



Review

Antioxidant Potential and Its Changes Caused by Various Factors in Lesser-Known Medicinal and Aromatic Plants

Sona Skrovankova * and Jiri Mlcek

Department of Food Analysis and Chemistry, Faculty of Technology, Tomas Bata University in Zlin, Vavreckova 5669, 76001 Zlin, Czech Republic; mlcek@utb.cz

* Correspondence: skrovankova@utb.cz; Tel.: +420-576031524

Abstract: The review focuses on the evaluation of antioxidant potential and its changes by various factors such as growing conditions, the use of fertilizers, the analyzed part of the plant, the solvent used, the extraction method, purifying procedures, and the determination method for selected medicinal and aromatic plants that are lesser-known as antioxidant sources. The lesser-known representatives of Lamiaceae family (*Lamium album*, *Leonurus cardiaca*, *Hyssopus officinalis*, *Scutellaria baicalensis*), Asteraceae family (*Artemisia absinthium*), Myrtaceae family (*Pimenta dioica*), and Rosaceae family (*Crataegus laevigata*) were selected. The most important factors affecting antioxidant potential are the used solvent and its polarity (water and its temperature, ethanol, mixture of these solvents, methanol, n-butanol, and ethylacetate), extraction techniques, essential oil preparation, and the type and conditions of antioxidant activity (AA) determination method (DPPH, ABTS, FRAP, etc.). The plant composition and the occurrence of biologically active compounds (BACs), such as phenolics (phenolic acids and flavonoids) that participate in their biological impacts and deactivate reactive oxygen species, are also described. This work thus provides a summary of this issue and an extension of information focused on factors that affect plant components' presence and thus have an impact on the overall antioxidant potential (total polyphenol content TPC, antioxidant activity) of lesser-known plant representatives with antioxidant effect.



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1. Introduction

Medicinal and aromatic plants, and herbs have been used in folk medicine and as foods for thousands of years due to their pharmacologic and therapeutic action and nutritional effect too. According to the World Health Organization (WHO), herbal medicines include “herbs, herbal materials, herbal preparations, and finished herbal products, that contain as active ingredients parts of plants, or other plant materials, or combinations” [1]. Whole plants or their parts, such as leaves, flowers, seeds, roots, or rhizomes, can be used in the fresh and dried condition, or extracted from fresh or dried form (juices, teas, decoctions, ethanolic and other alcoholic extract, tinctures, powdered plant material, essential oils, and resins).

Even nowadays much of the human population depends on traditional medicine for their health care needs, as WHO reports [2].

The medicinal and aromatic plants and herbs are important due to their composition structure, and biologically active substances present, mainly phenolic compounds and

alkaloids, which may be used as agents in the synthesis of drugs. Besides medical purposes, they are valued in the human diet because of the presence of dietary antioxidants such as phenolics, vitamins (vitamin C (L-ascorbic acid), tocopherols (vitamin E)), and carotenoids (β -carotene, etc.). Their use in the food industry and for culinary purposes, due to their flavoring properties in various meals and foods, is also worth mentioning. The usage is common also in cosmetics due to their preservative effect of antimicrobial constituents or efficient components of essential oils. The plants which are utilized for the production of cosmetic ingredients (antioxidant, anti-wrinkling, and anti-aging effects, UV protection, moisturizing, and smoothing activities) belong mainly to the families of Asteraceae, Lamiaceae, Fabaceae, Poaceae, Malvaceae, and Rosaceae [3].

Particularly important and known plants with high antioxidant potential are representatives of some plant families, mostly the Lamiaceae family (*Ocimum basilicum* L., *Origanum majorana* L., *Origanum vulgare* L., *Melissa officinalis* L., *Mentha × piperita* L., *Rosmarinus officinalis* L., *Salvia officinalis* L., *Satureja hortensis* L., *Thymus vulgaris* L.) that have been investigated by many researchers in a great extent [4–8]. Also, antioxidants of Apiceae family plants (*Foeniculum vulgare* Mill., *Angelica archangelica* L., *Carum carvi* L., *Coriandrum sativum* L., *Cuminum cyminum* L., *Pimpinella anisum* L.) [9–14], and Rosaceae family (*Alchemilla vulgaris* L.) [15,16] were studied widely.

Lipid oxidation may be one of the causes of several chronic diseases including cardiovascular diseases, cancer, and age-related diseases. The potential role of antioxidant compounds in the prevention of these and other chronic diseases has been investigated. Hydroxyl radicals, as products of lipid oxidation, can cause DNA damage and thus be involved in some diseases such as cancer, cardiovascular diseases, and other oxidative stress-induced diseases. The protective effect of flavonoids and other phenolic compounds on these illnesses through the mechanisms for the inhibition of proliferation, inflammation, and metastasis is also studied [17,18].

According to several epidemiological studies [19,20], the intake of dietary antioxidants, such as polyphenols, anthocyanins, quercetin, and rutin, could reduce the risk of cardiovascular diseases (coronary heart disease, heart failure, hypertensive heart disease, and stroke) with their high prevalence and mortality. The mechanism of their effects lies primarily in reducing blood pressure, improving the lipid profile, alleviating oxidative stress, and reducing inflammation [21]. Also, some in vivo studies focused on these substances in herbs such as *Ocimum sanctum* [22] and *Scutellaria baicalensis* [23] confirmed these findings.

The general recommendation of many studies for consumers is to increase the consumption of foods containing antioxidants. Mainly vegetables and fruits, drinks like tea or wine are recommended. Although the consumption of medicinal and aromatic plants, herbs, and their products and preparations, is much lower than the above-mentioned plant products, medicinal and aromatic plants could also contribute to an overall higher intake of dietary antioxidants [24]. Although it is assumed that frequent consumption of all antioxidant representatives could improve human health, the relevance of their intake is not fully proven due to the lower number of in vivo studies. Therefore, the effects of dietary antioxidants in vivo should be studied more intensively to know their real physiological effects on humans [25].

In recent years, much research has been carried out to find active compounds and their mixtures present in different types of plant material, medicinal and aromatic plants, and foods, that are effective against oxidation. Herbs contain high levels of antioxidants that can delay or inhibit the oxidation of lipids (or other present sensitive molecules). Many products of (lipid) oxidation are known to interact with biological materials to cause cellular damage as oxidation processes were found to be linked with human aging and the progression of several diseases, degenerative processes in the human body, and

mutagenesis. It is assumed that present antioxidants may reduce the aging process, the progression of several diseases, and prolong human life [26].

The presence and the content of many herbal components such as phenolic acids and flavonoids, alkaloids, anthocyanins, coumarins, saponins, and tannins, contribute to antioxidant and other protective properties of plants. They may act independently, or in their combination when synergism or antagonism can be seen. Usually, a stronger effect of multiple folders is customary. However, the *in vitro* conditions of common research determination are often different from the results of *in vivo* experiments [27–29].

Extracts, infusions, and essential oils of these plants are considered the most desirable and effective products. The most significant antioxidant representatives in the plants belong to phenolic compounds (phenolic acids and flavonoids) that contain an –OH group(s) on a benzene ring. Phenolic acids, such as caffeic acid and its esters and ferulic acid, together with flavonoids (flavones, flavonols, isoflavones, flavanones, flavan-3-ols, dihydroflavonols (flavanonols or catechins), and anthocyanidins) are the most important groups of these compounds. The known and abundant constituents of flavones include apigenin and luteolin; flavonols kaempferol, quercetin, rutin, and myricetin; flavanones naringenin, naringin, hesperidin, and hesperitin; to flavan-3-ols epicatechin, and anthocyanidins cyanidin delphinidin, malvidin, pelargonidin and peonidin [28,29].

Phenolic compounds have the ability to quickly extinguish all types of radicals, especially peroxy radicals (ROO•). The antioxidant reaction of these highly reactive radicals with phenolics is shown in Figure 1.

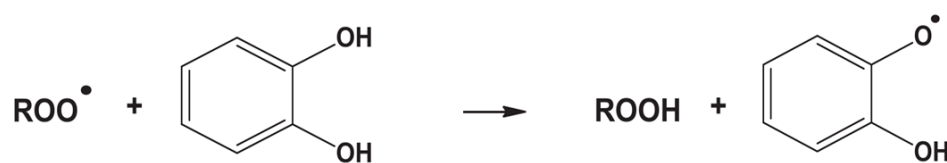


Figure 1. The antioxidant reaction of peroxy radical (ROO•) and phenolic compound.

The antioxidant action and the chemical composition of specific plants and herbs are partly variable, depending on the exact plant cultivar, genotype, and variety, and growing conditions such as environmental factors, location, precipitation, plant nutrition, cultivation techniques (conventional, organic), stage of ripening, and the harvest time. Storage conditions and processing techniques are also significant for the presence and content of each individual component and their quality [30].

For this review, there were selected from several families of medicinal and aromatic plants that are lesser-known as antioxidant sources. There were chosen the representatives of the Lamiaceae family *Lamium album*, *Leonurus cardiaca*, *Hyssopus officinalis*, *Scutellaria baicalensis*, the representative of Rosaceae family *Crataegus laevigata*, the representative of Asteraceae family *Artemisia absinthium*, and the representative of Myrtaceae family *Pimenta dioica*.

2. Methods Used to Evaluate the Antioxidant Potential of Plants

An exact quantity of individual phenolic compounds, which are presented in the plants, is often measured by chromatographic methods such as HPLC. However, these results will not give insight into the overall antioxidant potential and the antioxidant strength of plant extract. For this information, it is more appropriate to use a special method with spectral measurement such as the Folin–Ciocalteu (FC) method.

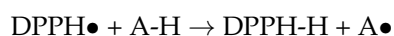
FC method is based on the chemical reduction of the FC reagent (a mixture of tungsten and molybdenum oxides) by the phenolic compounds present. It measures phenolic hydroxyl groups in the plant extract. The products of the reduction of the metal oxides are blue in color with a broad absorption band, with a maximum at 765 nm. The maximum

absorption of chromophores depends on the concentration of phenolic compounds and the alkaline solution. The intensity of the color is proportional to the concentration of the phenolic compounds (TPC—total phenolic content). This method, based on the oxidation of phenolic compounds by the FC reagent, is rapid, easy to perform, and low-cost. The reaction is suitable for several groups of phenolic compounds as many compounds change colors differently due to differences in reaction kinetics [31]. It is necessary to use a reference substance such as gallic acid, tannic acid, catechin, or pyrogallol. Gallic acid is the most often used standard. The result of measured absorbances is recalculated through the calibration curve with different gallic acid concentrations and expressed as gallic acid equivalents (GAE).

The antioxidant potential of medicinal and aromatic plants depends not only on individual phenolic compounds, effective oxygen radical scavengers, and their overall content but also on total antioxidant activity, measured by several methods. The most commonly used methods are DPPH (1,1-diphenyl-2-picrylhydrazyl radical), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid radical (ABTS⁺), oxygen radical absorbance capacity (ORAC) test, ferric (Fe³⁺) and cupric (Cu²⁺) ions reduction assays, and total radical-trapping antioxidant parameter (TRAP) method. Most of these methods use similar principles and techniques, based on a suitable standard spectrophotometer measurement. A newly developed method, enzymatic assay HAPX (Hemoglobin/Ascorbate Peroxidase Activity Inhibition) assay measures the capability of the extract components to quench the damage inflicted by hydrogen peroxide upon hemoglobin. That brings additional information as it involves the interaction of the antioxidants with a protein, i.e., the physiological-relevant ferryl hemoglobin species [32].

