis consisted in the use of high-performance liquid chromatography (HPLC), using the HPLC-DAD technique, in the identification, separation, and quantification of some compounds from the class of polyphenols present in the alcoholic extracts obtained from the species of *Ocimum basilicum*, *Ocimum citriodorum,* and *Agastache foeniculum*. Extracts subjected to separation, identification, and quantification of biologically active compounds were obtained using various solvents and different extraction methods.

3.4.3. Results and Discussion

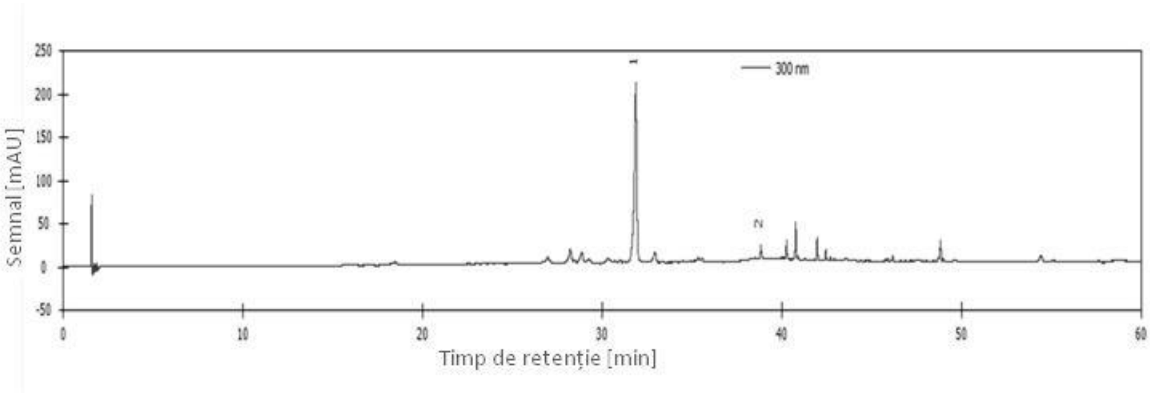
Chromatographic separation revealed the presence in the extracts from the analyzed Romanian basil varieties of six main constituents, using standard reference compounds for their identification and quantification, namely catechin, rutin, hyperoside, naringin, naringenin, and genistein (Figure 3.24).



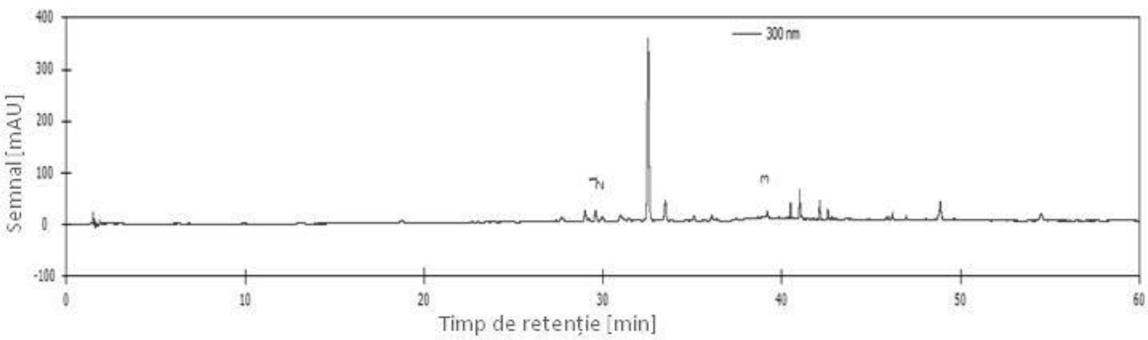
(a)



(b)



(c)



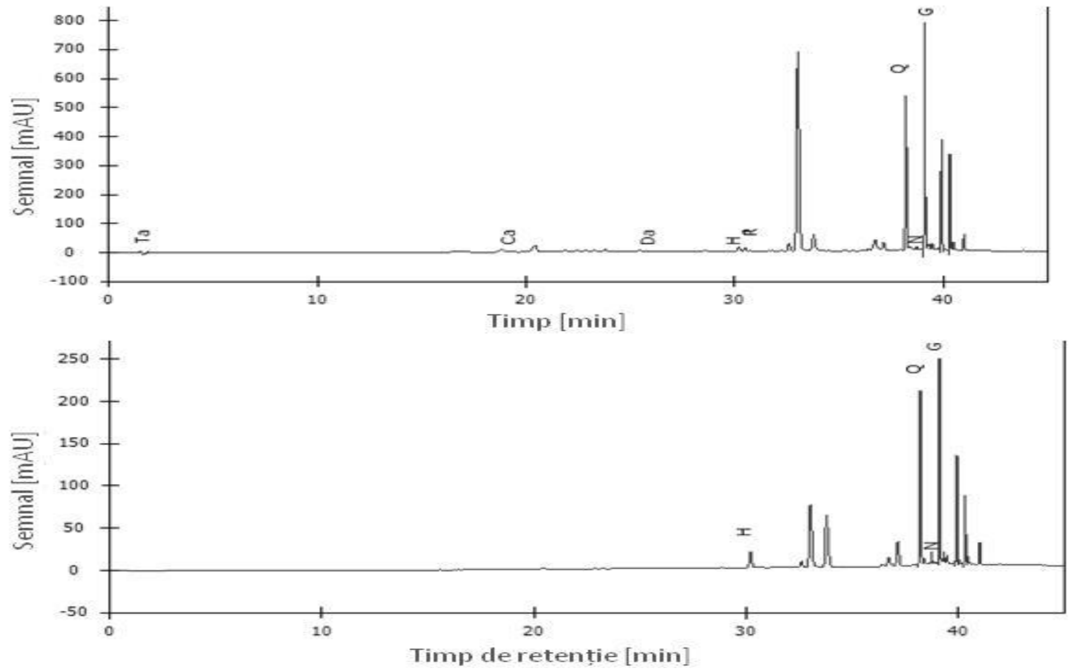
(d)

Figure 3.24. HPLC - DAD Chromatograms of the analyzed samples: (a) methanolic extract of „Aromat de Buzău" (1 – catechins, 2 – rutin, 3 – naringin, 4 – genistein), (b) ethanolic extract of „Aromat de Buzău" (1 – catechin, 2 – rutin, 3 – naringin, 4 – naringenin, 5 – genistein), (c) methanolic extract of „Macedon" (1 – naringin, 2 – naringenin) and (d) ethanolic extract of „Macedon" (1 – rutin, 2 – hyperoside, 3 – genistein) with detection at λ = 300 nm.

The quantification of the chemical compounds identified from the four methanolic and ethanolic basil extracts of the two Romanian varieties of *Ocimum* is summarized in Table 3.2. The results showed that naringin is the main compound of basil extracts, its concentration varying between 930.10 ± 0.15 µg/g and 2618.11 ± 0.23 µg/g. The highest concentrations of naringin were recorded for the methanolic extracts for both varieties of basil, the concentration being three times higher in the variety *O. basilicum* "Aromat de Buzău".

Genistein and quercetin were found in high concentrations in both ultrasound-assisted alcoholic extracts of A. foeniculum, while tannic acid was only quantifiable in the methanolic extract (Table 3.3 and Figure 3.25).

(a)



(b)

Figure 3.25. HPLC-DAD chromatographic separation of compounds from (a) *A. foeniculum* - methanolic extract (Ta-tannic acid, Ca-caffeic acid, Da-daidzein, H - hyperoside, R-rutin, Q-quercetin, N-naringenin, G - genistein); (b) A. foeniculum-ethanolic extract (Ta-tannic acid, H - hyperoside, Q-quercetin, N-naringenin, G-genistein).

3.4.4. Partial conclusions

- Alcoholic, ethanolic, and methanolic extracts of *Ocimum basilicum*, *Ocimum citriodorum,* and *Agastache foeniculum* were analyzed in this study.
- The main compounds with biologically active properties identified in the two Romanian varieties of *Ocimum* were different depending on the species, the method of extraction, and the solvent used.
- In the alcoholic extracts from the analyzed Romanian basil varieties, six main constituents were highlighted, namely catechin, rutin, hyperoside, naringin, naringenin, and genistein.
- In the methanolic extracts from the species *Ocimum basilicum* (AB), compounds such as catechin, rutin, naringin, and genistein were identified and in the ethanolic extracts of the same species, the compounds catechin, rutin, naringin, naringenin, and genistein were identified.
- In the methanolic extracts obtained from the species *Ocimum citriodorum* (MB) the compounds naringin and naringenin were identified, while in the ethanolic ones rutin, hyperoside, and genistein.
- In the *Agastache foeniculum* species, the phenolic p-coumaric and caffeic acids, the flavonols rutin, quercetin, and hyperoside, the flavanone naringenin and the isoflavone genistein were identified in both the methanolic and ethanolic extracts. Tannic acid was identified only in the methanolic extracts of *Agastache foeniculum*.
- Genistein and quercetin were found in high concentrations in both alcoholic extracts of A. foeniculum, with quercetin content ranging from 704.148 - 1073.637 µg/g s.u. and that of genistein between 2229.999 - 3171.823 µg/g s.u. Tannic acid was quantifiable only in the methanolic extract. Caffeic acid, p-coumaric acid, quercetin, rutin, and hyperoside were identified in both *A. foeniculum* extracts.

Chapter 4. Electrochemical analysis of the compounds with antioxidant activity from the studied plants

4.2. Main objectives

The research previously reported in the specialized literature, but also the studies carried out during the doctorate period, indicated the presence of some polyphenolic compounds in the studied aromatic plants.

- The main objective of this experimental study was to evaluate, through in vitro analyses, the antioxidant potential of alcoholic extracts and essential oils, obtained from new varieties of basil, *Ocimum basilicum*, *Ocimum citriodorum*, *Agastache foeniculum*, but also of commercial basil oil, by electrochemical methods (cyclic voltammetry). Different classes of compounds present in the studied samples indicate potential antioxidant properties, which induce different mechanisms of action.
- Electrochemical evaluation of the compounds present in the analyzed species such as eugenol, estragole, and linalool.
- Studying possible synergistic interactions between antioxidant compounds in plant material, considering the complex composition of medicinal plants.
- Characterization of the general antioxidant capacity (ox/red) of alcoholic extracts or essential oils obtained from them by using electrochemical methods.

4.4. Results and Discussion

4.4.2. Electrochemical evaluation of the antioxidant activity of chemical compounds present in essential oil samples from *Agastache foeniculum* (AdB)

Samples of 5 µL of essential oil from *Agastache foeniculum* solubilized in 20 mL of methanol were analyzed by OCP (open circuit potential), for freshly prepared solutions, solutions analyzed after 24 hours and after 5 days, being stored in a refrigerator at 4° C. The experimental conditions are similar to those used in the analysis of AB basil oil (4.4.1).

From Figure 4.29 (a) you can see the evolution of the potential (E) of the analyzed samples up to 10 min. For the sample analyzed after 24 hours, there is a difference of approximately +30 mV in the positive area compared to the fresh sample, a dissociation and structural arrangement between the biologically active compounds in the sample.

In the next 30 minutes, no visible differences are observed between the analyzed samples, at different time intervals from the time of preparation, but a shift towards positive values of the potential is recorded. The results indicate the availability of electronic exchanges between the chemical components of the *Agastache foeniculum* essential oil sample, being systems with dynamic evolution that register an increase in potential values up to 100 mV, and subsequent stability.

Cyclic voltammograms recorded when applying a potential of E = ±2 V/Ag/AgClsat, at a scanning speed of 100 $mV\cdot s^{-1}$, for the essential oil samples of *Agastache foeniculum*, evaluated at different time intervals are shown in Figure 4.30 (b). As the voltammograms are presented, the significant anodic peak with high intensity at a potential higher than 1.3 V can be observed, which has a slightly increased value in the samples, over time.

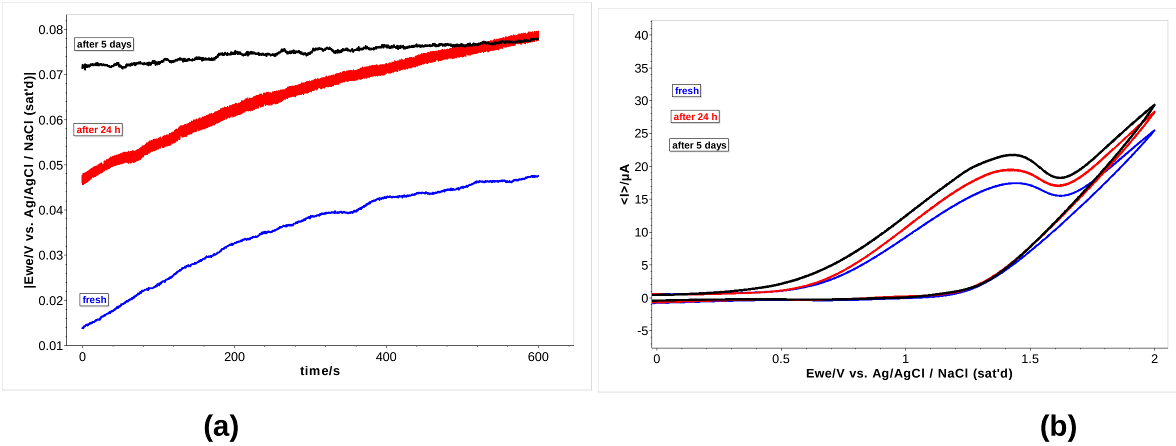


Figure 4.29. Essential oil samples of *Agastache foeniculum* („Aromat de Buzău"): **(a)** OCP values recorded, **(b)** cyclic voltammograms recorded, E = ±2 $V/_{Ag/Ag\ Clsat}$, at the scanning speed of 100 $mV{\cdot}s^{-1}$

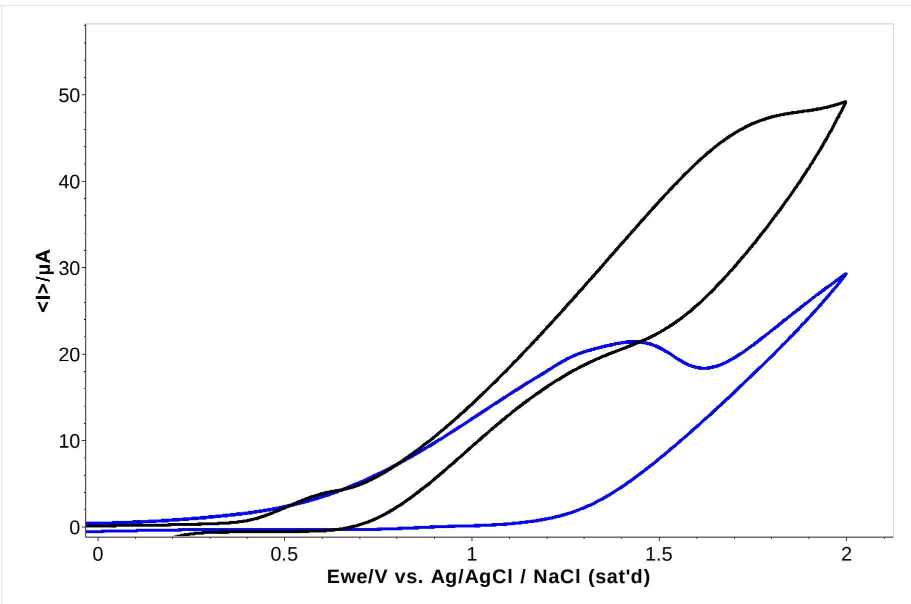


Figure 4.30. Cyclic voltammograms were recorded for essential oil samples of *Agastache foeniculum* („Aromat de Buzău") (blue) and the standard compound pure eugenol (black), E = ±2 $V/_{Ag/Ag\ Clsat}$, at the scanning speed of 100 $mV{\cdot}s^{-1}$

In Figure 4.31, the cyclic voltammograms of *Agastache foeniculum* oil samples are represented compared to the pure compound, eugenol, and it is noted that the profile of the voltammograms is similar. Eugenol was confirmed by chromatographic methods to be a phytoconstituent of the essential oil of *Agastache foeniculum* (Chapter 3). In both analyzed samples, from 0.5 V, some biologically active compounds started to show the intensity of active antioxidant potential. In the analyzed sample of *Agastache foeniculum* essential oil, a maximum of the anodic current of 20-22 µA was recorded at the potential between 1.32 and 1.42 V, a value comparable to the half-wave potential ($E_{½}$) of eugenol recorded at approx 1.35 $V_{/Ag/AgClsat}$.

4.4.3. Electrochemical evaluation of the antioxidant activity of alcoholic extracts obtained from hybrid species of *O. basilicum*

An electrochemical study was carried out for the methanolic and ethanolic extracts of the two hybrid basil species, AB ("Aromat de Buzău") and MB ("Macedon lămâios") [440].

The electrochemical evaluation included the OCP and CV method, sample preparation, and the experimental conditions are similar to those presented for AB basil oil samples (4.4).

The results obtained for OCP (open circuit potential) for hybrid basil species, methanolic and ethanolic extracts are shown in Figure 4.32.

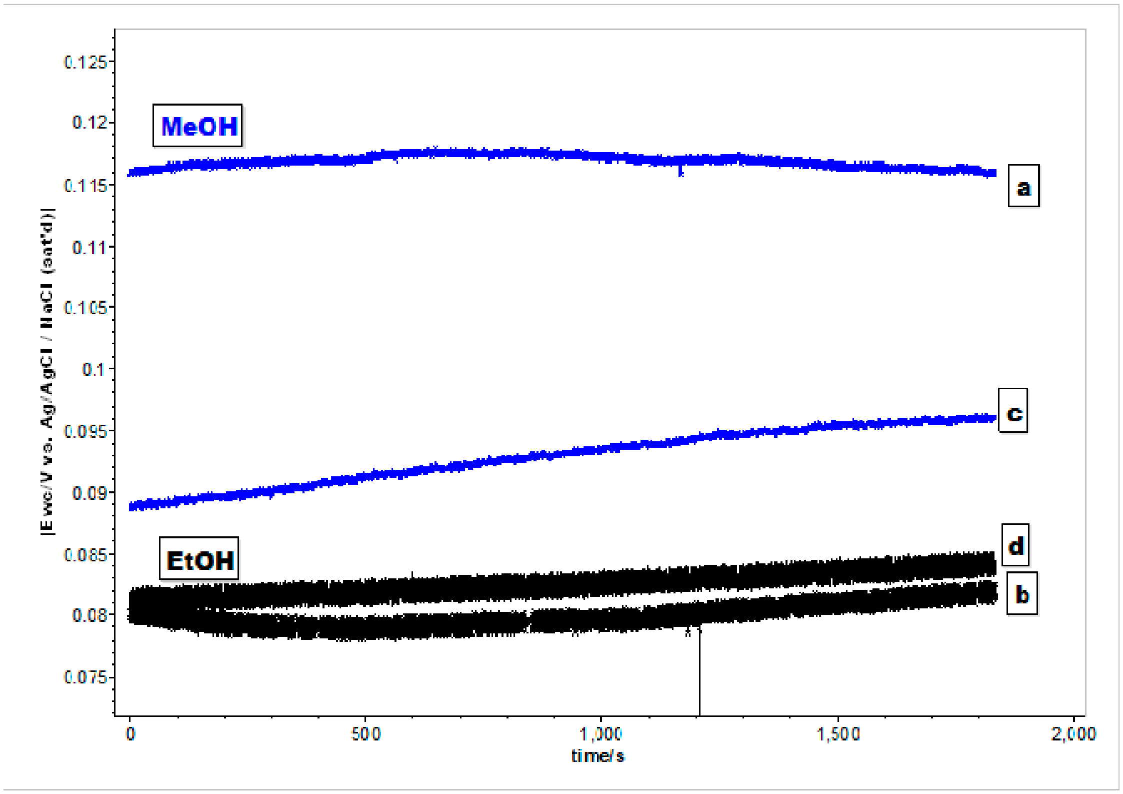


Figure 4.31. OCP values recorded for methanolic extracts of basil from AB (a) and MB (c) and, respectively, for ethanolic extracts from AB (b) and MB (d)

The OCP values recorded in both samples, hybrid species of basil, indicate a stable potential after 30 minutes with a small difference in the potential values depending on the solvents used. For the AB methanolic extract ("Buzău Aromat"), a potential with more positive values is observed, (E = 0,115 $V_{/Ag/AgClsat}$) compared to MB ("Macedon") methanolic extract, a potential (E= 0,095 $V/_{Ag/AgClsat}$). In the ethanolic extracts, there are no significant differences between the two hybrid species of basil, registering a potential of 0,080 $V_{/Ag/AgClsat}$.

Figure 4.32. and Figure 4.33. shows the cyclic voltammograms recorded for hybrid samples of basil, alcoholic extracts, and a different profile of the voltammograms is observed.

In the sample of *O. basilicum* AB ("Aromat de Buzău") the methanolic extract, at a potential of 0,5 V/Ag/$AgCl_{sat}$ but compounds with potentially intense activity, registering an anodic current I_a = 12 μA, about 50% more active than in the methanolic extract of *O. citriodorum* MB („Macedon”) (Figure 4.33).

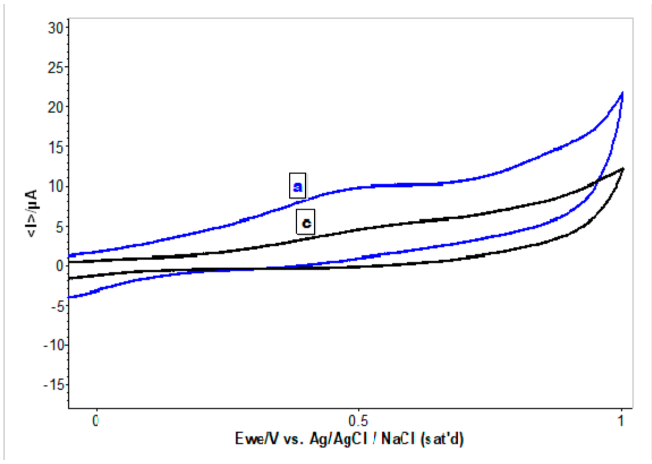


Figure 4.32. Cyclic voltammograms recorded for methanolic extracts of basil AB (a-blue) and MB (c-black), E = ±1 $V/_{Ag/Ag\ Clsat}$, la 100 mV·s^{-1}.

In ethanolic extracts, the potency activity is lower than in the methanolic extract, in the hybrid species AB ("Buzău Aromat") compared to the extract from the hybrid species MB ("Macedon"), a species that indicates a wider availability for the initiation of electronic changes, from a potential of 0,25 $V/_{Ag/AgClsat}$ to 0,80 $V/_{Ag/AgClsat}$ (Figure 4.36).

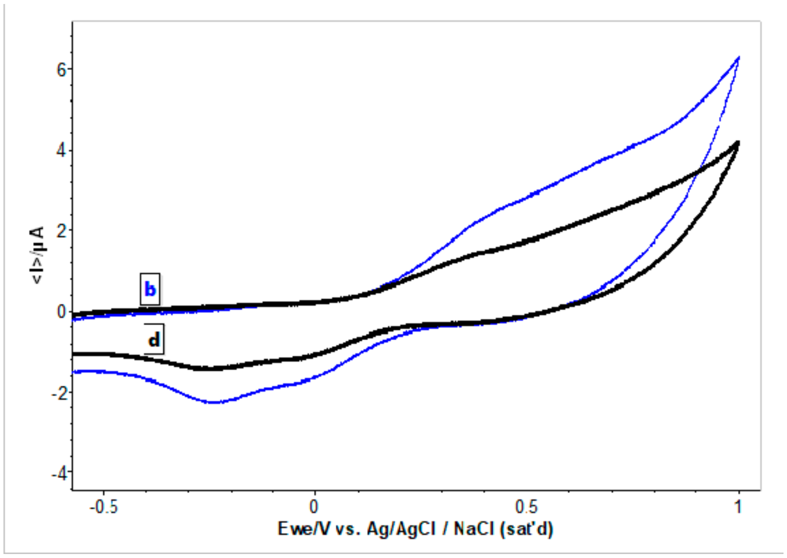


Figure 4.33. Cyclic voltammograms recorded for ethanolic extracts of basil AB (b-blue) and MB (d-black), E = ±1$V/_{Ag/AgClsat}$, the scan rate of 100 mV·s^{-1}.