Antioxidant effect should not be tested with a single method as it may not reflect antioxidant activity and, usually, it is quite problematic to compare the results of the methods with each other.

The DPPH method is a free radical scavenging assay that belongs to the most used tests. Its reagent solution has a violet color that disappears when the testing solution is mixed with a solution (extract) containing a substance or a mixture that can donate a hydrogen atom. The reduced form of the DPPH radical (DPPH•), hydrazine (DPPH-H), is formed and the color of the solution changes to yellow as a result of radical reduction by hydrogen atom of antioxidants (A-H) [33].



The absorbance drop is measured by UV-VIS spectroscopy, usually at 517 nm. To evaluate the results of the DPPH test, Trolox, as a standard radical scavenger compound, is used. It is a water-soluble analog of vitamin E (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). The calibration curve with Trolox is prepared and the results are recalculated as TE (Trolox equivalents). To determine the antioxidant concentration that scavenges 50% of the initial DPPH radical concentration IC₅₀ (inhibitory concentration) is used. It indicates the practicality of antioxidant testing using DPPH radicals. The lower the IC₅₀ values, the higher the DPPH radical-removing ability of the antioxidants. This method is used to determine the antioxidant activity of antioxidant molecules in herbal extracts [33,34].

The ABTS test is based on the same principle (free radical scavenging) as the DPPH assay. It measures the neutralization of 2,2'-azino-bis(3-ethylbenzthiazolin-6-sulfonic acid) (ABTS⁺) stable radical cation by antioxidant compound/mixture. The radical has a blue-green color that disappears in the presence of antioxidants. The discoloration of the ABTS radical in the presence of powerful antioxidant agents is measured by absorbance at 734 nm [35].

The ORAC test describes the antioxidants' ability to yield the hydrogen atom. It measures the splitting ability of the radical chain reaction by antioxidants by monitoring the inhibition of the oxidation of the peroxy radicals (free radicals). Peroxy radical, emitted by a generator, reacts with a fluorescent sample that leads to loss of fluorescence, measured by a fluorimeter. As a reference compound, similarly, for DPPH and ABTS tests, Trolox is used. The results are then described as Trolox equivalent (TE) [35].

The FRAP (Ferric Reducing Antioxidant Power) test is the method measuring the reduction of the complex of ferric ions (Fe^{3+}) to the blue ferrous complex (Fe^{2+}) due to the action of the present antioxidants, in acidic conditions (pH 3.6) to maintain iron solubility. Tripyridyltriazine (TPTZ), as the linking ligand to the iron ion, is often used. AA is determined as an increase of absorbance value at 593 nm. The results of AA are then expressed as micromolar equivalents of Fe^{2+} [35].

These spectrometric methods are the most used, at low cost, that can together give comprehensive insight into antioxidant power.

3. The Lesser-Known Plant Representatives with Antioxidant Effect of Lamiaceae Family

The representatives of the Lamiaceae family, formerly called Labiate, are traditional plants of European states and also other countries, western (North and South America) and eastern (Asian countries) ones too. The family includes subfamilies such as Aju-goideae, Chloranthoideae, Lamioideae, Nepetoideae, Pogostemonoideae, Scutellarioideae, Teucroideae, and Viticoideae. Nearly half of the representatives of the Lamiaceae family belong to the subfamily Nepetoideae. Lamiaceae includes more than 7000 species [36]. The family is an abundant source of antioxidants such as phenolic acids and flavonoids that are considered the most important groups of compounds with many biological functions in preventing health problems [26]. Representatives of the Lamiaceae family, which were chosen for this review are *Hyssopus officinalis*, *Lamium album*, *Leonurus cardiaca*, and *Scutellaria baicalensis*.

3.1. *Hyssopus officinalis*, Its BACs and Antioxidant Potential

The genus *Hyssopus* includes several known and lesser-known plant species such as, *H. ambiguus*, *H. cuspidatus*, *H. latilabiatum*, *H. macranthus*, *H. officinalis*, *H. seravschanicus*, and *H. subulifolius*, of which *Hyssopus officinalis* is one of the most common and utilized. *Hyssopus officinalis* L. (hyssop) is a perennial herb with a minty and slightly bitter flavor that is used as a spice in the gastronomy and food industry [37], the cosmetics industry, and in traditional medicine.

The hyssop herb and its preparations (infusions, extracts, and tinctures) are known also due to their carminative, tonic, antiseptic, diaphoretic, and stomachic effects. It is utilized as an expectorant, muscle relaxant, and cough reliever [38]. The experimental evidence also points to the sedative, anxiolytic [39], anti-ulcer [40], and anti-asthmatic impact [41]. *Hyssopus officinalis* may be considered also as a potential plant source with moderate antimicrobial properties. An antimicrobial activity was detected against *Staphylococcus aureus* and *Candida albicans* by Vlase et al. [32].

One of the factors that affect the antioxidant potential of plants is their concrete product. Extracts are often used as great sources of effective antioxidants. However, essential oils are also receiving a lot of attention due to their antioxidant properties. Essential oil is considered to be the most eminent and most frequently examined hyssop's product, containing very important BACs (biologically active compounds), as seen in Table 1.

The herb yields 0.3–1% essential oil from the cultivated or wild herb. As the dominant compound monoterpenoid *cis*-pinocamphone (48.98–50.77%) [42] was detected. Other

important substances are β -pinene (13.38–13.54%), *trans*-pinocamphone (5.78–5.94%), and β -phellandrene (4.44–5.17%). As the major group of BACs, there were oxygenated monoterpenes (61.69% of the total oil content) identified, followed by monoterpene hydrocarbons, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, phenylpropanoids, and other compounds. Also, Hajdari et al. [43] identified *cis*-pinocamphone, with a content of 30.44% to 57.73%, as the one of main compounds of the essential oil of wild-growing hyssop (*H. officinalis* subsp. *aristatus*) from five different localities in Kosovo. They also stated 1,8-cineole and *trans*-pinocamphone as significant constituents.

Fathiazad et al. [44] identified 20 main compounds representing the most important substances of hyssop essential oil. Myrtenylacetate was detected as the main compound. Other important constituents are camphor, germacrene, and spathulenol. Mićović et al. [45] found out that hyssop essential oil is rich in monoterpene hydrocarbons such as limonene (8.0–23.8%), β -pinene; oxygenated monoterpenes (1,8-cineole, 38.2–67.1%); and phenylpropanoids (methyl eugenol, up to 28.3%). The dominant and most abundant groups of hyssop volatiles were monoterpenes and their oxygenated derivatives of which 1,8-cineole and *cis*-pinocamphone were defined with the highest contents.

Fertilizers could be another important factor affecting the composition of plant essential oils or extracts, which can improve plant quality. Iranian researchers [44] reported that the most suitable procedure for obtaining the highest efficiency of antioxidant properties was the use of medium and high levels of cattle manure treatment compared to the control, and compared to poultry or sheep manure. The yield of essential oils was also increased by the use of cattle manure (by 42.5%, 61.6%, and 47.7% at low, medium, and high levels of treatment, respectively).

As was mentioned above, plant extracts contain very effective antioxidants. Hyssop herb extract contains very important BACs (Table 1) such as flavonoids. The major flavonoid apigenin 7-*O*- β -D-glucuronide was stated by Fathiazad et al. [44]. Other flavonoids such as quercetin 3-*O*-glucoside (isoquercitrin) and diosmetin 7-*O*-rutinoside (diosmin), flavonoid glycosides such as rutin, and free flavonoid aglycons as luteolin, in different concentrations, were also detected in extracts of hyssop herb. Phenolic acids, tannins, diterpene lactones such as marrubiin, and triterpenoid compounds (ursolic and oleanolic acids) were identified in this plant too [32]. Using chromatographic analysis of methanolic extracts of hyssop flowering aerial parts, originating from Montenegro, there were determined phenolic compounds, specifically benzoic acid derivative (syringic acid), hydroxycinnamic acid derivatives (chlorogenic (5-*O*-caffeoylquinic acid), feruloylquinic and rosmarinic acids, as well as caffeoyl pentoside) and flavonoids, derivatives of quercetin and diosmetin [46]. In ethanolic extracts of Italian hyssop aerial parts, similar compounds such as 5-*O*-caffeoylquinic and feruloylquinic acids, isoquercitrin and isorhamnetin, as well as rutin were identified [47].

Table 1. Phenolic profile and other significant components and different factors of selected Lamiaceae plants.

Plant	Part of Plant	Extract/Solvent	Phenolic Profile and Other Significant Components	Origin	Ref.
<i>Hyssopus officinalis</i>		essential oil	<i>cis</i> -pinocamphone, β -pinene, <i>trans</i> -pinocamphone, β -phellandrene	Bulgaria	[42]
		essential oil	<i>cis</i> -pinocamphone, 1,8-cineole, <i>trans</i> -pinocamphone, β -pinene, caryophyllene oxide	Kosovo	[43]
		essential oil	myrtenyl acetate, camphor, germacrene, spathulenol, β -caryophyllene, <i>cis</i> -sabinol, β -bourbonene, bornyl acetate	Iran	[44]
		essential oil	limonene, β -pinene, Z- β -ocimene, α -pinene, sabinene, 1,8-cineole, methyl eugenol, <i>cis</i> -pinocamphone	Montenegro	[45]

Table 1. Cont.