4.5. Partial conclusions

- Cyclic voltammetry (CV) is a versatile technique, intensively used for the general electrochemical characterization of natural compounds, providing data on their oxidation/reduction capacity, in the analyzed samples, and in different solvents, references from the specialized literature confirming that there is a correlation between the oxidation potential of different antioxidants and their antioxidant efficiency.
- Two electrochemical techniques, OCP (open circuit potential) and CV (cyclic voltammetry) were used in the evaluation of the antioxidant potential of basil oil samples, and important electrochemical parameters were evaluated, such as the intensity of the anodic current, the potential of half-wave, the diffusion coefficient.
- By evaluating the OCP of samples with different contents of basil oil from different origins, in methanol and ethanol solvents, a diversity was observed in the recorded potential, but positive values of 0.100 V/Ag/Ag Clsat can be considered as predominant, with good stability over time (samples being analyzed up to 30 minutes).
- Pure chemical compounds found in the analyzed plant oil species, confirmed by other instrumental techniques, such as eugenol, estragole, and linalool, were electrochemically analyzed and the results indicated that the samples could be differentiated, by the anodic current recorded, dependent on the concentration of the compound of interest.
- The evaluation of basil oil samples compared to eugenol and linalool (reference substances) indicated that at a potential of 0.5 V/Ag/AgClsat electronic exchanges begin, an anodic current is recorded, which reflects the antioxidant potential, being influenced by the analyzed species.
- Cyclic voltammograms (CV) recorded for the methanolic extract for samples with *O. basilicum* AB ("Buzău Aromat") oil indicated an anodic current, I_a, with a maximum value of 12 μA, at a potential of 0.5 V/Ag/$AgCl_{sat}$.
- The cyclic voltammograms (CV) recorded indicated the anodic current, Ia, in different solvents, variable values, in the samples of basil oil (AB, MB, CB), hybrid species (AB, MB), *Agastache foeniculum* oil, which they change with the oil concentration, and depend on the applied potential.
- The recorded anodic current, therefore superior antioxidant capacity, is obtained in samples with basil oil, when the applied potential is greater than 1 V/Ag/$AgCl_{sat}$, which confirms the activity of some chemical compounds, which interact effectively in electronic exchanges, such as would be the hydroxyl groups, from polyphenols, with the oxygen input from the samples.
- Through the recorded voltammograms, the samples with different oil content, from different species can be differentiated by the anodic current recorded, so by the active antioxidant potential for the chemical species present.
- The study of electrochemical parameters is useful for the general characterization of the antioxidant potential of the samples and the differentiation between them.
- Electrochemical methods can be widely used in the evaluation of antioxidants in plant oil samples, and extracts from other plant products and foods.
- Our study provides a comprehensive solution for the use of cyclic voltammetry, through electrochemical parameters, in exploring the structure and behavior of basil oil, to advance the research, and to find appropriate utility for this product, with real beneficial antioxidant properties.

The results of these researches, obtained in this chapter, were partially published in impactful scientific articles, as **first author**: Molecules, *Flavonoid profiles of two new approved Romanian Ocimum hybrids*, 2020; IJMS, *Bridging the Chemical Profile and Biological Activities of a New Variety of Agastache foeniculum (Pursh) Kuntze Extracts and Essential Oil*, 2023.

Chapter 5. Quantification of some classes of organic compounds present in the studied species and evaluation of some biologically

active properties

5.2. Main objectives

The main objectives of this chapter were to identify and quantify different classes of compounds from the extracts obtained from selected medicinal plants, cultivated in Romania, and to investigate their biologically active properties.

5.4. Results and Discussion

5.4.1. Total polyphenol content

Among the most important natural antioxidants with important biological activities are polyphenols, compounds that have attracted the interest of researchers for the prevention and treatment of various conditions [553]. The total content of polyphenols based on the Folin–Ciocalteu microspectrophotometric method was determined for the ethanolic and methanolic extracts of basil and *Lophantus* and the results are presented in Table 5.3. The analyzed extracts were prepared by ultrasound-assisted solvent extraction, which favors cell wall disruption and improved extraction yield [554].

5.4.2. Total flavonoid content

The evaluation of the extraction efficiency of flavonoids, expressed as quercetin equivalents (QEq), was carried out for the ethanolic and methanolic extracts of basil and *Lophantus*. The results of the four basil extracts showed a higher amount of total flavonoid content than total phenolic compound content. Some variation was observed depending on the plant species analyzed and the extraction solvents used (Table 5.3).

The total content of phenolic compounds and flavonoids was determined for both methanolic and ethanolic extracts of dried stems, flowers, and leaves of *O. basilicum*, *O. citriodorum,* and *A. foeniculum*.

The extraction efficiency of phenolic compounds was evaluated for two different solvents, ethanol and methanol, based on the total phenolic acid content expressed as gallic acid equivalents (GA). The results showed that, of the four basil extracts, the methanolic extract of "Aromat de Buzău" contains the highest amount of phenolic compounds (936.2 ± 0.23 mg EqAG/g), while the ethanolic extract of the same variety contains a lower concentration of phenolic compounds (114.7 ± 0.12 mg EqAG/g). By comparison, the phenolic compounds present in the 'Macedon' basil extracts showed a lower content than the methanolic extract of *O. basilicum*, but higher concentrations than the ethanolic extract of the same variety. Some variation was observed depending on the plant species and the extraction solvents used. The methanolic extract "Aromat de Buzău" (*O. basilicum*) presented a quantity of flavonoids of 619.34 ± 4.98 mg EqQ/g, while the ethanolic extract of 598.78 ± 4.98 mg EqQ/g. The total amount of flavonoids in the methanolic extract of the variety "Macedon" (*O. citriodorum*) was 689.05 ± 5.78 mg EqQ/g, while the flavonoid content for the variety "Macedon" (*O. citriodorum*)) of the ethanolic extract was 505.20 ± 3.34 mg EqQ/g. The methanolic extract of *A. foeniculum* showed a total content of 485.1 ± 0.05 mg EqAG/g higher than the ethanolic one of 403.9 ± 0.06 mg EqAG/g. Also, the total flavonoid content in the methanolic extract (367.32 ± 0.01 mg EqQ/g) was higher than for the ethanolic extract of the same cultivar (355.94 ± 0.01 mg EqQ/g).

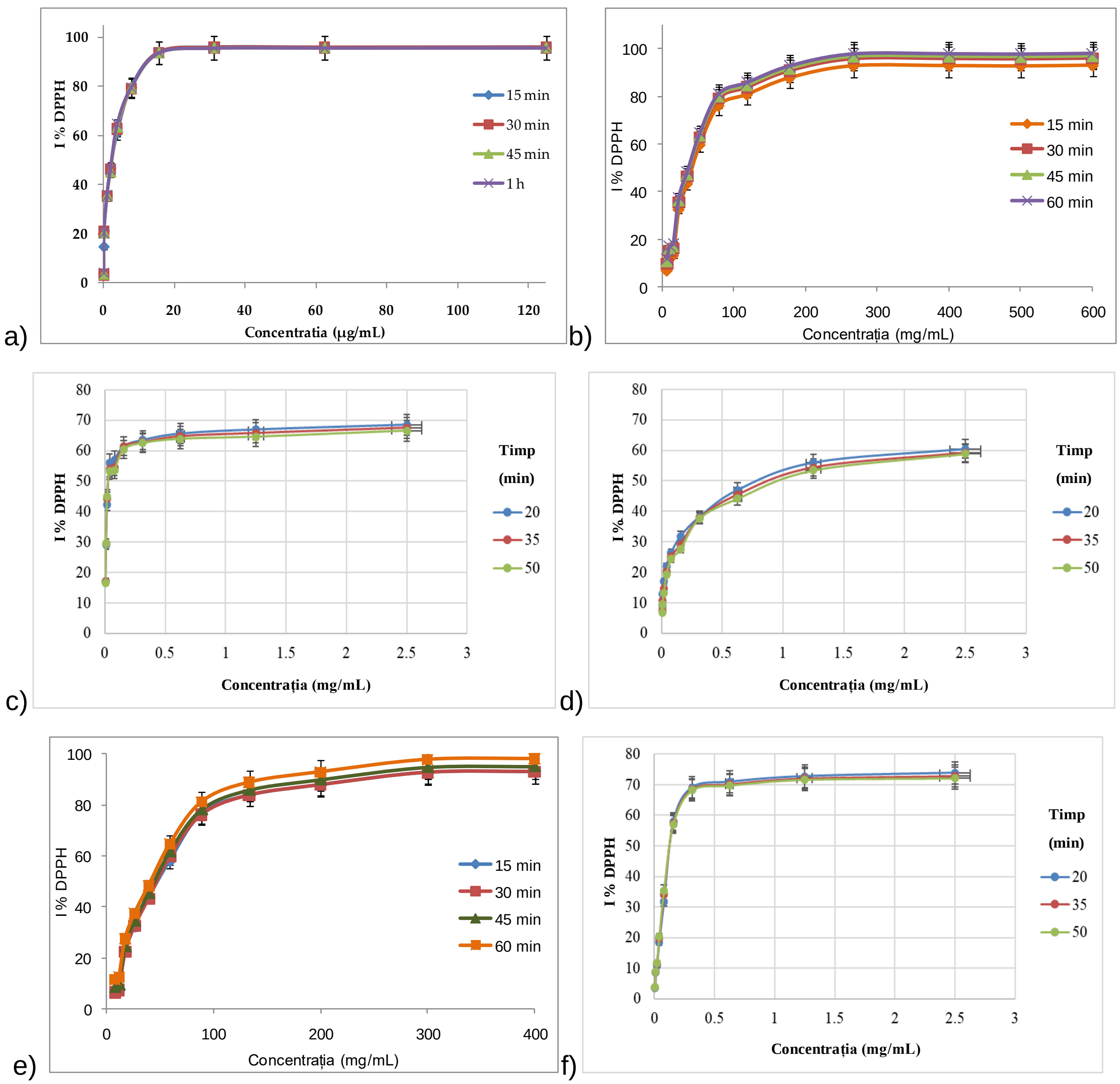
Methanolic extracts were characterized by the highest content of phenolic compounds and flavonoids. Therefore, methanol is a solvent that leads to an optimized extraction of phenolic compounds and flavonoids compared to ethanol, these results being similar to other data in the literature [419].

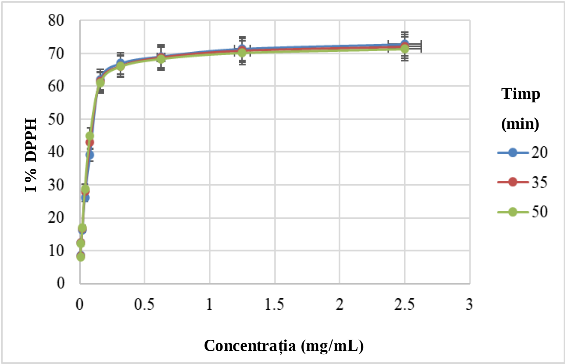
5.4.3. Antioxidant potential

5.4.3.1. Assessment of antioxidant activity by inhibition of DPPH free radical

Plants with a high content of secondary metabolites may exhibit antioxidant activity due to their redox properties [314]. Polyphenolic compounds of plant origin have been shown in many studies to exert antioxidant effects [555]. By increasing the concentration of phenolic compounds and the degree of hydroxylation of phenolic compounds, their DPPH radical scavenging activity also increases and can be defined as antioxidant activity [556].

Extracts and essential oils of *O. basilicum*, *O. citriodorum,* and *A. foeniculum* showed antioxidant activity against DPPH● free radicals (Figures 5.9 and 5.10). Also, the essential oils of *C. carvi*, *I. verum,* and *P. anisum* showed a very good DPPH radical inhibition activity, higher than the other samples analyzed from the plant species of the Lamiaceae family. The fractionated extracts of *A. foeniculum* showed varied antioxidant activities, due to the varied composition of compounds belonging to different classes.