Plant	Part of Plant	Extract/Solvent	Phenolic Profile and Other Significant Components	Origin	Ref.
<i>Hyssopus officinalis</i>	aerial parts	hydromethanolic, hydroethanolic extracts	apigenin 7-O- β -D-glucuronide, isoquercitrin, diosmin, rutin, isoquercitrin, quercitrin, luteolin, quercetin	Iran	[44]
	aerial parts	ethanolic extracts	caftaric acid, gentisic acid, caffeic acid, <i>p</i> -coumaric acid, chlorogenic acid, ferulic acid, rutin, isoquercitrin, quercitrin, luteolin, quercetin	Romania	[32]
	flowering aerial parts	methanol extracts	syringic acid, chlorogenic acid, rosmarinic acid, feruloylquinic acid, caffeoyl pentoside, quercetin O-hexoside, diosmetin O-deoxyhexosyl-hexoside	Montenegro	[45]
	aerial parts	ethanol extracts	5-O-caffeoylquinic acid, feruloylquinic acid, isoquercitrin, isorhamnetin, rutin, 3-O-caffeoyl quinic acid, caffeoyl methylquinic acid, quercetin-3-O-hexoside, dicaffeoylquinic acid	Italy	[44]
	aerial parts	ethanol extracts	chlorogenic acid, caffeic acid, rutin	Moldova	[48]
<i>Lamium album</i>	stems, leaves, flowers	ethanol extracts	verbascoside, isoverbascoside, isoscutellarein-7-O-allosyl(1 \rightarrow 2)glucoside, isoscutellarein-O-allosyl glucoside, O-methylisoscutellarein-7-O-allosyl(1 \rightarrow 2)glucoside, luteolin-7-O-glucoside, apigenin-7-O-glucoside, apigenin-7-O-rutinoside, naringenin-7-O-rutinoside	Portugal	[49]
	flowers	methanol extracts	caffeic acid, chlorogenic acid, ferulic acid, gallic acid, <i>p</i> -coumaric acid, protocatechuic acid, syringic acid, gentisic acid, vanillic acid, rutoside, quercetin, isoquercetin	Poland	[50]
	flowers	methanol extracts	chrysin, pinostrobin, myricetin, <i>trans</i> -3-hydroxycinnamic acid, quercetin, rutin, galangin, apigenin, syringic acid, vanillic acid	Poland	[51]
		essential oil	thymol, germacrene D, borneol, <i>trans</i> -caryophyllene, γ -guaiaiolacetate, phytol, β -phellandrene.	Iran	[52]
<i>Leonurus cardiaca</i>		essential oil	epicedrol, α -humulene, dehydro-1,8-cineole, germacrene D, spathulenol	Iran	[53]
	aerial parts	ethanol extracts	quercetin, rutin, caffeic acid, chlorogenic acid, verbascoside, lavandulifolioside, ursolic acid, caffeoylmalic, <i>trans</i> -ferulic acid, leonurine, harpagide	Poland	[54]
	aerial parts		ferulic acid, chlorogenic acid, caffeic acid, cichoric acid, rutoside, lavandulifolioside, verbascoside, isoquercitrin, stachydrine	Germany	[55]
	aerial parts	ethanolic extracts	lavandulifolioside, verbascoside, leucoseptoside A, leonoside B, quercetin-3-O-glucoside, rutin, quercetin-3-O-sophoroside	Portugal	[56]
	officinal motherwort tincture	dry extract	chlorogenic and caffeic acids, ellagic acid, catechin, hyperoside, rutin	Ukraine	[57]

Antioxidant Potential of *Hyssopus officinalis*

For the antioxidant properties of herbs, such as hyssop, several groups of antioxidant effective compounds are responsible. Phenolic compounds (phenolic acids and flavonoids) are the most important and abundant. Also, antioxidant vitamins (L-ascorbic acid and tocopherols) and some carotenoids (hydrocarbons carotenes, such as α -carotene, and especially β -carotene; and xanthophylls containing oxygen atoms, such as β -cryptoxanthin, and especially lutein, and zeaxanthin) could be significant antioxidants for some herbs.

To determine the antioxidant potential of a plant, such as hyssop, it is proper to analyze the plant by the selected method simultaneously together with other plants, so they might be compared with each other under the same assay conditions. Thus, it can give a real insight into the antioxidant strength in the context of comparison with other plants.

By comparing the amount of polyphenols (TPC, caffeic acid derivatives content) in several plants from Romania (*Hyssopus officinalis*, *Teucrium chamaedrys*, *Ocimum basilicum*) [32], it was found that hyssop herb was not the most effective, *T. chamaedrys* had better antioxidant values. However, the higher consumed amount of hyssop could increase its importance as a dietary antioxidant. The exact measured caffeic acid derivatives concentration for hyssop was 9.25 mg GAE/g compared to a better value of 12.51 mg GAE/g for *T. chamaedrys* and basil (*Ocimum basilicum*) with mean antioxidant parameters. The highest

antioxidant activity of ethanol extracts prepared from these plants (hyssop, basil, and wall germander) by the DPPH radical bleaching method was determined again (similarly as for TPC) for *T. chamaedrys*, with the lowest IC50 concentration and, therefore highest AA. Lower AA was stated for the basil and hyssop extracts with similar IC50 values without statistically significant differences between them in terms of radical scavenging activity. For the comparison of AA results another method (TEAC) was also used. The highest AA was again (similar to DPPH results) stated for wall germander extract, lower antioxidant values were determined for hyssop extract (around 2/3 of the value of wall germander extract AA), and the lowest for basil extract (lower than 1/3 of the highest AA value). These results are in agreement with the DPPH values. The results of enzymatic assay HAPX for these samples, as the authors found out, were negatively correlated with DPPH and TEAC methods. The statistical results showed no significant difference between the three analyzed extracts in terms of AA [32].

Antioxidant potential of methanolic extracts prepared from eight aromatic and medicinal plants (*Hyssopus officinalis*, *Angelica panicii*, *Angelica sylvestris*, *Laserpitium latifolium*, *Achillea grandifolia*, *Achillea crithmifolia*, *Artemisia absinthium*, and *Tanacetum parthenium*), originated in southeast Serbia, was analyzed by the ABTS and DPPH (by Inactivation, I) methods, and TRP (Total reducing power assay) by the iron (III) to iron (II) reduction assay, and the ferric reducing antioxidant power assay (FRAP) [58]. Results of antioxidant activities from all methods demonstrated a similar sequence of activity: *A. crithmifolia* > *A. grandifolia* > *H. officinalis* > *A. absinthium* > *T. parthenium* > *L. latifolium* > *A. panicii* > *A. sylvestris*. The representatives of the Asteraceae family (*A. crithmifolia*, *A. grandifolia*, *A. absinthium*) thus showed the highest antioxidant properties; hyssop demonstrated moderate to high AA. The total content of polyphenols and flavonoids in the methanol extracts of the studied species positively correlated with their antioxidant properties, confirming their major role in the antioxidant activity of these species [58].

Significant factors influencing the amount of presented phenolic compounds and AA include the part of the plant used to prepare the extract (Table 2). The content of phenolics (TPC) in different parts of the plant, flowers, leaves, and stems of *H. officinalis* L. var. *angustifolius*, measured by the FC method, in ethanol extracts determined Alinezhad et al. [59]. There were evaluated slightly higher values for plant stems than for flowers and leaves. Values of AA of flowers, leaves, and stem extracts demonstrated good antioxidant activity of this plant. However, stem extract showed better DPPH radical scavenging activity in IC50 values (the lowest IC50 therefore the highest AA) than what was discovered for flowers or leaves.

In the flowering aerial parts of *H. officinalis* subsp. *aristatus* (Godr.) Nyman, in prepared methanolic extracts, determined by Mićović et al. [45] and originated from Montenegro, the most abundant compounds chlorogenic and rosmarinic acids were identified. The content of chlorogenic acid was much higher, in the range between 23.35 and 33.46 mg/g than the amount of rosmarinic acid (3.53–17.98 mg/g). TPC for hyssop samples was in a wider range of values that could be given due to various factors mentioned earlier in the review. For six hyssop methanolic extracts was evaluated antioxidant activity was less effective in DPPH radical scavenging with moderate antioxidant efficacy (IC50 < 100 µg/mL) for analyzed samples. They also expressed moderate to weak antioxidant activity measured by FRAP (0.667–0.959 mmol Fe²⁺/g). Compared to standard substances such as rutin (4.11 mmol Fe²⁺/g) and ascorbic acid (8.18 mmol Fe²⁺/g), analyzed hyssop extracts were less effective [45].

Pink, white, and blue flowers of hyssop herb from the Republic of Moldova, prepared as ethanol extracts, were analyzed by Benea et al. [48]. The chlorogenic and caffeic acids were identified in all three evaluated genotypes of hyssop herba. The highest total content of

hydroxycinnamic acids was determined in white flower plant extracts and dried ethanolic extracts (1.48 mg/g; 3.01 mg/g of caffeic acid, respectively), followed by hyssop with pink flowers (1.19 mg/g; 2.91 mg/g) and blue flowers (1.01 mg/g; 2.85 mg/g). The highest polyphenol content they detected was in the extract of *H. officinalis* L. with pink flowers (Table 2).

Used solvent for plant extraction affects the isolated antioxidants and their content in the final extract too. Comparing the values of the polyphenolic content in Iranian hyssop of angustifolius variety [44], higher TPC values measured by the FC method were found in n-butanol extracts than in ethylacetate extracts. These findings are in correlation with the polarity of the solvents used for extraction and the solubility of phenolic compounds in them. Extracts prepared with n-butanol solvent also had better antioxidant activity (lowest IC50 concentration) than ethylacetate extract and in comparison with apigenin 7-O- β -D-glucuronide, purified flavonoid that showed weak radical scavenging activity. Their results showed a direct linear correlation between TPC and AA; therefore, it is supposed that phenolic compounds contribute directly to AA. Also, other antioxidant compounds, phenolics, vitamins, minerals such as Se and Zn, and others, could be responsible for AA, to some extent.

In the studies evaluating the content of total polyphenols and AA, variable results can be observed (Table 2). As mentioned above, several factors, not only regarding genetic and growing conditions but also the methods of preparing samples (exact conditions), solvents used, experimental conditions, and procedures of determination method, can affect the results and they may be inconsistent.

Table 2. Antioxidant potential (TPC and AA) and different factors of selected Lamiaceae plants.

Plant	Part of Plant	Extract/ Solvent	TPC	AA	Origin	Ref.
<i>Hyssopus officinalis</i>	stems, leaves, flowers	ethanolic extracts	stems (374.60 mg GAE/g) > flowers (337.30 mg/g) > leaves (348.0 mg/g)	DPPH: stems (IC50 79.9 μ g/mL) > flowers (148.8 μ g/mL) > leaves (208.2 μ g/mL)	Iran	[59]
	aerial parts	ethanolic extracts	77.72 mg GAE/g	DPPH: IC50 125.44 μ g/mL ABTS: 57.39 μ mol TE/mg HAPX: 16.17%	Romania	[32]
	flowering aerial parts	methanol extracts	64.1–112.0 mg GAE/g	DPPH: IC50 56.04–199.89 μ g/mL FRAP: 0.667–0.959 mmol Fe ²⁺ /g	Montenegro	[45]
	aerial parts with pink, white, and blue flowers	ethanol extracts	blue flowers (12.256 mg GAE/g) > pink flowers (8.114 mg GAE/g) > white flowers (8.012 mg GAE/g),	DPPH: pink flowers (IC50 34.172 mg/mL) > white flowers (34.774 mg/mL) > blue flowers (38.091 mg/mL)	Moldova	[48]
	aerial parts	n-butanol and ethylacetate extracts	n-butanol (246 mg GAE/g) > ethylacetate (51 mg GAE/g)	DPPH: n-butanol (IC50 25 μ g/mL) > ethylacetate (IC50 103 μ g/mL)	Iran	[44]
	aerial parts	methanolic extracts	80 mg GAE/g	DPPH: I (83%) TRP: 56 mg AAE/g FRAP: 0.73 mmol Fe/g	Serbia	[58]
<i>Lamium album</i>	flowering aerial parts, roots	methanolic extracts	aerial parts (242.7 mg GAE/g) > roots (135.0 mg GAE/g)	DPPH: aerial parts (IC50 238.4 μ g/mL) > roots (257.0 μ g/mL) aerial parts chelating activities (IC50 1.13 mg/mL) > roots (1.32 mg/mL)	Iran	[60]
	aerial parts	n-butanol extracts	2.9 mg GAE/mL	DPPH: IC50 19.29 mg/mL	Romania	[61]
	aerial part of in vivo and in vitro	methanolic, ethanolic, and chloroform extracts	21.45–103.12 mg GAE/g	DPPH: IC50 20.62–274.35 μ g/mL ABTS: 0.12–0.65 TEAC	Bulgaria	[62]
	flowers	methanol extracts	234.17–650.17 mg GAE/g	DPPH: IC50 20.62–274.35 μ g/mL ABTS: 0.12–0.65 TEAC	Poland	[51]

Table 2. Cont.