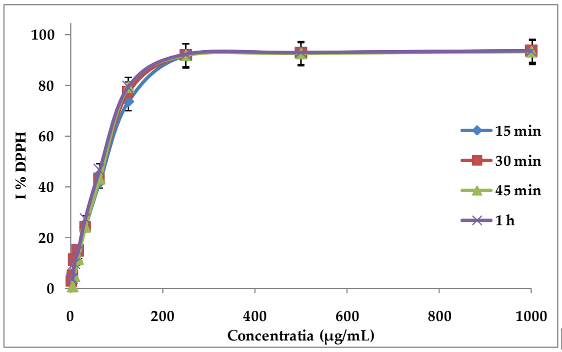


g)

Figure 5.9. DPPH radical inhibition percentage for (a) gallic acid, (b) *O. basilicum* essential oil, (c) *O. basilicum* methanolic extract, (d) *O. basilicum* ethanolic extract, (e) *O. basilicum* essential oil .citriodorum, (f) methanolic extract of *O. citriodorum* and (g) ethanolic extract of *O. citriodorum* after different time intervals of incubation. Each value represents the mean of three individual experiments and error bars with standard deviation.

The high content of phenolic and flavonoid compounds justifies the antioxidant activity of the alcoholic extracts of the analyzed plants. Compounds with redox properties in the composition of essential oils may also be responsible for their high antioxidant activity. The percentage of DPPH radical inhibition by the extracts did not vary significantly with time (from 15 min to 60 min), unlike the essential oils where the same concentration was found to increase its DPPH radical inhibition activity over time (Figures 5.9 and 5.10). The results obtained through the analysis of the samples showed that the activity of inhibiting DPPH radicals is highly influenced by the solvent used for the extraction and, consequently, by the phytochemical composition of the extract (Figures 5.9 and 5.10). The antioxidant capacity of the analyzed species is a useful parameter that correlates with phytochemical determinations. The obtained results are consistent with literature data that showed strong DPPH radical scavenging activity for extracts and essential oils from selected plant species [70,253,265,289,409,527,528].

a)



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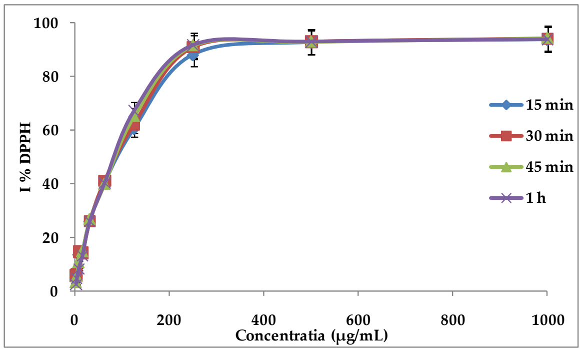


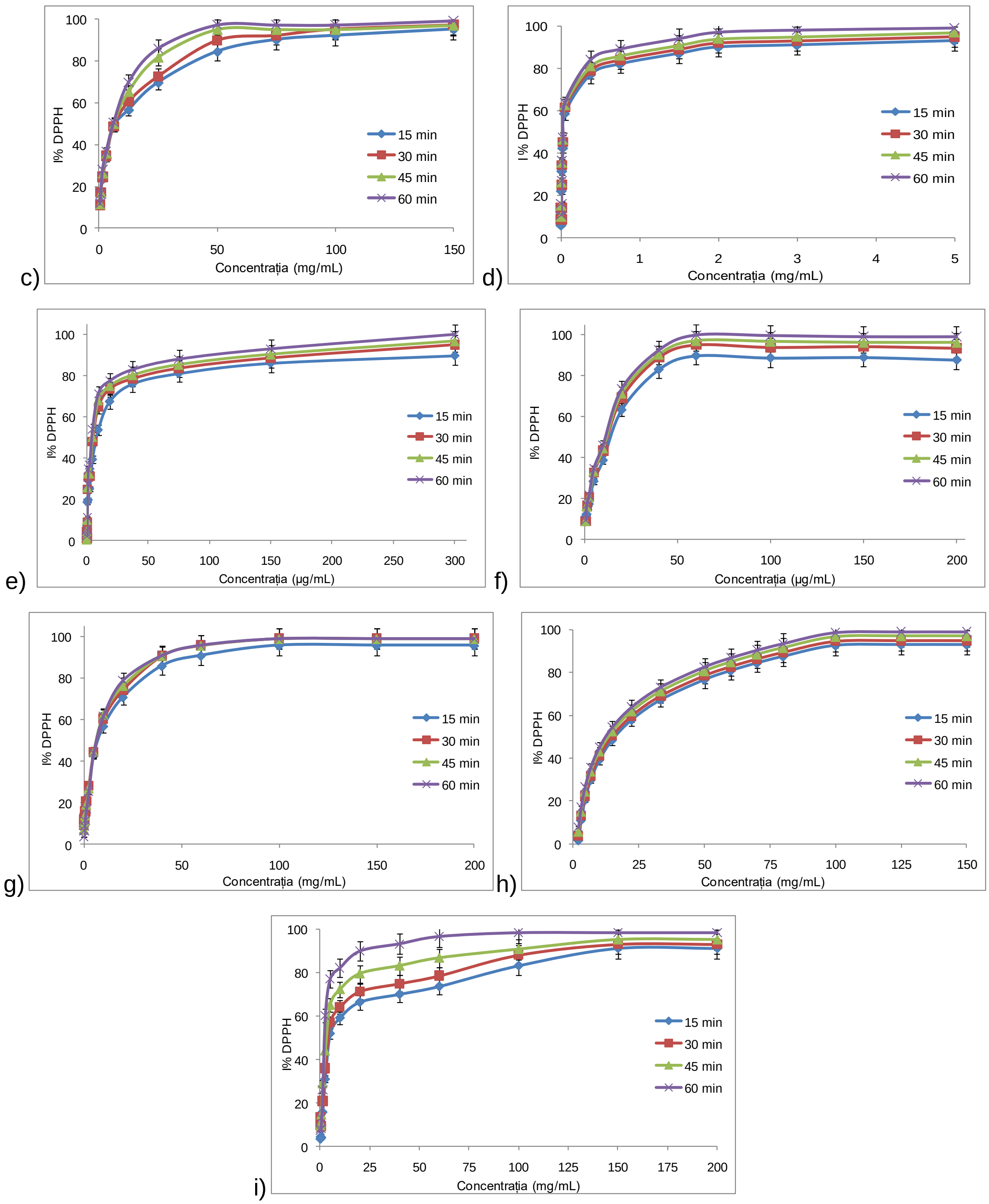


Figure 5.10. DPPH radical scavenging activity of (a) methanolic extract of *A. foeniculum*, (b) ethanolic extract of *A. foeniculum*, (c) essential oil of *A. foeniculum*, (d) dichloromethane fractionated extract of *A. foeniculum*, (e) fractionated extract in ethyl acetate of *A. foeniculum* (f) fractionated extract in ethanol of *A. foeniculum*, (g) essential oil of *C. carvi*, (h) essential oil of *P. anisum*, (i) essential oil of *I. verum* after different incubation time intervals. Each value represents the mean of three individual experiments and error bars with standard deviation.

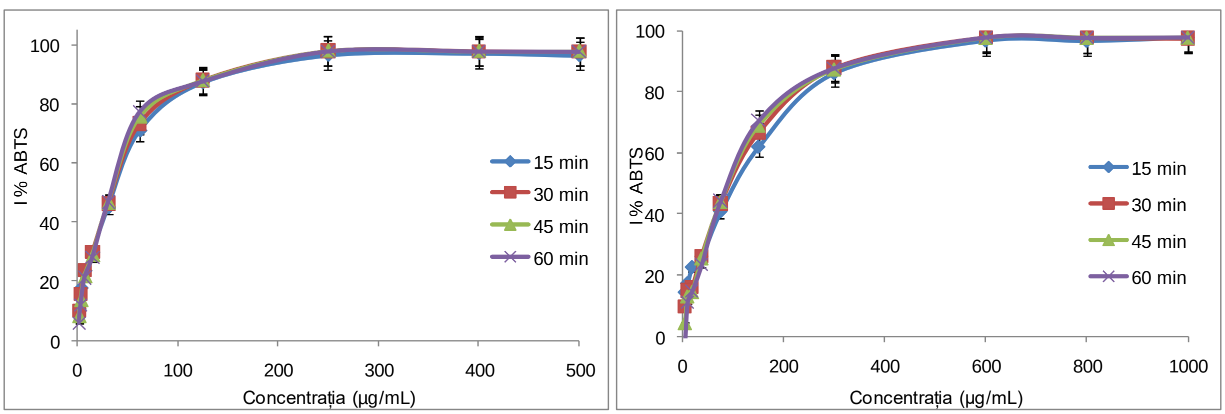
The increased free radical-reducing activity of the natural compounds also justifies the results of this study in which extracts with a high total flavonoid content showed high antioxidant activities. Several studies have demonstrated a relationship between high flavonoid concentration and modulation of cellular redox homeostasis processes, such as scavenging ROS [512,557]. The plant extracts and essential oils in this study demonstrated antioxidant effects that may justify and encourage some of their uses for the prevention of several conditions and further clinical trials.

The potential biological activities of the analyzed species differ between subspecies, as each variety has a varied chemical profile. The variation in the composition of essential oils and extracts of medicinal plants is due to their genetic variations, plant growth stages, climatic conditions, fertilizers, irrigation regimes, and stage of maturity at the time of harvest [360].

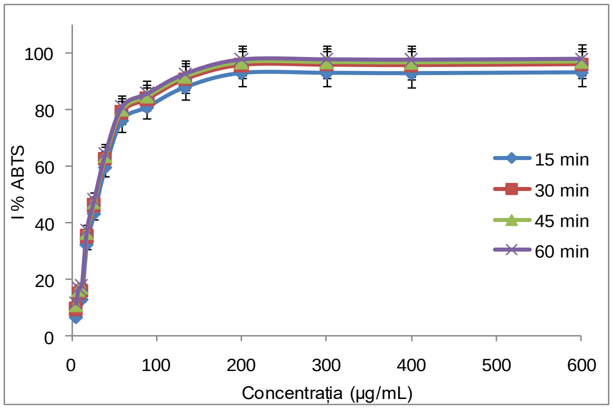
Due to their bioactive components, the varieties analyzed (*Ocimum basilicum* ”Aromat de Buzău”, *Ocimum citriodorum* ”Macedon lămâios”, *Agastache foeniculum* ”Aromat de Buzău”, *Carum carvi*, *Pimpinella anisum,* and *Illicium verum*) could be promising therapeutic agents for human health with antioxidant, anti-inflammatory, analgesic, antimicrobial, antihypertensive, vasorelaxant, antiviral, nutraceutical, anticarcinogenic and antidiabetic properties [108,109,382]. Further research studies are needed to explore the full synergistic/antagonistic potential of the many possible combinations not determined in this thesis. In this sense, further studies are needed to evaluate their mechanism of action and the optimal dose for the prevention and treatment of various conditions generated by oxidative stress.

5.4.3.2. Antioxidant activity using the ABTS method

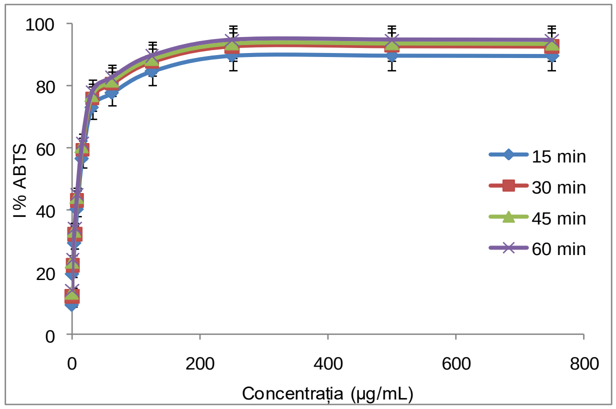
Powerful antioxidants prevent biomolecules (proteins, sugars, nucleic acids, polyunsaturated lipids) from undergoing oxidative damage through free radical-mediated reactions [564]. The decolorization of the ABTS solution is determined by inhibition of the $ABTS^{•+}$ radical cation as a function of sample concentration and time (Figures 5.14 and 5.13).