Plant	Part of Plant	Extract/ Solvent	TPC	AA	Origin	Ref.
<i>Leonurus cardiaca</i>	aerial parts	water extracts	44.21 mg GAE/g	DPPH: 3.32 mg TE/g ABTS: 5.12 mg TE/g FRAP: 265.92 µM FeSO ₄ /g	Poland	[63]
	aerial parts	ethanol extracts	500 mg GAE/g	DPPH: IC50 18.3 µg/mL TRP: 94.7 µg/mL	Portugal	[56]
	aerial parts	ethanol extracts	66.90–132.73 mg GAE/g	DPPH: 187–471 mg TE/g ABTS: 176–583 mg TE/g CUPRAC: 328–991 mg TE/g	Poland	[54]
	leaves	ethanol and methanol extracts	17.5–38.1 mg GAE/g	ABTS: 63.5–403.7 µM TE/g	USA	[64]
	aerial parts	chloroform, ethylacetate, n-butanol, and methanolic–aqueous extracts	buthanolic fraction > methanolic–aqueous > ethylacetate > chloroform 4.9–48.37 mg GAE/g	DPPH and ABTS: buthanolic fraction > methanolic–aqueous > ethylacetate > chloroform DPPH: IC50 53.79–1814.35 µg/mL	Iran	[65]

3.2. *Lamium album*, Its BACs and Antioxidant Potential

The genus *Lamium* includes more than 40 species of flowering plants that are a source of phenolic compounds with various biological activities. *Lamium album* L., known as “white dead nettle”. The plant is common in European countries and is widely used in folk medicine in the case of kidney problems as it has diuretic properties. Further, the plant is effective for menstrual problems and has antispasmodic effects in reducing muscle spasms. These impacts are partly attributed to the high amount of phenolics such as phenolic acids and flavonoids, as important antioxidants [61].

Kovalyova et al. [66] referred that a dry extract of white dead nettle herb shows psycho-sedative properties at a dose of 100 mg/kg, and tends to anti-hypoxic effect and reduce anxiety action at a dose of 10 mg/kg. Also, the antibacterial properties of lipophilic and phenolic complexes and dry extract of *L. album* were studied. While white dead nettle dry extract showed weak antibacterial activity, chloroform, and ethylacetate extracts presented a strong antibacterial activity against *S. aureus*. The phenolic complex had the highest activity for *P. aeruginosa* and the lipophilic complex for *C. albicans* [66].

Phenylpropanoids (verbascoside and phlinoside D), as well as iridoids (lamalbid, and shanzhiside methylester), and flavonoids have been proven to be more significant inhibitors of IL-8 secretion than TNF- α . *L. album* may limit non-infectious inflammation, vaginal and cervical inflammation [67].

The plant is also common in gastronomy as a base component of some vegetarian dishes such as “white dead nettle frittata” or “dead nettle soup” [49].

As mentioned above, many plant extracts are characterized by the presence of very effective antioxidants. White dead nettle herb extract contains very important BACs (Table 1) such as verbascoside, in an amount of approximately 50% of the phenolic content of aerial plant parts (flowers, leaves, and stems) of *L. album* ethanolic extracts. Generally, the main components and important biologically active substances of white dead nettle extracts are phenylethanoids and several isoscutellarein derivatives. Other effective compounds of this plant include flavonoids and phenolic acids, terpenes, iridoids, and essential oil [37].

Flowers, as one of the important parts of this plant, could be used for the methanol extract preparation. The presence of several flavonoids in *L. album* flowers was confirmed, of which rutoside, quercetin, and isoquercetin were the most valuable. Tannins were detected in epidermis cell vacuoles, papilla vacuoles, and protoplasts of the glandular trichomes. Phenolic acids were identified in the protoplasts of epidermis cells, the protoplasts, and the cell walls of glandular trichomes. Protocatechuic, vanillic, and caffeic acids were in both forms there, as free acids and as glycosides. Caffeic acid was found also in an esterified form, protocatechuic and ferulic acids were detected as esters [50].

Important factors influencing the presence of plant components include the type of isolation of bioactive substances and the exact conditions. Besides classic extraction, supercritical-CO₂ extraction could be used as an alternative method for extracting these compounds from white dead nettle flowers, as confirmed by Uwineza et al. [51]. Methanol as a co-solvent and three temperatures (40, 50, and 60 °C) were applied, and resulted in the different amounts of individual presented compounds. The highest content of all extract components was evaluated for the temperature of 50 °C. Chrysin, pinostrobin, myricetin, and *trans*-3-hydroxycinnamic acid were identified as the significant constituents, which may be responsible for the high content of polyphenols and high antioxidant activity.

Antioxidant Potential of *Lamium album*

As was mentioned before (Section 3.1), mainly phenolic compounds, together with some other representatives (antioxidant vitamins and carotenoids), belong to the most important and abundant antioxidants of medicinal and aromatic plants.

In Table 2, data related to the antioxidant potential of white dead nettle are presented. Comparing information on some *Lamium* species can give better insight into the antioxidant strength of each plant species. Romanian plants of *L. album* and *L. purpureum* were studied by Bubueanu et al. [61]. The content, of total phenols was explored by the Folin–Ciocalteu method in butanol extracts. Both plants had similar values for total phenolic content; however, *L. purpureum* had a higher value compared to *L. album* extract. The antioxidant effect of butanol extracts of both species was estimated by two methods, DPPH and chemiluminescence. The results showed strong antioxidant activity of both extracts for both methods, in a concentration-dependent manner.

Significant factors influencing the amount of presented phenolic compounds and AA include the part of the plant used to prepare the extract (Table 2). Both flowering aerial parts and roots belong to the often used parts of the white dead nettle plant. The antioxidant potential of these parts was studied by Fathi et al. [60]. They evaluated the total phenolic content (TPC) of aerial and root extracts, prepared with methanol as a solvent. Aerial parts of the plant were detected to be much better sources (almost double the value) of polyphenols than the roots. Similar results were shown for total flavonoid content, where aerial parts had more than twice the flavonoid value (79.8 mg QE/g) compared to roots (30.3 mg QE/g). The method with DPPH was used for the analysis of the scavenging rate of this radical. The aerial organs were a little more antioxidant effective. Reducing power assay with Fe³⁺, as an indicator of electron-donating activity, was also used for the determination of antioxidant action strength. The extracts had shown poor reducing power with insignificant differences among them. Also, weak chelating efficiency, of ferrozine-Fe²⁺ chelating complex evaluation, was detected for both extracts. The flowering aerial parts are confirmed as a slightly better antioxidant source than white dead nettle roots [60].

Relevant factors influencing the presence of plant components include the type and conditions of isolation of bioactive substances. Bulgarian researchers [62] investigated in vitro and in vivo (explants were inoculated on basal MS medium containing sucrose and agar without any supplementation of growth regulators) extracts of *L. album*. Eight combinations of extraction methods and solvents were used: Soxhlet extraction and chloroform extract of in vivo and in vitro plants, methanol extraction of in vivo and in vitro plants; second type of extraction—thermostat extraction at 40 °C and methanol extraction of in vivo and in vitro plants; ethanol extraction of in vivo and in vitro plants. The highest TPC was detected in in vivo methanol extracts, obtained by both thermostat and Soxhlet extractions. In vitro cultivated extracts of *L. album* plants showed approximately a two-fold decrease in total phenols compared to in vivo extracts. Flavonoids, a group of secondary plant phenols, were represented in the white dead nettle samples in the highest concentration in in vivo

methanol extracts, by both extractions (62.05 and 82.11 mg QE/g, respectively), similar to polyphenols. Comparably, in in vivo methanol extracts of both extractions, the highest DPPH scavenging potential was detected. In vitro ethanol and methanol (of both extraction types) extracts showed the weakest antioxidant activity. The results of the ABTS method correlate with the data obtained by DPPH radical scavenging activity determination. The highest ABTS scavenging potential was set in in vivo methanol extracts for both extraction types. Also, ethanol extract of thermostat extraction had similar AA values; other samples reached low values, only a third of the highest [62].

Supercritical-CO₂ extraction with methanol was used by Uwineza et al. [51] as an alternative extraction method for bioactive compounds of white dead nettle flowers, at three temperatures (40, 50, and 60 °C). The extracts showed high TPC while the extract at 50 °C showed the highest TPC, followed by 60 °C, and 40 °C that showed the lowest value. The antioxidant activity of these extracts was determined by three assays: DPPH, ABTS, and FRAP. For the DPPH scavenging method, IC₅₀ values were obtained and the best AA value (lowest IC₅₀) was evaluated for the extract prepared at the temperature of 50 °C, then 60 °C, and the least effective of 40 °C. Similar results of effectivity were determined for ABTS evaluation, with the lowest value again for 40 °C temperature. This trend was stated for the FRAP method too. The values fluctuated within the range of 19.48 to 44.74 µmol TE/g, the lowest value was observed at a temperature of 40 °C. Therefore, the temperature of 50 °C/250 bar was the most efficient for high antioxidant activity values and the best content of various bioactive compounds.

Used solvent for plant extraction affects the isolated antioxidants and their content in the final extract too. Methanol and ethylacetate extracts of white dead nettle plants were evaluated by the free radical DPPH scavenging method. The methanol extract was rich mainly with flavonoids and phenolic acids and was able to reduce the radical by about 29% at the highest applied concentration (225 µg/mL). Ethylacetate extract contained triterpenes, in particular, and did not exhibit DPPH radical scavenging activity [68].

3.3. *Leonurus cardiaca*, Its BACs and Antioxidant Potential

The genus *Leonurus* includes several plant species of which *Leonurus cardiaca*, typical for European regions, is one of the most popular. Except for this species, there are *Leonurus japonicus*, *Leonurus chaituroides*, *Leonurus glaucescens*, or *Leonurus persicus*.

Leonurus cardiaca L., known as motherwort, is a perennial herb favored as a mild cardiac tonic. The plant contains a number of flavonoids and phenolic glycosides with antioxidant and anti-free radical activities important for cardioprotective prevention too. *L. cardiaca* extract could be a useful drug with cardioprotective function as it protects cardiac muscles from the effects of pathogenic processes. It is due to the decreasing of ROS (reactive oxygen species) production in mitochondria by antioxidant effective compounds (chlorogenic acid, quercetin, rutin) [69]. Other known therapeutic applications include the treatment of neurological, gynecological, and digestive disorders as well as thyroid dysfunctions. It is also used in homeopathy [70]. Also, due to the research results it may be beneficial in preventing the development of inflammatory lesions within chronically infected tissues [71].

The essential oil of *L. cardiaca* provides different main components and different amounts of these components within the composition than extracts obtained with polar or non-polar solvents (Table 1). The essential oil obtained from the aerial parts *L. cardiaca* L. subsp. *persicus*, grown in Iran, was analyzed by Mazooji et al. [52]. They identified forty-six compounds of which the major ones were thymol (35.25%), germacrene D (7.62%), and borneol (6.69%). Other constituents were present in the amount under 5%. The most abundant compound group of the oil (48.86%) was oxygenated monoterpenes, fol-

lowed by sesquiterpene hydrocarbons (17.58%). The most amount fraction was a group of monoterpenoid (57.01%). As major constituents of the essential oil from the aerial parts of motherwort herb, detected by Iranian researchers [60], different compounds, specifically epi-cedrol (9.7%), α -humulene (9.2%), dehydro-1,8-cineole (8.9%), germacrene D (8.9%), and spathulenol (8.8%), were identified.

Ethanol is an effective solvent used for the extraction of significant components presented in medicinal plants. It was also used for the isolation of bioactive compounds in *L. cardiaca* aerial parts. Important groups of components in extracts were identified as alkaloids, labdane diterpenes, phenylethanoid glycosides, and iridoids. Antioxidative relevant compounds include flavonoids quercetin and rutin, phenolic acids such as caffeic acid and chlorogenic acid, phenylethanoid glycosides (verbascoside and lavandulifolioside), and ursolic acid. The potent biological activity compounds are harpagide, leonurine, and forskolin, too [54]. Similar compounds quantified in different parts of *L. cardiaca* Kuchta et al. [55]. The presence of ferulic acid, chlorogenic acid, caffeic acid, cichoric acid, rutoside, lavandulifolioside, verbascoside, and isoquercitrin, as well as betains like stachydrine was detected. Stachydrine ((2S)-1,1-dimethylpyrrolidinium-2-carboxylic acid) may be regarded also as an essential active principle which is used in Traditional Chinese medicine (TCM). Stachydrine content [72] in drug samples of *L. cardiaca* aerial parts was determined in the amount from 0.6 to 1.5%, in the fruits of *L. cardiaca* contained an average of 0.2%. *L. cardiaca* hydroethanolic extract, obtained from the aerial parts (leaves and stems) of plants grown under organic cultivation in Portugal, was rich in phenylethanoid glycosides, especially lavandulifolioside and verbascoside (254 and 137.4 $\mu\text{g}/\text{mg}$, respectively), leucoseptoside A and leonoside B were present in much lower amount. From the group of flavonols quercetin-3-O-glucoside, and rutin (24.9 and 15.8 $\mu\text{g}/\text{mg}$, respectively) were determined, much less of quercetin-3-O-sophoroside [56].

The officinal motherwort tincture (Vishpha Pharmaceutical Factory, Ukraine) was analyzed by Koshovyi et al. [57]. The principal hydroxycinnamic acids were defined as chlorogenic and caffeic acids, and the dry extracts had a great amount of ellagic acid. Among flavonoids, catechin, hyperoside, and rutin were confirmed. As the main iridoid glycosides of motherwort herb, the following were identified: ajugol (leonuride), ajugoside, galiridoside, harpagide, and harpagide acetate. 5-caffeoylquinic (chlorogenic) acid, caffeoyl-glucosyl-rhamnosyltyrosol (verbascoside), and verbascoside arabinoside (lavandulifolioside) dominated the dihydroxycinnamic (caffeic) acid derivatives [73]. As main flavonoids of motherwort herb were evaluated rutin, hyperoside, isoquercitrin (quercetin-3-O-glucoside), and apigenin-7-O-glucoside [73].

Antioxidant Potential of *Leonurus cardiaca*

Comparison of values determining antioxidant potential (especially the content of polyphenols and AA) is important for determining the antioxidant power of *L. cardiaca* and other plants (Table 2). The antioxidant potential of four water herb extracts obtained from yarrow (*Achillea millefolium* L.), knotweed (*Polygonum aviculare* L.), horsetail (*Equisetum arvense* L.), and motherwort (*Leonurus cardiaca* L.) was analyzed by Telichowska et al. [63]. They determined that the highest content of phenolic compounds had the horsetail extract followed by motherwort herb (*L. cardiaca*), knotweed, and yarrow herbs contained much less of polyphenols. The highest activity against the DPPH radical was detected for the extract from horsetail and knotweed herbs; the motherwort herb had the least AA value. Also, horsetail extract showed the highest AA measured with the ABTS radical, followed by yarrow and motherwort herbs. These results were confirmed also by FRAP tests whereas the horsetail extract showed 40% higher activity compared to the lowest yarrow herb extract. *L. cardiaca* reached the second highest value.

A comparison of the antioxidant potential of ethanolic extract of *L. cardiaca*, *Mentha aquatica*, and *Lavandula dentata*, obtained from the aerial parts (leaves and stems) of plants grown under organic cultivation in Portugal, was determined by Pereira et al. [56]. The total phenolic content of plant extracts followed the sequence order of *L. cardiaca* > *M. aquatica* > *L. dentata*, with values of 500, 307, and 94 µg/mg. The ability of herbal ethanolic extracts to reduce iron (III) and scavenge DPPH radical was evaluated. The ethanolic extracts of the plants exhibited good antioxidant potential with the order of *M. aquatica* > *L. dentata* > *L. cardiaca*, IC₅₀ values corresponding to about 3–7 times higher than that of ascorbic acid, and about two to three times higher than that of BHA as a standard, respectively [56].

Another factor, the solvent used can also significantly affect the antioxidant potential of *L. cardiaca*. Ethanol extracts and purified extracts, prepared from the aerial parts of *L. cardiaca*, were analyzed for total phenolic and flavonoid content and antioxidant activities by Angeloni et al. [54]. Purified extracts were obtained through different adsorption resins (XAD7-HP and SP207). The highest levels of total phenolic and flavonoid compounds were detected in purified samples; crude extracts contained about half the values of purified extracts. (DPPH, ABTS, FRAP, CUPRAC, and ferrous chelating assays) were applied to these samples too. Antioxidant activity measured by DPPH showed better results for purified extracts (about 2.5 times higher) than in crude extracts. The ABTS test, similar to the DPPH evaluation, pointed out that the scavenging abilities of the extracts were higher in purified extracts (nearly 3 times higher) than in crude extracts. The highest values in other assays (reducing power assays, CUPRAC, and FRAP) were also observed for the purified extracts. The strongest antioxidant extracts in both free radicals and reducing power assays showed also the highest level of total phenolics, as phenolic compounds are the main contributors to the antioxidant properties what was confirmed by correlation analysis (linear correlation between TPC and all assays).

Also, the exact procedure of antioxidant isolation from the sample is an important factor influencing antioxidant potential values. Extracts prepared by different procedures (fresh, sun-dried, 40 °C oven-dried, and 70 °C oven-dried) and two solvents (methanol, ethanol) of motherwort samples, were analyzed by Yi and Wetzstein [64]. They found out that both drying and extraction conditions significantly impacted the TPC and antioxidant activity of motherwort herbal extracts. Sun-dried or 40 °C oven-dried herbs exhibited significantly higher TPC and AA than fresh samples. Motherwort extracts had the highest TPC values after dried at 40 °C. Used 70 °C oven-drying procedure caused significant antioxidant loss. The extraction procedure with fresh tissue and 80% ethanol was much more effective with higher TPC and AA than using 80% methanol. The highest values of AA they detected for samples that were sun-dried, followed by fresh ones and ones after exposure to 70 °C and 40 °C oven-dried herbs that exhibited lower AA [64].

The solvent is also a significant factor that may influence polyphenolic content and antioxidant activity. Four different solvents (chloroform, ethylacetate, n-butanol, and methanolic–aqueous 50:50) of aerial parts of cultivated motherwort were used by Jafari et al. [65] for TPC and AA analysis. Buthanolic fraction showed the highest phenolic content, followed by methanolic–aqueous extraction. Ethylacetate extract reached half the values, and chloroform was the least effective (tenth of the highest value). The highest total flavonoid content was revealed in the methanolic–aqueous fraction. Buthanolic and methanolic–aqueous fractions showed the highest DPPH antioxidant activity. Ethylacetate extraction was much more effective (up to half the best values), and chloroform solvent was at least suitable for antioxidant potential. Similarly, it was for FRAP results. A linear correlation between TPC and AA was confirmed; therefore, polyphenols could be considered as the main antioxidants of *L. cardiaca* extracts.

Another factor that may affect antioxidant potential is the usage of fertilizers. Total phenolics content was significantly increased upon optimum concentrations of employed treatments with 250 mg/L of nZVI (foliar-applied nanoscale zero-valent iron) fertilizer that was the most effective [74]. Among the extracts, ones obtained from plants treated with 250–500 mg/L nZVI revealed strong antioxidant activity by scavenging free radical (DPPH) test and chelating ferrous ions assay too.

3.4. *Scutellaria baicalensis*, Its BACs and Antioxidant Potential

Species of *Scutellaria*, subfamily Scutellarioideae, are known as skullcap. The generic name of the plant describes the shape of the calyx at the base of the flowers. Other names include hoodwort, quaker bonnet, or helmet flower. *Scutellaria* is a large genus, comprising more than 300 species, such as well-known and popular baical or Chinese skullcap *Scutellaria baicalensis*, *Scutellaria alpina*, *Scutellaria barbata*, *S. nervosa*, *S. orientalis*, and others. They are growing nearly worldwide, mainly in countries with temperate climates [75].

Scutellaria baicalensis is extensively used in folk medicine in European countries, North America, eastern countries, Korea, Japan, India, and China, in Traditional Chinese Medicine (TCM). It is listed in the European Pharmacopoeia, Chinese Pharmacopoeia, Japanese Pharmacopoeia, and Korean Pharmacopoeia. Raw or dry roots of this plant are, due to the presence of many biologically active compounds with good antioxidant activity, widely used for the treatment of diseases such as cardiovascular diseases, inflammation problems, and hypertension. The most common clinically applied form in TCM is the aqueous extract of *S. baicalensis*. Extracts and main flavonoids of *Scutellaria baicalensis* have been found to possess anticancer effects in multiple cancer cell lines both in vitro and in vivo and this species is promising for anticancer therapy [76].

The plant demonstrates also antioxidant and anti-inflammatory properties [77]. The constituents responsible for these qualities were identified as wogonin, wogonoside, and baicalin for anti-inflammatory properties; baicalein, baicalin, and viscidulin III exhibited free radical-scavenging activities measured by DPPH and ABTS assays.

The most pharmaceutically active components include flavonoids, of which the main flavonoids are baicalin and its aglycon baicalein, wogonoside, and its aglycon wogonin. Baicalin is among the most studied compounds due to its effects. Baicalin, as Chen et al. [78] reported, has the capacity to protect IEC-6 cells.