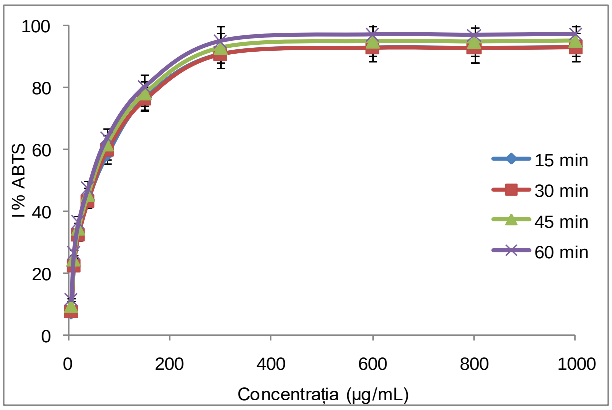
(a) (**b**)



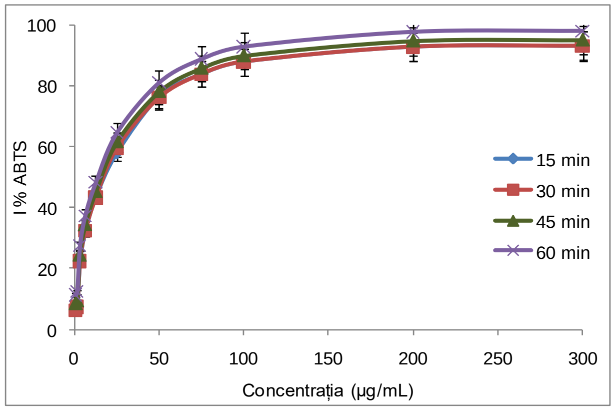
(c)



(**d**)



(e)

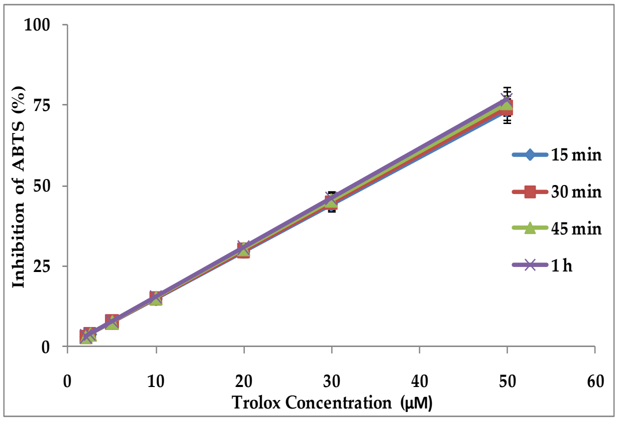


(**f**)

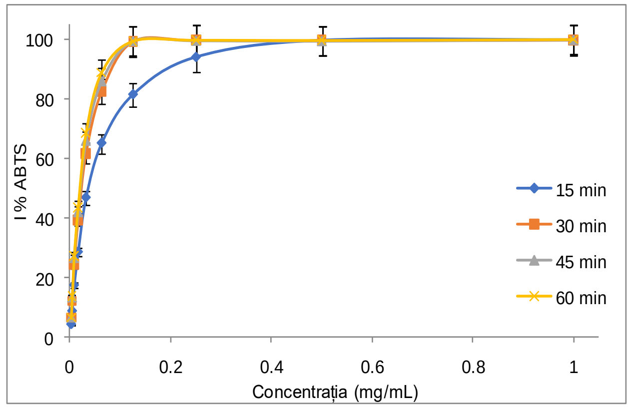
Figure 5.14. ABTS radical inhibition activity of (a) methanolic extract, (b) ethanolic extract, (c) the volatile oil of *Ocimum basilicum*; (d) the methanolic extract, (e) the ethanolic extract, (f) the volatile oil of *Ocimum citriodorum*. Each value represents the mean of three experiments and error bars with standard deviation.

Antioxidant compounds are abundantly available in plants and play an important role in scavenging free radicals, thus providing the human body with important benefits against oxidative damage. The extracts and essential oils analyzed demonstrated antioxidant properties due to the presence of biologically active compounds such as anethole, estragole, carvone, linalool, eugenol, rutin, quercetin, genistein, daidzein, and hyperoside. The antioxidant activities of the analyzed samples showed an antioxidant effect correlated with the concentration of the samples but also with other results from the specialized literature for all the plant varieties analyzed [107,391,392,409,529,530].

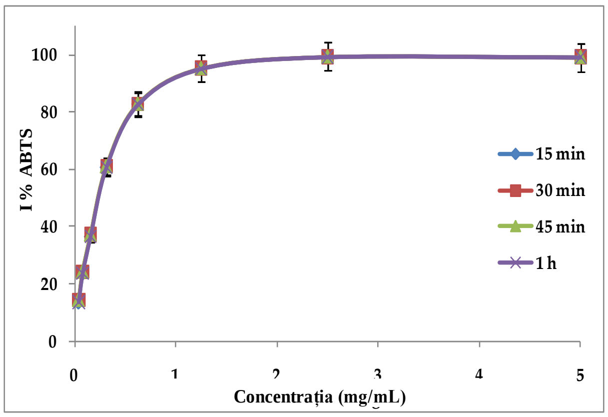
The best antioxidant activities among the analyzed essential oils were found for the one extracted from *C. carvi* seeds and in the case of the extracts the methanolic extract for all varieties of plants from the Lamiaceae family (Figures 5.14 and 5.15).


Inhibition of ABTS (%)
Trolox Concentration (μM)
15 min
30 min
45 min
1 h

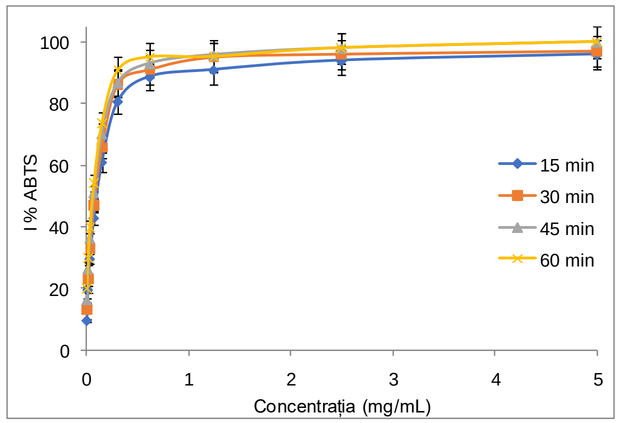
(a)


I % ABTS
Concentrația (mg/mL)
15 min
30 min
45 min
60 min

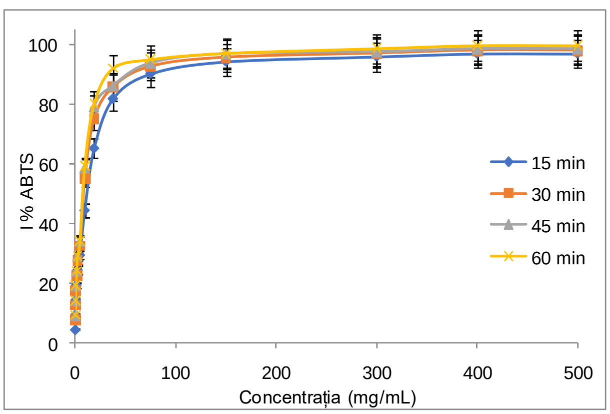
(b)


I % ABTS
Concentrația (mg/mL)
15 min
30 min
45 min
1 h

(c)


I % ABTS
Concentrația (mg/mL)
15 min
30 min
45 min
60 min

(d)


I % ABTS
Concentrația (mg/mL)
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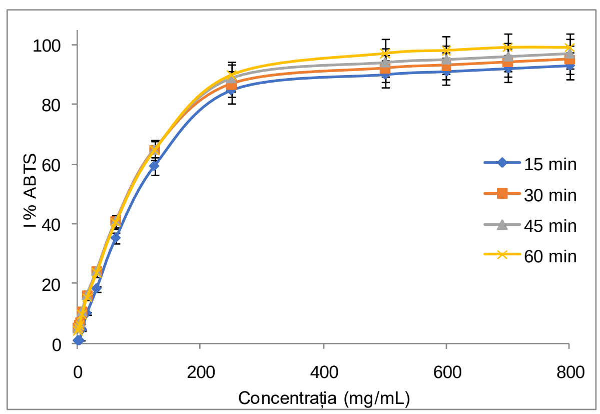
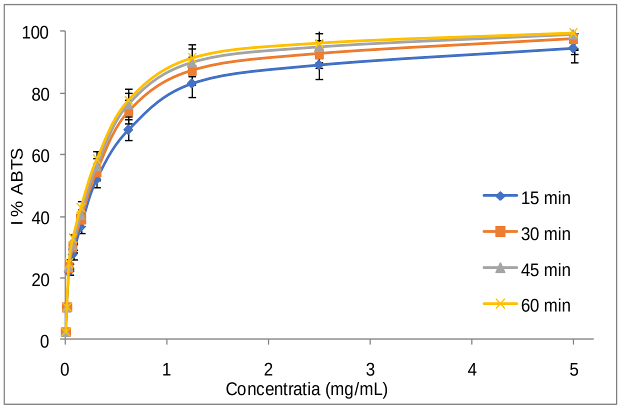
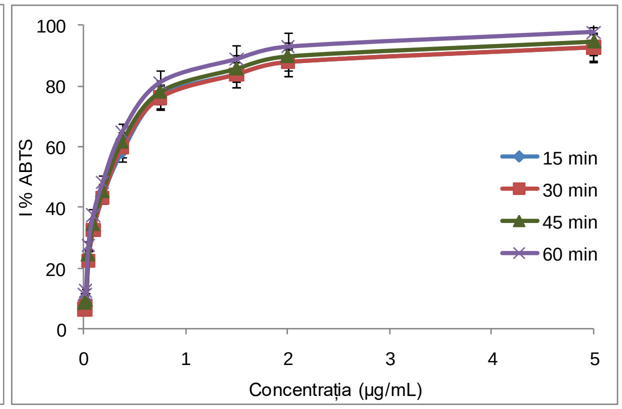
(e)

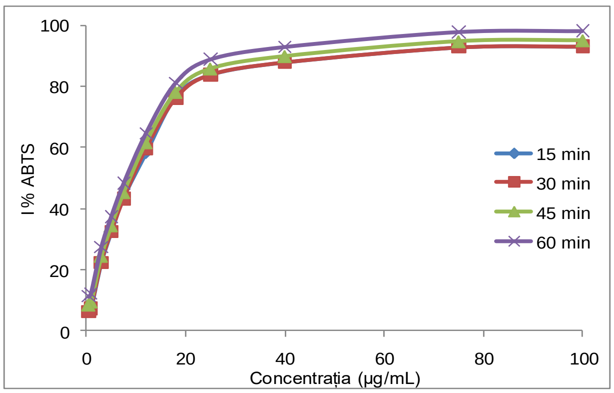

I % ABTS
Concentrația (mg/mL)
15 min
30 min
45 min
60 min

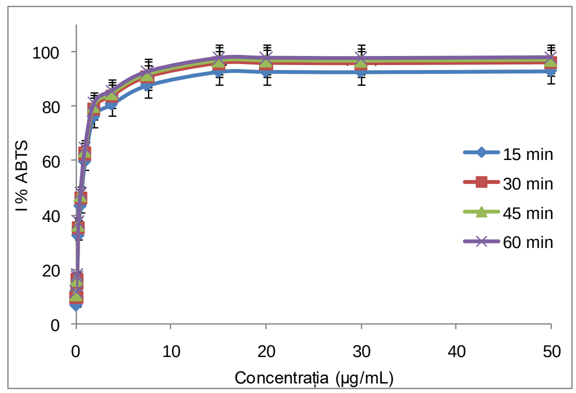
(f)





(g) **(h)**





(i) **(j)**

Figure 5.15. ABTS radical scavenging activity of (a) a standard-Trolox, (b) methanolic extract of *A. foeniculum*, (c) ethanolic extract of *A. foeniculum*, (d) dichloromethane fractionated extract of *A. foeniculum*, (e) fractionated extract in ethyl acetate of *A. foeniculum*; (f) fractionated extract in ethanol of *A. foeniculum*; (g) volatile oil of *A. foeniculum*; (h) *C. carvi* essential oil; (i) *I. verum* essential oil; (j) *P. anisum* essential oil. Each value represents the mean of three determinations and error bars with standard deviation.