The most known active ingredients of the genus *Scutellaria* belong to flavonoids such as baicalin, baicalein, and wogonin, as it was mentioned above. Besides them, in the plant occur compounds such as phenylethanoid glycosides, diterpenes, flavones, and essential oil. Other significant constituents in the roots of *S. baicalensis* belong to scutellarin, di-hydroxybaicalein-7-*O*-glucuronide, norwogonin-7-*O*-glucuronide, oroxylin A-7-*O*-glucuronide, chrysin-7-*O*-glucuronide and wogonoside. Two new flavonoids from the roots of *S. baicalensis* were detected by Long et al. [79]. In the stems and leaves of *S. baicalensis*, isocarhamidin-7-*O*-glucuronide and carthamidin-7-*O*-glucuronide were also found as the two main components. The compounds that were also identified in the stems and leaves are luteolin, apigenin, pinocembrin, naringenin-7-*O*-glucuronide, apigenin-7-*O*-glucuronide, in the leaves there are catechin, calycosin, casticin, plantagin, and kaempferol too [80,81]. Also, carotenoids belong to the group of strong antioxidants. Tuan et al. [82] analyzed baical skullcaps (roots, leaves) for the presence of these bioactive compounds. Carotenoids were present mainly in the leaves of *S. baicalensis*, and in roots there were only trace amounts of them. The predominant carotenoids in the herb were lutein and β -carotene, with abundant amounts in the leaves.

Antioxidant potential of *Scutellaria baicalensis*

As there was mentioned before, baicalein, baicalin, and viscidulin III have been marked as significant antioxidant constituents. They demonstrated the highest activity in both the DPPH and ABTS free radical-scavenging activity assays, with the lowest half-maximal inhibitory concentration (IC₅₀) for baicalin. It was presented with the lowest values of IC₅₀ (15.1 and 10.8 μ M for the assays, respectively). The standard Trolox had IC₅₀ values of 38.1 and 12.8 μ M, respectively, so the efficiency of baicalin as an antioxidant is high. viscidulin III (16.4 and 15.3 μ M, respectively) and baicalein (17.0 and 16.5 μ M, respectively) possess similar, good antioxidant strength [77].

The impact of different solvents used could be, due to their different polarity, significant for TPC and AA values. Distilled water, methanol, 70% aqueous–methanol, ethanol, and 70% aqueous–ethanol were used as solvents of *S. baicalensis* hairy root extracts studied by Lim et al. [83]. They evaluated an amount of kaempferol, an important antioxidant component; the highest was presented in 70% aqueous–methanol extract, followed by 70% aqueous–ethanol, ethanol extract, methanol, and distilled water extract. The content decreased in the sequence: quercetin, rutin, epicatechin, benzoic acid, and gallic acid. Most of the individual phenolic compounds were highest after aqueous–methanol extraction, followed by aqueous–ethanol extraction, whereas the other solvents did not show significant differences. Thus, aqueous alcoholic solvents were more effective for the extraction of phenolics from *S. baicalensis*, whereas water extraction showed low efficiency. In the DPPH assay, the aqueous–ethanol extract showed the highest scavenging activity, followed by aqueous–methanol, methanol, ethanol, and distilled water. The ABTS assay of *S. baicalensis* extracts showed a similar trend to that of DPPH. The highest antioxidant activities, of DPPH, ABTS, and SOD-like scavenging activities and reducing power, were achieved in the aqueous–ethanol extract compared to the other solvent extracts, whereas distilled water was the least suitable. Hot water extraction, 80% ethanol, and 80% methanol were used in the study of Lim et al. [84]. The highest total phenolic and total flavonoid contents were in the methanol extract, followed by ethanol extraction; the least effective was hot water. A comparable situation was for the DPPH and ABTS radical scavenging activities, with methanol and ethanol solvents as more effective than hot water extraction.

Plant parts that are selected for analysis are important for the values of TPC and AA. Separated organs, inflorescences, stems, and leaves of ethanolic *Scutellaria baicalensis* extracts were studied by Ukrainian researchers [85]. They determined the total content of polyphenol compounds in the range of 28.06–96.76 mg GAE/g dw, depending on the part of the plant. The highest content of polyphenols was presented in leaves, followed by inflorescences and stems (about one-third to two-thirds of the value in leaves). Total flavonoid contents in ethanol extracts were with similar distribution as for polyphenols. The best values were for the leaves, followed by inflorescences (about half of the value in leaves), and the least content of flavonoids was found in the stems (about one-sixth to two-thirds of the value in leaves). Total phenolic acid content was found in the range from 2.60 to 23.4 mg CAE/g (caffeic acid equivalents), stems of both samples accumulated the least content of phenolic acids and leaves had the higher content of it. Therefore, the highest content of polyphenol compounds was identified in the leaves of the herb. The results of the DPPH method indicated an antioxidant ability of 7.63–8.83 mg TE/g; TEAC values were similar for all structural parts of the plant, inflorescences, stems, and leaves. Results of reducing power measuring of ethanolic extracts showed the values of this parameter similar for inflorescences and stems (a third to a half of the values in the leaves), so the highest reducing power values exhibited again extracts from the leaves.

Traditional Chinese medicinal plants (*Scutellaria baicalensis*, *Coptis chinensis*, and *Sonchus oleraceus*) in the form of aqueous extracts were studied and compared by

Li et al. [86]. The highest phenolic content was found in the plant of *S. oleraceus*, similar to *S. baicalensis*, with significantly lower amount (half the amount of others) for *C. chinensis*. The highest TFC was found again in the extract of *S. oleraceus*, followed by *C. chinensis* (half the amount), and *S. baicalensis*. Higher total antioxidant capacities of CUPRAC analysis were found in *S. baicalensis* and *S. oleraceus*, the lowest in *C. chinensis* (half the value of others). Hydroxyl free radical-scavenging ability with a lower IC₅₀ value, which means better scavenging activity, was detected for *S. oleraceus* and *C. chinensis*. The IC₅₀ value of *S. baicalensis* was lower than that of other herbs.

4. The Lesser-Known Representatives of Plants with Antioxidant Effect of Rosaceae, Asteraceae, and Myrtaceae Families

Much less information is available on the lesser-known members with antioxidant effect of the Rosaceae, Asteraceae, and Myrtaceae families than on members of the Lamiaceae family, which includes a large number of representatives with antioxidant activity. As the lesser-known representatives of these families for this review, there were selected *Crataegus laevigata* of the Rosaceae family, *Artemisia absinthium* of the Asteraceae family, and *Pimenta dioica* of the Myrtaceae family.

4.1. *Crataegus laevigata*, Its BACs and Antioxidant Potential

The genus *Crataegus* belongs to the subfamily of Maloideae, family Rosaceae. The family Rosaceae includes herbs, shrubs, and trees which are economically important due to edible fruits (raspberries and strawberries, cherries, peaches, and plums) important for their antioxidant properties. *Crataegus* contains up to three hundred species, of which *Crataegus laevigata*, hawthorn, is one of the most popular. Many members of this family have been used as natural ingredients that are utilized in cosmeceutical preparations in the cosmetic industry, or in the pharmaceutical industry [87].

Also, it possesses anti-inflammatory and anti-hypotensive effects. It is used traditionally as a cardiac tonic with diuretic and astringent properties. The plant is in the homeopathic medicine system for the cardiovascular system as it is a rich source of procyanidins. [88]. The results of Karapetyan et al. [89] research indicate that *C. laevigata* extract shows antioxidant properties and therefore can be used to prevent the deteriorating effects in the brain caused by acute oxygen deficiency.

The main phenolic compounds of hawthorn are mainly from the group of flavonoids. Hawthorn phenols include epicatechin and catechin, procyanidin B2, chlorogenic acid, quercetin, isoquercetin, rutin, hyperoside, and *p*-coumaric acid [90]. Antioxidant phytochemicals found in *Crataegus* extracts include procyanidins, flavonoids, flavonols, glycosylated flavanones, and triterpene pentacyclic acids. Also, quercetin-3-*O*-glucoside, quercetin-3-*O*-rhamnoside, quercetin 3-*O*-rhamnosyl-(1,6)-glucoside and quercetin 3-*O*-rhamnosyl-(1,2)-[rhamnosyl-(1,6)]-glucoside are of high importance [90,91].

Solvent selection is important for the composition of antioxidants in plants such as hawthorn. Methanol and ethanol extracts of the *Crataegus* plant both exhibited high levels of total flavonoids and polyphenols contents. Acetone solvent, ethylacetate, trichloromethane, and petroleum ether were less effective. The most abundant phenolic compounds were determined as epicatechin and procyanidin B2, with concentrations up to 281.6 and 243.5 mg/100 g, respectively. The levels of other constituents such as chlorogenic acid and quercetin were much lower, up to one-third. Besides them there were quercetin, catechin, *p*-coumaric acid, hyperoside and isoquercitrin. Their content was up to 30 mg/100 g. The highest total flavonoid content was obtained in methanol extract, followed by ethanol, acetone, ethylacetate, trichloromethane, and petroleum ether extracts. The total polyphenols contents showed a significant difference and varied in the range

from 2.66 to 38.40 mg GAE/g. Similar to total flavonoids, the total polyphenol contents decreased along with decreasing order of solvent polarity (methanol > ethanol > acetone > ethylacetate > trichloromethane > petroleum) [90].

The type of cultivar and its origin is also a significant factor. Hawthorn fruits (*Crataegus* spp.) of 22 Chinese cultivars and origins belonging to four species/varieties, *C. pinnatifida*, *C. brettschneideri*, *C. pinnatifida*, *C. scabrifolia*, were determined by Liu et al. [92]. Major phenolic compounds such as hyperoside, isoquercitrin, chlorogenic acid, ideain, epicatechin, procyanidin (PA) dimers, and PA trimers were quantified in the fruits. All compounds (except ideain) were detected in all the samples. Vitexin-4''-O-glucoside and vitexin-2''-O-rhamnoside have been described as major flavonoids presented in hawthorn leaves. The contents of vitexin-4''-O-glucoside and vitexin-2''-O-rhamnoside in the analyzed product were increased 8.44-fold and 8.43-fold from 0.72% and 2.63% to 6.08% and 22.2% [93]. Hyperoside, chlorogenic acid, and isoquercetin were found to be the most abundant phenolic compounds in the extracts of hawthorn fruits. The fruits of different *Crataegus* species [91] (especially *C. pseudomelanocarpa* and *C. pentagyna*) grown in Iran showed a high level of TPC and AA. However, a considerable variation in the results of TPC and AA of hawthorn species was shown.

Methanol extracts of leaves and flowers of 14 hawthorn species (*Crataegus* spp.) from wild-growing genotypes from Iran were analyzed by Alirezalu et al. [94]. Significant phenolic compounds included chlorogenic acid, vitexin 2''-O-rhamnoside, vitexin, rutin, hyperoside, quercetin, and isoquercetin. The content of phenolic compounds was significantly variable both among species and in different plant organs; therefore, both these factors affected TPC and AA in these samples. Chlorogenic acid, vitexin, and vitexin 2''-O-rhamnoside were found to be the most abundant phenolic compounds analyzed in the extracts of hawthorn leaves. In the extracts of hawthorn flowers in most of the species, the most abundant were found to be chlorogenic acid, hyperoside, and rutin. Quercetin was found in very low quantities both in leaves and in flowers, or it was not detected.