All extracts obtained as a result of sequential extraction with solvents of increasing polarity (dichloromethane, ethyl acetate, and ethanol) from dry *A. foeniculum* leaf powder showed high antioxidant activity by the ABTS method. The best activity was observed for the fraction in ethyl acetate. Depending on the solvent used in the fractional extraction, the chemical composition of the extracts is varied and some classes of compounds are only found in certain solvents. The chemical composition of extracts with dichloromethane can be made up of compounds from the classes of alkaloids and terpenoids, with ethyl acetate terpenoids, saponins and alkaloids can be extracted and with ethanol compounds from the classes of flavonoids, phenolic compounds, saponins, and sugars can be extracted [565]. Therefore, this type of extraction is useful in evaluating the compounds responsible for the antioxidant activity of the extracts. Further studies will also be carried out to obtain fractionated extracts for basil species.

Extracts and essential oils from the analyzed plants were found to have antioxidant effects against $ABTS^{\bullet+}$ radical cations that are due to the unique phytochemical composition of each analyzed sample. These results may justify and therefore encourage the use of the plants in this study as sources of compounds with antioxidant properties.

5.4.3.3. Antioxidant activity using the FRAP method

The test consists of the reduction of ferric ions to ferrous at low pH, giving a colored complex. Ferric tripyridyltriazine (TPTZ 2,4,6-Tris(2-pyridyl)-s-triazine) has a light yellow color which, when reduced to the ferrous form by antioxidants, turns to a violet-blue color [232]. The change in absorbance is proportional to the total reducing power of the antioxidants in the analyzed sample.

Table 5.4. The antioxidant potential of plants analyzed by the FRAP method

The species of the analyzed plant	Samples analyzed	FRAP IC_{50} (μM Eq Fe(II) / g extract)
O. basilicum	MeOH	54.178 ± 0.051 [a]
	EtOH	49.685 ± 0.024 [b]
O. citrodorum	MeOH	51.582 ± 0.030 [b]
	EtOH	46.314 ± 0.018 [c]
A. foeniculum	MeOH	45.721 ± 0.014 [c]
	EtOH	39.483 ± 0.017 [d]

Values followed by the same letter (a, b, c, d) in the same column do not show statistically significant differences (p < 0.01). Each value represents the mean of three individual experiments ± standard deviation.

For basil species, methanolic extracts were found to have a higher IC50 concentration than ethanolic ones. In the case of *A. foeniculum* species, the methanolic extract demonstrated a higher total ferric complex reducing power (IC50 = 45.721 ± 0.014 μM Fe(II) equivalents/g extract) compared to the ethanolic extract (IC50 = 39.483 ± 0.017 μM equivalents Fe(II)/g extract), indicating that the methanolic extracts contain a higher amount of antioxidant compounds (Table 5.4). The results obtained by evaluating the antioxidant activity by the FRAP method are similar to other results in the specialized literature for the analyzed plant varieties [265,527,529,531–533].

5.4.4. Cytotoxic potential on tumor cells

The cytotoxic activity of the essential oils was evaluated in MCF-7 breast cancer cells and normal dermal fibroblasts, HDF. The analyzed volatile oils are complex mixtures of compounds and each potential compound enhances or modifies the effects of other constituents. In this study, essential oils were used with all the constituents, complex phytochemical mixtures, to maximize the potential anticancer effect and to properly assess the potential risks to healthy cells and tissues.

The cytotoxic activity of *A. foeniculum* essential oil was evaluated by MTT assay. At lower concentrations (1 μL/mL), high cytotoxicity was observed against MCF-7 breast tumor cells, but not healthy human dermal fibroblasts (HDF), indicating selectivity for tumor cells and suggesting the presence of biologically active components that contributes to the observed high cytotoxic effect. The results of the present study provide new insights into the use of *A. foeniculum* essential oil as a potential source of bioactive compounds and a good candidate for herbal pharmaceuticals.

The current study of *A. foeniculum* essential oil from Romania on the MCF-7 tumor cell line indicated a greater cytotoxic effect at lower concentrations (1 μL/mL), which suggested the presence of other biologically active components that contributed to the high cytotoxic effect. In addition, using healthy cells, HDF human dermal fibroblasts, the cytotoxic effect appears to indicate improved selectivity for breast tumor cells at concentrations higher than 0.2 μL/mL (Figure 5.17). *A. foeniculum* essential oil exhibits statistically significant cytotoxic activity on MCF-7 breast tumor cells at concentrations higher than 0.2 μL/mL ($p < 0.001$). The IC50 values obtained using the statistical program GraphPad Prism (vs. 5.0) were on MCF-7 breast tumor cells IC50=0.24±0.09 µL/mL and on healthy human HDF dermal cells IC50=0.47±0 .1 µL/mL.

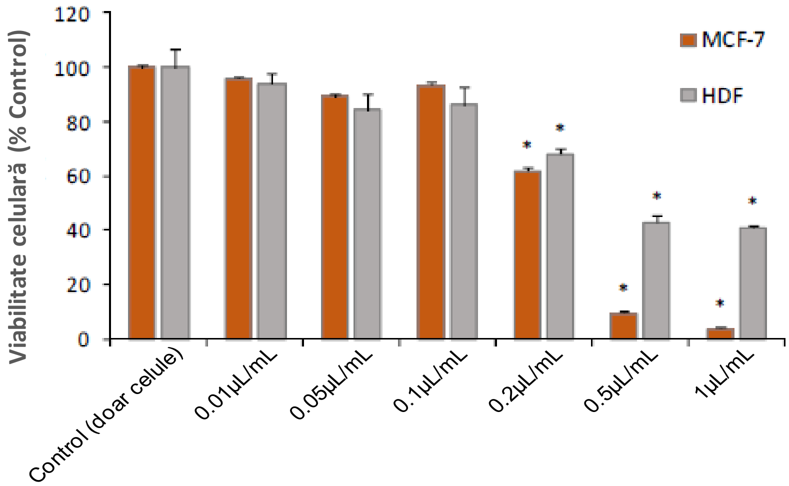


Figure 5.17. Cytotoxic activity of *A. foeniculum* essential oil against MCF-7 and HDF cell lines. Control = only cells without treatment. Data represent the mean ± SD of two independent experiments with at least four replicates per condition. *Significantly different (Dunnett test) from baseline conditions ($p < 0.001$).

Our studies evaluating Romanian *A. foeniculum* volatile oil on MCF-7 breast cancer cell lines indicated a high cytotoxic effect at low concentrations (<0.5 µL/mL), which means that a mixture of biological compounds actives contributed to the observed high cytotoxic effect. For healthy HDF dermal cells only at concentrations higher than 0.5 µL/mL, loss of viability was observed, but not as much as that observed for cancer cells. Other studies reported that agastinol and agastenol, lignans detected in *Agastache* species, inhibited apoptosis in U937 leukemia cells [124]. Estragole, the main component of the volatile oil, has demonstrated anticancer properties but also other important biological activities such as antimicrobial, bradycardic, muscle relaxant, anesthetic, anticonvulsant, vasoprotective, anti-inflammatory, and antioxidant [108].

The strongest action of decreasing tumor cell viability on MCF-7 cells was obtained for *Carum carvi* essential oil, whose major constituent is carvone (Figure 5.18).

5.4.5. Inhibition of xanthine oxidase by some compounds from the analyzed species

Xanthine oxidase (XO) is involved in purine degradation in humans by forming xanthine from hypoxanthine and is ultimately converted to uric acid by an enzymatic reaction catalyzed by xanthine oxidase [575]. Uric acid is eliminated in the urine, but excessive uric acid formation can consequently lead to hyperuricemia and gout [575,576]. EO from *A. foeniculum* demonstrated high inhibition of xanthine oxidase activity in vitro (84.077 ± 0.031%) at a concentration of 20 µg/mL, which is comparable to that of allopurinol 30 µg/mL, a well-known XO inhibitor [576]. EO and alcoholic extracts demonstrated significant inhibitory activities at concentrations higher than 2.5 µg/mL ($p < 0.001$) and higher than 0.25 mg/mL ($p < 0.001$), respectively (Figures 5.15).

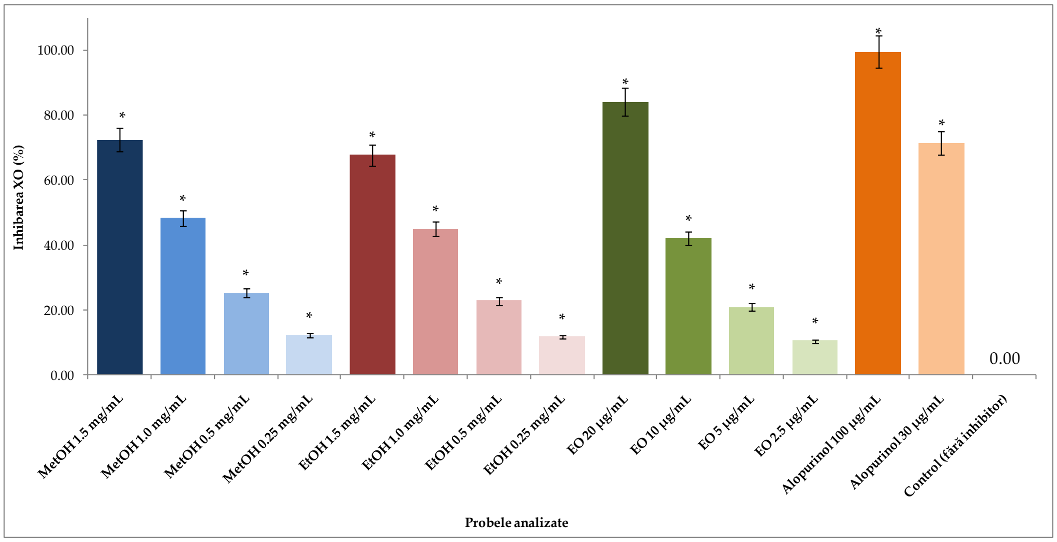


Figure 5.15. Xanthine oxidase (XO) inhibitory activity by allopurinol, extracts, and volatile oil (EO) of *A. foeniculum* evaluated at different concentrations. The results shown are means of three individual experiments and error bars with standard deviation. *Significantly different (Dunnett t-tests) from control (no inhibitor) conditions (p < 0.001).

These results suggest that *A. foeniculum* extracts and essential oil may have multiple mechanisms of action and diverse biological activities that contribute to their biological activity. Further studies are needed to evaluate the XO inhibition potential of alcoholic extracts of basil and essential oils of basil, caraway, and anise.

5.5. Partial conclusions

- Extracts and essential oils of *O. basilicum*, *O. citriodorum,* and *A. foeniculum* showed antioxidant activity against DPPH• free radicals. Also, the essential oils of *C. carvi*, *I. verum,* and *P. anisum* showed a very good DPPH radical inhibition activity, higher than the other samples analyzed from the plant species of the Lamiaceae family. The fractionated extracts of *A. foeniculum* showed varied antioxidant activities, due to the varied composition of compounds belonging to different classes.
- Through the FRAP method for evaluating the antioxidant activity, for basil species, it was found that the methanolic extracts have a higher IC50 concentration than the ethanolic ones. In the case of *A. foeniculum* species, the methanolic extract demonstrated a higher total ferric complex reducing power (IC50 = 45.721 ± 0.014 µM Fe(II) equivalents/g extract) compared to the ethanolic extract (IC50 = 39.483 ± 0.017 µM equivalents Fe(II)/g extract).
- The strongest action of decreasing tumor cell viability on MCF-7 cells was obtained for *Carum carvi* essential oil, whose major constituent is carvone.
- Essential oils and alcoholic extracts demonstrated significant inhibitory activities at concentrations greater than 2.5 µg/mL ($p < 0.001$) and greater than 0.25 mg/mL ($p < 0.001$), respectively.
- These results suggest that extracts and essential oils from *A. foeniculum* may have multiple biological activities due to their chemical composition. This study intends to provide new contributions to the pharmacologically relevant effects of alcoholic extracts and EOs of *A. foeniculum*, such as antioxidant and xanthine oxidase inhibitory activities, as well as cytotoxic properties of EOs on cancer cells, which can be considered as evidence of the effectiveness of this medicinal plant.

Capitolul 6. Analysis of some isoflavonoids separated from the species analyzed by *in silico* studies (molecular modeling)

6.2. Main objectives

In this study, the main objective was to evaluate the results obtained from the in silico analysis of the bioactive potential of some isoflavones (genistein and daidzein) identified in the plant species analyzed through molecular modeling studies in which two proteins were used, PI3Kα (phosphatidylinositol 3-kinase alpha) and XO (xanthine oxidase), proteins with an important enzymatic role and which are involved in mitigating various inflammatory diseases.

It was also proposed to investigate the inhibition capacity of two enzymes, PI3kα kinase and xanthine oxidase (XO) by the two compounds.

6.4. Results and Discussion

The results obtained by applying the computational method were successfully achieved. The optimized molecular structures of the genistein and daidzein compounds, the comparison of the X-ray structure with the optimized molecular structure for genistein, the evaluation of the electronic parameters, the frontier molecular orbitals, the molecular electrostatic potential map, the calculation of some molecular parameters with the SwissADME and AdmetSAR programs, as well as the docking are highlighted of the studied compounds on two enzymes, protein kinase PI3Kα, and xanthinoxidase XO.

Electrostatic molecular potential (ESP) maps were plotted for each investigated compound to illustrate the relative polarity of the molecule and identify the active sites of electrophilic/nucleophilic attacks. ESP maps (Figures 6.10 and 6.11) indicate areas of negative electrostatic potential in red and positive in blue. The regions with negative electrostatic potential are located in the area of the C = O, OH, or pyranic ring groups that contain oxygen. These areas are favorable sites for attack by electrophilic agents, while areas with positive potential favor attack by nucleophilic agents.

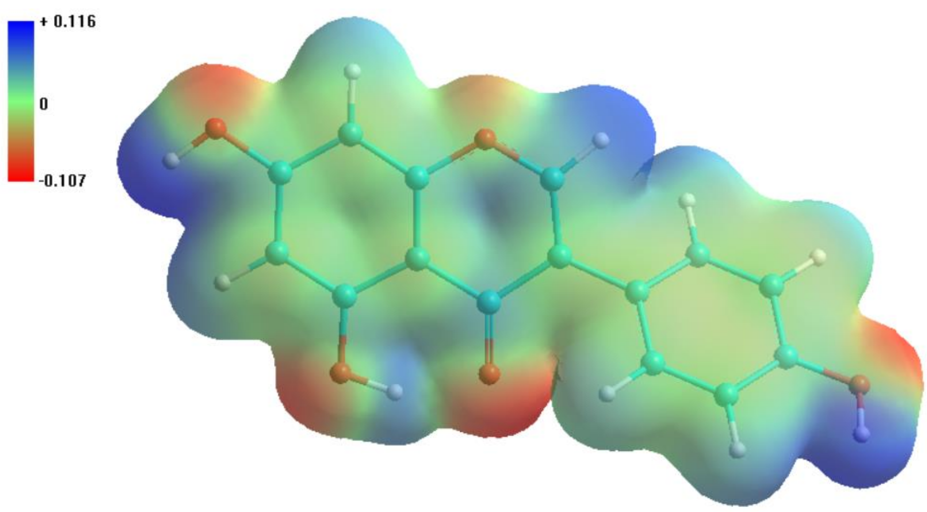


Figure 6.10. ESP for the compound 5,7-dihydroxy-3-(4-hydroxyphenyl)chromene-4-one (genistein)

Using ESP maps, it was identified that genistein has five active sites for electrophilic attack while daidzein has four such sites.

6.4.4. Evaluation of some parameters regarding the bioavailability of the studied compounds

The bioavailability of a drug indicates its effectiveness and represents the amount of active substance released from the drug, absorbed and distributed in the body to reach the site of action where the therapeutic effect is manifested, as well as the speed with which this process takes place [618].

Table 6.6. Calculation of some parameters regarding the oral bioavailability of the investigated compounds using the SwissADME and AdmetSAR programs

No.	Compound name	LogS	LogPapp (cm/s)	BBB	Gastrointestinal Absorption	BA Score
1	Genisteine	-3,72	1,0095	No	High	0,55
2	Daidzeine	-3,53	1,1564	Yes	High	0,55

*BBB- the blood-brain barrier

6.4.5. Molecular docking results

Molecular docking of studied compounds on the PI3Kα receptor

The PI3Kα protein structure (PDB ID: 4JPS) was downloaded from the Protein Data Bank website (http://www.pdb.org/pdb/home/home.do). In docking assays, isoflavones were used as ligands while PI3Kα kinase was used as a receptor. All water molecules and other small molecules in the protein crystal structure were excluded, and polar hydrogen atoms and Gasteiger charges were added. After setting the default parameters, docking studies were performed between PI3Kα and isoflavones and the standard ligand. To generate the most conclusive docking results, multiple analyzes were performed to find the most suitable site, by using several grid boxes (x, y, z).

Comparing the docking results (Table 6.7) for genistein and daidzein with those of the standard ligand, alpelisib (Figures 6.12), it is found that the investigated isoflavones form a less stable complex with the PI3Kα kinase than in the case of the standard alpelisib in that the binding energy for the kinase -standard (-9.1 kcal/mol) is lower than for isoflavones (-7.8 kcal/mol for genistein and -8 kcal/mol for daidzein). Therefore, the inhibition activity of isoflavones (inhibition constant Ki = 1.48 μM for daidzein, Ki = 2.09 μM for genistein) is lower than that of the standard compound (Ki = 0.21 μM). We noted that alpelisib is known from the experiment [613] to be a drug with intense inhibitory activity. In conclusion, the investigated compounds can be considered potential PI3Kα kinase inhibitors with medium activity.

6.5. Partial conclusions

➢ Two isoflavones, genistein and daidzein, present in the analyzed plants, were analyzed from a structural and chemical potential point of view, through molecular modeling. The compounds were optimized thus obtaining the equilibrium geometries, frontier MO (HOMO, LUMO), molecular electrostatic potential map, and a series of electronic parameters. The validation of the B3LYP/6-311G(d,p) optimization method was achieved by comparing the optimized structure of genistein with the XRD analysis. The very good agreement of the molecule geometries (bond length, bond angle) obtained theoretically and experimentally confirmed that the B3LYP/6-311G(d,p) modeling method chosen for optimization is very efficient.

- From the analysis of the electronic parameters (ΔE_{H-L} energy, chemical rigidity, chemical flexibility, electrophilicity index, etc.), it appears that both genistein and daidzein have medium chemical potential. Regarding the charge distribution for the frontier orbitals, it was noted that the double bond C2 = C3 allows a more pronounced delocalization of the electric charge when going from the HOMO orbital to the LUMO orbital for the investigated compounds.
- Molecular electrostatic potential maps identified for the compound genistein the existence of five active sites for electrophilic attack, unlike the compound daidzein where only four active sites for electrophilic attack were noted.
- From the analysis of oral bioavailability parameters, it was found that both investigated compounds comply with Lipinski's extended rules and have a bioavailability score of 0.55, thus demonstrating that they have good oral bioavailability.
- Verification of the inhibition potential of genistein and daidzein on the receptors, PI3Kα and XO, was done by molecular docking and using standard ligands with known inhibitory activity. It was found that the investigated ligands have medium inhibition potential (in the order of μM) both on PI3Kα kinase and on xanthine oxidase, a fact confirmed by the experimental data. So, genistein and daidzein may be potential drugs (adjuvants) for treating cancer and gout, further investigation is needed for compliance and future applications.

The results of these researches, obtained in this chapter, were partially published in impactful scientific articles, as first author: *Separations*, ”*Comparative Study of Natural Antioxidants from Glycine max, Anethum graveolens and Pimpinella anisum Seed and Sprout Extracts Obtained by Ultrasound-Assisted Extraction,* 2022” [441].

Chapter 7. General conclusions and originality of contributions

7.1. General conclusions

The research carried out during this doctoral thesis was complex and branched, addressing the phytochemical study of some new/cultivated varieties of aromatic plants in Romania, the studies focusing mainly on two extraction methods, with the help of ultrasound and hydrodistillation. The identification, separation, and quantification of natural organic compounds was carried out from alcoholic extracts (ethanolic and methanolic), obtained by ultrasound, and from oily extracts, obtained by hydrodistillation. The original scientific contributions resulting from the studies are summarized below.

- Aromatic plant species, new hybrid varieties of basil "Aromat de Buzau" (*Ocimum basilicum*) and "Macedon" (*Ocimum citriodorum*) but also Lophantus (*Agastache foeniculum* (Pursch) Kuntze "Aromat de Buzau"), as well as plants grown in Romania, anise seeds (*Pimpinella anisum*) and caraway (*Carum carvi*) and star anise (*Illicium verum*), were taken from the Buzau Vegetable Research and Development Station, Romania.
- The extraction of natural organic compounds from aromatic plants was carried out for the first time, for hybrid varieties, by solvent and ultrasound-assisted (US) extraction methods, using ethyl alcohol and methanol as solvents. For the first time, extracts from the species *Agastache foeniculum* (Pursch) Kuntze "Aromat de Buzau" were obtained using fractional US extraction, using solvents with different polarities. Volatile organic compounds were extracted from the six aromatic plants studied by hydrodistillation extraction.
- The separation, identification, and quantification of the natural compounds from the studied species were achieved, for the first time, for the new basil species "Aromat de Buzău" (*Ocimum basilicum*) and "Macedon" (*Ocimum citriodorum*) and *Agastache foeniculum* (Pursch) Kuntze " Aromat de Buzau".
- The chemical composition of extracts obtained by hydrodistillation from species of aromatic plants grown in Romania from the Lamiaceae family was analyzed for the first time by GC-MS chromatography: *Ocimum basilicum*, *Ocimum citriodorum*, *Agastache foeniculum*, *Pimpinella anisum*, *Carum carvi* and *Illicium verum* in which it was confirmed the presence of the major compounds linalool, methyl chavicol (estragole), eugenol, methyl-eugenol, D-germacrene, 1,8-cineole, β-caryophyllene, trans-anethole, ɣ-himachalene, carvone, limonene, and estragole.
- The main component detected by GC-MS analysis in the essential oil of *Ocimum basilicum* "Aromat de Buzau" (34.87±1.05%) was the acyclic terpenoid linalool (3,7-dimethylocta-1,6-dien-3-ol), in the essential oil of *Agastache foeniculum* also called "Buzău Scent" was estragole (1-allyl-4-methoxybenzene), in the analyzed essential oil of caraway (*Carum carvi*) it was rich in carvone (68.49±1, 41%), and the main component identified in the analyzed essential oils of star anise (*Illicium verum*) and anise berry (*Pimpinella anisum*) was trans-anethole.
- High-performance liquid chromatography was used for the identification, separation, and quantification of compounds from the class of polyphenols, flavonoids, and lignans from alcoholic, ethanolic, and methanolic extracts of *Ocimum basilicum*, *Ocimum citriodorum,* and *Agastache foeniculum*.
- The main compounds with biologically active properties identified were different depending on the species, the method of extraction, and the solvent used. Thus, in the alcoholic extracts from the analyzed Romanian basil varieties, six main constituents were highlighted, namely catechin, rutin, hyperoside, naringin, naringenin and genistein.
- The quantification of the chemical compounds identified in the alcoholic extracts was

carried out and the results showed that naringin is the main compound of the basil extracts, its concentration varying between 930.10 ± 0.15 µg/g and 2618.11 ± 0.23 µg/g. The highest concentrations of naringin were recorded for the methanolic extracts for both varieties of basil, the concentration being three times higher in the *O. basilicum* "Aromat de Buzău" variety.