Antioxidant Potential of *Crataegus laevigata*

Very significant factor affecting antioxidant potential is the type of analyzed part of the plant. Hawthorn bark, leaves, and berries growing in Iran were analyzed by Rezaei-Golmisheh et al. [95]. The highest total phenol content of ethanolic extracts had the bark extract followed by the extracts of leaves (more than half of the highest value) and berries (more than a quarter of the highest value). Quercetin, as a significant representative of polyphenols, was found at the highest level in the bark extract, followed by leaves (two-thirds of the bark value) and berries (one-third of the highest value). The highest DPPH free radical scavenging potency was evaluated in the bark extract, followed by leaves and berries extract, which confirmed the polyphenol and quercetin content results.

The type of cultivar and plant part are also significant factors. Hawthorn leaves and flowers of hawthorn species (*Crataegus* spp.) from wild-growing genotypes of Iran, prepared as methanol extracts, were analyzed for the amount of phenolics and flavonoids, and AA [94]. TPC was in the range from 7.21 to 87.73 mg GAE/g dm of the plant. Total phenolic content was highest in the flowers of *C. pseudomelanocarpa*, followed by *C. meyeri* and *C. atrosanguinea*, whereas the lowest value was found in the flowers of *C. monogyna*. Phenolic content was the highest (82.74 mg GAE/g dm) in the leaves of *C. pentagyna*, then *C. pseudoheterophylla* and *C. azarolus*, whereas leaves of *C. monogyna* (12.41 mg GAE/g dm) ranked the lowest. Both leaves and flower organs of *C. pseudomelanocarpa* species exhibited a high level of total phenolic content. The amount of total flavonoids was significantly variable both among species and in different plant organs, ranging from 2.27 to 17.40 mg/g dm. Differences between the species and the parts of plants were highly significant ($p \leq 0.01$).

In most hawthorn species, flower organs possess a higher total flavonoid content than the leaf organs. The *Crataegus* plants were assessed also due to antioxidant activity, FRAP method. Antioxidant activity significantly varied in terms of both different plants' organs and species, in the range from 0.9 to 4.65 mmol Fe²⁺/g dm. The highest AA value was observed in the leaves of *C. pentagyna*, whereas the lowest activity was determined in the leaves of *C. azarolus* var. *aronia*. The highest (2.84 mmol Fe²⁺/g dm) and the lowest (0.96 mmol Fe²⁺/g dm) antioxidant activity in the flowers was detected in *C. monogyna* and *C. meyeri*, respectively.

The type of cultivar, as mentioned above, is a factor that may influence antioxidant potential. The total phenolic content of the wild-growing hawthorn fruits from seven provinces of Iran was analyzed by Alirezalu et al. [91]. They determined TPC in the range of 21.19–69.12 mg GAE/g dm, with the highest value in the fruits of *C. pentagyna*, followed by *C. pseudomelanocarpa*, *C. atrosanguinea* and *C. pseudoheterophylla*. The lowest amount was determined for *C. turkestanica* and *C. azarolus* (23.89 mg GAE/g dm). Total flavonoid content was presented in the range from 2.44 to 6.08 mg QE/g dm, with the highest value in the fruits of *Crataegus meyeri*, followed by *C. pentagyna* and *C. pseudomelanocarpa*, the lowest one for *C. szovitsii* and *C. azarolus* (2.74 mg GAE/g dm). Antioxidant activity, measured by FRAP, manifested values of 0.32–1.84 mmol Fe²⁺/g dm. The highest power showed *C. pentagyna* with a high content of polyphenols and flavonoids too. High AA values had *C. atrosanguinea* and *Crataegus meyeri*, whereas *C. persica* and *C. orientalis* achieved only low AA values. According to these results, TPC, TFC, and AA can be significantly influenced by both the species and the sampling location. Similar findings were also reported by Özyürek et al. [96], who evaluated flowers and leaves belonging to 17 taxa of 14 *Crataegus* species, naturally growing in Turkey. According to the results of the polyphenol assays, among the flower samples, the species with the highest content was *C. × sinaica* Boiss. nothosubsp. *sinaica*, *C. monogyna* Jacq. var. *monogyna*, *C. rhipidophylla* Gand. var. *rhipidophylla*, and among the leaf samples, *C. pentagyna* Waldst et Kit. ex Willdenow and *C. monogyna* Jacq. var. *monogyna*. Comparable observations were noticeable for AA values. The most antioxidative active species were *C. × sinaica* Boiss. nothosubsp. *sinaica*, *C. monogyna* Jacq. var. *monogyna*, and *C. rhipidophylla* Gand. var. *rhipidophylla*. Leaf extracts of *C. pentagyna* Waldst et Kit. ex Willdenow and *C. monogyna* Jacq. var. *monogyna* manifested the highest antioxidant activity. Therefore, as the authors referred, *Crataegus monogyna* samples have exhibited markedly high antioxidant activity. Though there are differences in antioxidant capacity between the same species collected from different regions, these differences can be due to factors such as land characteristics, unpolluted air, oxygen concentration, and height. Their study indicates that *Crataegus* species originating from nature cannot be standardized for medicinal use.

4.2. *Artemisia absinthium*, Its BACs and Antioxidant Potential

Genus *Artemisia*, from the family Asteraceae (Compositae), subfamily Asteroideae, is one of the most widely distributed, containing up to 500 species. *Artemisia absinthium*, with the common name wormwood, is a perennial herb with a characteristic, sage odor. Wormwood is growing in temperate regions, in Europe, Asia, and America. The flowers and aerial parts of the plants are utilized in the cosmetic industry and used as an ingredient in the spirit (absinthe). The herb is used in pharmacological preparations due to its cholagogue, choleric, stomachic, and carminative properties. It exhibits anti-inflammatory and anti-helminthic effects. In European and Asian folk medicine, it is used for gastrointestinal problems and dyspepsia [97,98]. Also, *A. absinthium* essential oil was found to inhibit the growth of both Gram-negative and Gram-positive bacteria strains [99].

The substances that were found to be responsible for wormwood biological activities are components of the essential oil, bitter constituents like sesquiterpenoid lactones, azulenes, flavonoids and phenolic acids, and tannins. The group of flavonoids in the wormwood plant contain quercetin, kaempferol, and apigenin; and the group of phenolic acids contain representatives such as gallic, caffeic, vanillic, and chlorogenic acids. Bitter substances specific to this plant are from the group of sesquiterpene lactones, absinthin, anabsinthin, and anabsin. Also, compounds of essential oils such as α -thujone and β -thujone, isothujone, thujylalcohol and its esters, camphene, guaiazulene and α -cadinene, azulenes such as chamazulene, prochamazulenogen, and azulene are significant [100].

In the distilled essential oil of wormwood, growing in Saudi Arabia, 34 volatile constituents were identified by Mohammed [101]. Among them, *cis*-davanone was found at the highest percentage (52.51%) of the total volatile constituents of the plant. Furthermore, there was α -gurjunene (7.15%), and under 5%, chamazulene, and camphene. The identified constituents were mainly categorized as oxygenated sesquiterpenes with a total percentage of 63.61%, followed by nonoxygenated sesquiterpenes (14.69%) of the total identified compounds. The monoterpenes were calculated as 20.86%, distributed as 9.82% of the non-oxygenated monoterpenes, and 11.04% of the oxygenated monoterpenes.

According to the findings of Naimi et al. [98], the major components of the studied *Artemisia absinthium* essential oils were camphor (36.22%) and α -thujone (30.28%). The aerial parts of *A. absinthium* showed the presence of alkaloids, flavonoids, tannins, and glycosides too. The composition of wormwood oil in more detail is composed also of the compounds: chamazulene (8.02%), arborescin (6.82%), terpinen-4-ol (6.80%), sabinene (5.81%), and linalool (1.18%); followed by substances with the low content (below 1%): *p*-mentha-1,4(8)-diene, *o*-cymene, ζ -terpinene, α -terpineol, germacrene, D4-Vinylcholestan-3-ol, and geranyl-*p*-cymene.

The vegetation stage is a factor that may modify the antioxidant potential of wormwood plants, *A. abrotanum*, and *A. absinthium* from Lithuania, at several vegetation stages (intensive growth, butonization, the beginning of flowering, intensive flowering, and the end of flowering). It has been analyzed by Saunoriūtė et al. [102]. The phenolic compounds are of two groups, the phenolic acids group (caffeoylquinic and hydroxycinnamic acids) and flavonoids (flavonols, flavones). As important identified compounds, there were determined 4,5-dicaffeoylquinic acid, 4-*O*-caffeoylquinic acid, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, caffeic acid, chlorogenic acid, neochlorogenic acid, isorhamnetin-3-rutinoside, luteolin-7-glycoside, luteolin-7-rutinoside, and rutin. The most common compounds identified in all extracts were chlorogenic acid, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, and rutin. Chlorogenic acid was the predominant compound in the extracts.

The part of the plant as a factor of the differences in the wormwood extracts was investigated by Moacă et al. [103]. Ethanolic extracts, obtained from leaves and stems of *A. absinthium*, contain phenolic acids such as gentisic acid, chlorogenic acid, caffeic acid, *p*-coumaric acid, isoquercitrin, rutin, quercitrin, luteolin, and apigenin. Chlorogenic acid was the most abundant polyphenolic quantified compound. Isoquercitrin, rutin, and quercitrin were also detected, but in smaller concentrations, accompanied by traces of gentisic acid, caffeic acid, *p*-coumaric acid, luteolin, and apigenin. In terms of their concentration in the two types of extract, stem extract, slightly richer, is similar to leaf extract.

Antioxidant Potential of *Artemisia absinthium*

The differences in the composition of essential oil or extract prepared using different solvents are also due to the various types of constituent isolation. Components of essential oils and methanolic extracts of *Artemisia absinthium* aerial parts from several regions of

Tunisia were compared in their study by Ksibi et al. [99]. Camphor was the main compound of essential oil with a level of 33.63% to 49.70%. Other relevant compounds are β -copaene, α -humulene, germacrene-D, and trans-caryophyllene. As for methanolic extracts, total polyphenolic content was within the range of 6.93 to 26.80 mg GAE/g dm. The highest total flavonoid content was detected in the sample of the same region with the highest polyphenol content, the lowest value was in the amount of two-thirds of the highest content. Quercetin and isorhamnetin were the main compounds and their percentages were region-dependent. The highest scavenging ability of DPPH (IC₅₀ 31.46 μ g/mL) had methanolic artemisia extract of the same region as mentioned before with the highest TPC and TFC.

The comparison of four plant extracts, *Artemisia absinthium*, *Melia azedarach*, *Trigonella foenum-graecum*, and *Peganum harmala* was evaluated by Naimi et al. [98]. Methanolic extract of *M. azedarach* revealed the highest total phenolic content (5.63 mg GAE/g), followed by *A. absinthium* (3.39 mg GAE/g). The lowest values were observed in *T. foenum-graecum* and *P. harmala* extracts. The highest total flavonoid content showed *M. azedarach*, higher amount contained also *A. absinthium*. The other plants had lower amounts of flavonoids. The observed variations in total phenol and flavonoid contents could be related to the different phytogeographical areas and the harvest seasons that affect the contents of secondary metabolites.