- In the *Agastache foeniculum* species, the phenolic p-coumaric and caffeic acids, the flavonols rutin, quercetin, and hyperoside, the flavanone naringenin and the isoflavone genistein were identified in both the methanolic and ethanolic extracts. Tannic acid was identified only in the methanolic extracts of *Agastache foeniculum*.
- Genistein and quercetin were found in high concentrations in both alcoholic extracts of A. foeniculum, with quercetin content ranging from 704.148 - 1073.637 µg/g s.u. and that of genistein between 2229.999 - 3171.823 µg/g s.u. Tannic acid was quantifiable only in the methanolic extract. Caffeic acid, p-coumaric acid, quercetin, rutin, and hyperoside were identified in both *A. foeniculum* extracts.
- The presence of polyphenolic and flavonoid compounds (coumaric acid, caffeic acid, catechin, rutin, hyperoside, quercetin, naringenin, naringin, and genistein) was also confirmed by UHPLC/MS analysis, in different fractions of *Ocimum basilicum*, *Ocimum citriodorum,* and *Agastache foeniculum*.
- UHPLC/MS analysis of *Agastache foeniculum* fractions revealed the presence of polyphenolic and flavonoid compounds (p-coumaric acid, chlorogenic acid, rutin, quercitin, daidzin, genistein), but also other specific compounds from the class of flavonoids (pinocembrin, formonetine, ononine, glycetine, prunetine, pseudobaptigenine, tectorigenin, irisolidone), compounds from the class of triterpenoids (oleanoic acid, betulinic acid), coumestans (glycoside of coumestrol), alkaloids (hordenine, flindersine and salsolinol) and amino acids (S-Lactoylglutathione, L-aspartic acid, citrulline).
- NMR spectrometry was used for the first time in the analysis of essential oils obtained from plant species at the SCDL Buzău research station, oils obtained by the hydrodistillation method. Thus, through the 1H NMR analysis of the essential oil of *Agastache foeniculum* variety "Aromat de Buzău", it was possible to identify the presence of the main component, estragole. The proton NMR spectrum of the essential oil of *A. foeniculum* in the fresh state also shows the same signals after 1 year, with the same intensity, finding that under the mentioned storage conditions the essential oil did not change its chemical composition. The presence of estragole in high concentrations leads to the conclusion that the variety *A. foeniculum* "Aromat de Buzău" can be considered to be the estragole chemotype. In the essential oil of *Carum carvi*, the presence of the major chemical compound, carvone, and in the essential oil of *P. anisum* and *I. verum*, the presence of the major chemical compound, trans-anethole, was identified. It has been shown that NMR analysis can help to identify the components of essential oils helping to identify and differentiate between species and to comply with government regulations in the adulteration of commercial essential oils.
- Two electrochemical techniques, OCP (open loop potential) and CV (cyclic voltammetry) were used for the first time, and important electrochemical parameters such as anodic current, half-wave potential, and diffusion coefficient were evaluated to evaluate extracts obtained from plants grown in Romania from the Lamiaceae family: *Ocimum basilicum*, *Ocimum citriodorum*, *Agastache foeniculum*, *Pimpinella anisum*, *Carum carvi* and *Illicium verum*.
- Pure chemical compounds found in the analyzed plant oil species, confirmed by other instrumental techniques, such as eugenol, estragole, and linalool, were electrochemically analyzed and the results indicated that the samples could be differentiated, by the anodic current recorded, dependent on the concentration of the compound of interest.

- The evaluation of basil oil samples compared to eugenol and linalool (reference substances) indicated that at a potential of 0.5 V/Ag/AgClsat electronic exchanges begin, an anodic current is recorded, which reflects the antioxidant potential, being also influenced by the analyzed species.
- Cyclic voltammograms (CV) recorded for the methanolic extract for samples with *O. basilicum* AB ("Buzău Aromat") oil indicated an anodic current, Ia with a maximum value of 12 µA, at a potential of 0.5 V/Ag/$AgCl_{sat}$.
- The recorded cyclic voltammograms (CV) indicated the anodic current, I_a, in different solvents, variable values, in the samples of basil oil (AB, MB, CB), hybrid species (AB, MB), *Agastache foeniculum* oil, which changes with the oil concentration and depends on the applied potential.
- The registered anodic current, therefore superior antioxidant capacity, is obtained in samples with basil oil, when the applied potential is greater than 1 V/Ag/$AgCl_{sat}$, which confirms the activity of some chemical compounds, which interact efficiently in electronic exchanges, such as be the hydroxyl groups, from polyphenols, with the oxygen input from the samples.
- Through the recorded voltammograms, the samples with different oil content, from different species can be differentiated by the recorded anodic current, so by the active antioxidant potential for the chemical species present, the study of the electrochemical parameters is useful for the general characterization of the antioxidant potential of the samples and the differentiation between them.
- Extracts and essential oils of *O. basilicum*, *O. citriodorum,* and *A. foeniculum* showed antioxidant activity against $DPPH^{\bullet}$ free radicals. Also, the essential oils of *C. carvi*, *I. verum,* and *P. anisum* showed a very good DPPH radical inhibition activity, higher than the other samples analyzed from the plant species of the Lamiaceae family. The fractionated extracts of *A. foeniculum* showed varied antioxidant activities, due to the varied composition of compounds belonging to different classes.
- Among the combinations of volatile oils analyzed by the DPPH radical inhibition microspectrophotometric method, the mixtures between *C. carvi* and *A. foeniculum* essential oil proved to have a higher synergistic potential than in the case of combinations with basil volatile oils, the value of the synergistic effect being the highest for the combination of 25:15 mg/mL *C. carvi* essential oil and *A. foeniculum* essential oil, with a value of 1.14 ± 0.04.
- Through the FRAP method for evaluating the antioxidant activity, for basil species, it was found that the methanolic extracts have a higher IC50 concentration than the ethanolic ones. In the case of *A. foeniculum* species, the methanolic extract demonstrated a higher total ferric complex reducing power (IC50 = 45.721 ± 0.014 µM Fe(II) equivalents/g extract) compared to the ethanolic extract (IC50 = 39.483 ± 0.017 µM equivalents Fe(II)/g extract).
- The strongest action of decreasing tumor cell viability on MCF-7 cells was obtained for *Carum carvi* essential oil, whose major constituent is carvone.
- Essential oils and alcoholic extracts demonstrated significant inhibitory activities at concentrations greater than 2.5 µg/mL ($p < 0.001$) and greater than 0.25 mg/mL ($p < 0.001$), respectively.
- These results suggest that extracts and essential oils from *A. foeniculum* may have multiple biological activities due to their chemical composition.
- Two isoflavones, genistein, and daidzein, present in the analyzed plants were analyzed from a structural and chemical potential point of view, through molecular modeling, the basis of this modeling is the relationship between the biological activity and the physicochemical parameters that define the molecular structure of the compounds chemicals. The compounds were optimized thus obtaining the equilibrium geometries, boundary OM, ESP, and a series of electronic parameters.

- The analysis of the electronic parameters showed that both genistein and daidzein have medium chemical potential.
- Molecular electrostatic potential maps identified for the compound genistein the existence of five active sites for electrophilic attack, in contrast to the daidzein compound where only four active sites for electrophilic attack were noted.
- From the analysis of oral bioavailability parameters, it was found that both investigated compounds obey Lipinski's extended rules and have a bioavailability score of 0.55, thus demonstrating good oral bioavailability.
- Verification of the inhibition potential of genistein and daidzein on the receptors, PI3Kα and XO, was done by molecular docking and using standard ligands with known inhibitory activity. It was found that the investigated ligands have medium inhibition potential (in the order of μM) both on PI3Kα kinase and on xanthine oxidase, a fact confirmed by the experimental data. So, genistein and daidzein may be potential drugs (adjuvants) for treating cancer and gout, further investigation is needed for compliance and future applications.

7.2. Prospects for further research

The experimental results obtained during the doctoral studies lead us to want to deepen the research started, the research perspectives being able to focus on activities such as:

- ✓ Evaluation of the antimicrobial and antiviral activity of the obtained essential oils;
- ✓ Evaluation of the antitumor activity on other cell lines and in vivo of the extracts and essential oils from the studied plants,
- ✓ Continuing research on the synergistic action of the categories of bioactive compounds through in vitro and in vivo tests;
- ✓ Continuation of in silico studies to confirm the activities analyzed in vitro by researching the mechanisms of action of the majority of compounds identified in the studied plants;
- ✓ Encapsulation of essential oils obtained from the species of aromatic plants studied to obtain cosmetic, food, and pharmaceutical products;
- ✓ Formulation of pharmaceutical preparations with the extracts and oils obtained from the studied plants;
- ✓ Continuing research with interdisciplinary approaches to carry out clinical studies, to identify the benefits on the human body;
- ✓ Other in silico studies of the compounds identified in the plants analyzed with biomolecules of interest;
- ✓ Biosynthesis of Au, Ag, Cu, Pt, and Sn nanoparticles and obtaining thin films with antimicrobial properties with possible applications in various fields such as pharmaceutical, medical, or food.

Chapter 8. Dissemination of results

8.1. List of original publications (Cumulative impact factor =14,479)

1. **Bălănescu, Fănică,** Andreea Veronica Botezatu, Fernanda Marques, Anna Busuioc, Olivian Marincaş, Costel Vînătoru, Geta Cârâc, Bianca Furdui, and Rodica Mihaela Dinica. ”Bridging the Chemical Profile and Biological Activities of a New Variety of *Agastache foeniculum* (Pursh) Kuntze Extracts and Essential Oil”, *Int. J. Mol. Sci.* 2023, *24*, 828, doi:10.3390/ijms24010828, F.I.=6,208.

2. **Balanescu, Fanica,** Anna Cazanevscaia Busuioc, Andreea Veronica Dediu Botezatu, Steluta Gosav, Sorin Marius Avramescu, Bianca Furdui, and Rodica Mihaela Dinica. ”Comparative Study of Natural Antioxidants from Glycine Max, Anethum Graveolens and *Pimpinella anisum* Seed and Sprout Extracts Obtained by Ultrasound-Assisted Extraction”, *Separations* 2022, *9*, 152, doi:10.3390/separations9060152, F.I.=3,344.

3. **Balanescu, Fanica,** Maria Daniela Ionica Mihaila, Geta Cârâc, Bianca Furdui, Costel Vînătoru, Sorin Marius Avramescu, Elena Lacramioara Lisa, Mihaela Cudalbeanu, and Rodica Mihaela Dinica. ”Flavonoid Profiles of Two New Approved Romanian Ocimum Hybrids”, *Molecules* 2020, *25*, 4573, doi:10.3390/molecules25194573, F.I.=4,927.

ISI works published in collaboration during doctoral studies (Cumulative impact factor = 8,39)

1. Botezatu (Dediu) Andreea Veronica; Horincar Georgiana; Ghinea Ioana Otilia; Furdui Bianca; Bahrim Gabriela-Elena; Barbu Vasilica; **Balanescu Fanica**; Favier Lidia, Dinica Rodica-Mihaela- *Whole-Cells of Yarrowia lipolytica Applied in ”One Pot” Indolizine Biosynthesis*, Catalysts, 2020, Volume 10, Issue 6, Article Number 629, DOI 10.3390/catal10060629, WOS:000551908500001, F.I. 4,501.
2. Dinica Rodica-Mihaela, Sandu Cristina, Botezatu Dediu Andreea Veronica, Busuioc Anna Cazanevscaia, **Balanescu Fanica**, Ionica Mihaila Maria Daniela, Dumitru Caterina Nela, Furdui Bianca, Iancu Alina Viorica- *Allantoin from Valiable Romanian Animal and Plant Sources with Promising Anti-inflammatory Activity as a Nutricosmetic Ingredient*, Sustainability, 2021, Volume 13, Issue 18, Article Number 10170, DOI 10.3390/su131810170, F.I. 3,889.

8.2. Participation in international and national conferences

- International Conference from Constanța -CHEMISTRY 2018 - "New Trends in Applied Chemistry", 2018;
- Scientific Conference of Doctoral Schools SCDS-UDJG 2018, 6th Edition 2018, GALAțI;
- IasiCHEM 2018 Conference, Chemistry Faculty Days "Alexandru Ioan Cuza University" Iași, 2018;
- Scientific Conference of Doctoral Schools SCDS-UDJG 2018, 7th Edition 2019, GALAțI;
- IUPAC International Chemistry Congress, Paris, 2019;
- Natural Products in Drug Discovery and Human Health, Lisbon, 2019;
- SCDS-UDJG Doctoral Schools Scientific Conference 2018, 8th Edition 2020, GALAțI;
- Scientific Conference of Doctoral Schools SCDS-UDJG 2018, 9th Edition 2021, GALAțI;
- Scientific Conference of Doctoral Schools SCDS-UDJG 2018, 10th Edition 2022, GALAȚI;
- National Chemistry Conference XXXVI edition, Călimănești-Căciulata, 2022.