Also, vegetation stages may provide different compositions and content of the substances present. Two wormwood plants, *A. abrotanum* and *A. absinthium* from Lithuania, at several vegetation stages (intensive growth, butonization, the beginning of flowering, intensive flowering, and the end of flowering), have been analyzed by Saunoriūtė et al. [102]. The highest content of phenolic compounds was found in the samples at the beginning of the flowering stage, and they significantly differed from the quantities found in the remaining vegetation stages. Other high values were detected during intensive flowering and the end of flowering. The lowest amount of phenolic compounds was found in the intensive growth stage. The highest total flavonoid content was found in *A. abrotanum* extracts of the butonization stage, similar to the beginning of flowering, followed by intensive flowering, while the lowest quantity was determined in *A. abrotanum* extracts of the intensive growth stage. The antioxidant activity (DPPH) showed that the strongest scavenging activity (14.23 μ mol TE/g dm) was observed in *A. abrotanum* herbal extracts of the intense flowering stage and at the beginning of flowering. The weakest antiradical activity (9.01 μ mol TE/g dm) was determined in the extracts during the intensive growth vegetation stage and butonization.

Solvent efficiency, as another factor, was studied in ethylacetate and aqueous extracts of *A. absinthium* L. leaves from Morocco [104]. The highest polyphenol content results were shown for ethylacetate extract compared to aqueous extract (half of the ethylacetate TPC amount). The flavonoid contents in extracts were in the same trend as TPC. Ethylacetate extract showed good DPPH radical scavenging, with a lower IC₅₀ value (0.167 mg/mL) than the aqueous extract (0.352 mg/mL). Similarly, it is for FRAP activity. Ethanolic extracts obtained from leaves and stems of *A. absinthium* L. were investigated by Moacă et al. [103]. The results showed that the leaf extract had a higher phenolic content (54.68 mg GAE/g) compared to the extract from the stems (44.15 mg GAE/g). The extracts presented mild antioxidant activity, the leaves extract had better AA than the stem extract (IC₅₀ concentrations were for wormwood leaves extract 0.4993 mg/mL, for stems extract 0.4865 mg/mL).

The antioxidant potential (TPC and AA), measured by the technique with the same method conditions, of individual plants in the same family or different ones, can show differences in antioxidant power between them. The antioxidant characteristics of eight aromatic and medicinal plants (*Hyssopus officinalis*, *Angelica panicicii*, *Angelica sylvestris*, *Laserpitium latifolium*, *Achillea grandifolia*, *Achillea crithmifolia*, *Artemisia absinthium* and

Tanacetum parthenium) prepared as methanol extracts evaluated Stanković et al. [58]. They determined that total polyphenol content was in the order: *A. crithmifolia* > *A. grandifolia* > *A. absinthium* > *T. parthenium* > *H. officinalis* > *L. latifolium* > *A. panicii* > *A. sylvestris*. The phenolic values were in the range of 50–175 mg GAE/g, and flavonoids in the range of 30–95 mg RE/g. The representatives of the Asteraceae family (*A. crithmifolia*, *A. grandifolia*, and *A. absinthium*) had the highest values of total polyphenols and flavonoids. Results of antioxidant activities (DPPH, ABTS, FRAP) demonstrated a similar sequence of the activity: *A. crithmifolia* > *A. grandifolia* > *H. officinalis* > *A. absinthium* > *T. parthenium* > *L. latifolium* > *A. panicii* > *A. sylvestris*. Wormwood manifested medium TPC and AA values. The total content of polyphenols and flavonoids in the methanol extracts of the studied species positively correlated with their antioxidant properties.

4.3. *Pimenta dioica*, Its BACs and Antioxidant Potential

Myrtaceae, myrtle family, is a family of several popular and notable members, spices such as allspice, eucalyptus, and clove or guava. They are woody with flower parts, native to America, Mexico, and India. *Pimenta dioica* is a genus of flowering plant members of the Myrtaceae family, subfamily Myrtoideae, commonly known as allspice or pimento. The dried fruits of allspice, in the whole or ground form, are used as condiments in the foods and have characteristic flavor and aroma [105]. Besides its utilization in gastronomy and food flavoring production, the spice is valuable due to its essential oils commercially used for perfumes, cosmetics, and in some pharmaceutical preparations for its therapeutic properties. The main commercial product of this spice is its essential oil, due to its utilization in different industries, as mentioned above, with beneficial effects for health too. Allspice is used also as a natural pesticide. In allspice, present glycosides and polyphenols show antibacterial, hypotensive, anti-neuralgic, and analgesic properties. Eugenol and gallic acid have selective antiproliferative and anti-tumor properties on human cancer cells and their animal models. In folk medicine, all parts of *P. dioica* are utilized for antiseptic, antihelmintic, and anesthetic activities. It has antioxidant, antimicrobial, tonic, and hypotensive activity [105–107].

Essential oils of pimento berry (*Pimenta dioica* (L) Merr.) from Jamaica were analyzed by Padmakumari et al. [108]. The major constituents of the oils were detected as eugenol (73.75–74.71%), the most abundant compound, followed by methyl eugenol, 4.08–9.54%, and caryophyllene, 3.30–4.90%. Monoterpene hydrocarbons constituted up to 5% of the oil, and sesquiterpene hydrocarbons, 6.64–9.38%. Also, in Guatemala essential oils [109], the major component was eugenol (71.4% for leaves oil and 65.9% for fruits oil), β -myrcene was the second major component, followed by (*E*)-caryophyllene, β -ocimene, 1,8-cineole, chavicol, (*E*)-caryophyllene, and α -humulene.

The presence of significant compounds such as phenols, flavonols, triterpenes, saponinins, and polyalcohol in aqueous, ethanolic, and acetonic extracts of *Pimenta dioica* leaves collected in Mexico was detected by Sánchez-Zarate et al. [110]. In aqueous extract, gallic acid, kaempferol, quercetin, catechin, caffeic acid, chlorogenic acid, and epicatechin were identified. In ethanolic extract phenols or flavonoids (gallic acid, myricetin, pedunculagin, epicatechin-3-*O*-gallate, kaempferol, and quercetin), aretriterpenoids (brachycarpone and glaucalactone), and saponin (hecogenin acetate), were evaluated. In the acetonic extract, catechin, polyalcohol (pentaerythriol tetrapropionate), and saponin (hecogenin acetate) were presented. The presence of ten important groups contained in various *P. dioica* leaf extracts was analyzed by Murali et al. [111]. Carbohydrates, proteins, steroids, alkaloids, flavonoids, phenols, and terpenoids are confirmed in ethanolic extract. Similarly, in the aqueous extract were present carbohydrates, alkaloids, flavonoids, steroids, saponins, tannins, and terpenoids. Only proteins, phenols, and terpenoids were stated in diethyl ether extract.

Antioxidant Potential of *Pimenta dioica*

Antioxidant potential (the quantity of phenolic compounds, flavonoids, and antioxidant activity) of allspice is evaluated in various extracts, such as aqueous, ethanolic, or acetic extracts, or in essential oils. All of them belong to significant factors that may change antioxidant potential values due to their usage.

Essential oils of pimento berry (*Pimenta dioica* (L) Merr.) from Jamaica were analyzed by Padmakumari et al. [108]. They determined eugenol is the main constituent which contributes to the antioxidant activity of the oil. However, the antioxidant potential of the oil was found to be higher than that of pure eugenol which may be due to the synergistic effect of other oxygenated compounds present in the oils. Essential oils of mature leaves of allspice, mature bark of cinnamon, mature green color capsules of cardamom, mace and seed of nutmeg, and unopened flower buds of clove, originated from Sri Lanka, were analyzed by De Soysa et al. [112]. They evaluated the total polyphenol content with the highest value for clove (310.4 mg GAE/g) essential oil, followed by allspice oil at half TPC value (134.3 mg GAE/g), and cinnamon oil (67.5 mg GAE/g). Other essential oils, nutmeg seed, and mace had much lower TPC, and cardamom the least (3.5 mg GAE/g). Significantly higher antioxidant activity was again observed in clove oil (974.340 mg TE/g), followed by allspice (344.917 mg TE/g) in one-third value, then cinnamon, and both nutmeg oils. Cardamom presented again the lowest value of AA (3.2 mg TE/g). Allspice had significantly higher AA and total phenolic values when compared to all selected spice species except clove.

Total phenolic and flavonoid contents of allspice in powdered form were determined using both water and methanol extraction. The total phenolic content was higher in methanol compared to water extracts of allspice, while the content of flavonoids was higher in water extracts compared to methanol extracts of allspice. The total phenolic and flavonoid contents were higher in methanol extracts compared to water extracts of allspice. A comparison of the antioxidant activity of water and methanol extracts of allspice was conducted using different assays (DPPH, TEAC, NO, ORAC, and FRAP). The IC₅₀ (DPPH) values were higher in methanol extracts compared to water extracts of allspice (52.5 and 48.96 mg/mL). The FRAP assay is based on the ability of the allspice extract to reduce colorless Fe³⁺ to blue-colored Fe²⁺. Methanol extract showed similar results to water extract of allspice. NO activity and ORAC of allspice were higher in methanol extracts compared to water extracts of allspice. TEAC, FRAP, and DPPH activity was therefore higher in methanol extracts compared to water extracts of allspice [113].

Aqueous, ethanolic, and acetic extracts of *Pimenta dioica* L. leaves from Mexico were studied by Sánchez-Zarate et al. [110]. The acetic extract manifested the highest phenolic content (626.29 mg GAE/g), phenolic content of the aqueous and ethanolic extracts did not show a significant difference. Likewise, acetic extract had the highest antioxidant activity (lowest IC₅₀) for DPPH, ABTS, and FRAP assay, respectively.

5. Conclusions

Medicinal and aromatic plants that are lesser-known as antioxidant sources and were selected for this review (*Lamium album*, *Leonurus cardiaca*, *Hyssopus officinalis*, *Scutellaria baicalensis*, *Crataegus laevigata*, *Artemisia absinthium*, and *Pimenta dioica*) are good sources of biologically active compounds (BACs) such as antioxidants. BACs participate in their biological impacts, and deactivate reactive oxygen species, and thus affect the overall antioxidant activity (AA). The most important BACs in selected plants belong to phenolic compounds such as phenolic acids, and flavonoids like flavonols, flavanols, and tannins. Even though they are not present in the highest amounts of selected plants, herbs consumed more often may act as strong antioxidants and thus, contribute as a remedy in

the prevention and supportive treatment of several diseases such as cardiovascular diseases and inflammation disorders, or some types of cancer. Their utilization, due to the presence of the active compounds, can also be significant in the food and cosmetic industries. This review thus gives a comprehensive summary of information about important BACs present in selected plants, the content of total polyphenols, and antioxidant activity that are influenced by different factors such as the used solvent and its polarity, extraction technique, and the procedure and conditions of AA determination method.

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