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Evaluation of the Antioxidant Potential of Extracts from Aromatic Plants of the *Lamiaceae* Family

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Abstract

Antioxidants protect the human body from oxidative stress caused by reactive oxygen and nitrogen species, which damage biomolecules and contribute to chronic diseases such as cancer, cardiovascular, metabolic, and neurodegenerative disorders. They act through both endogenous (*e.g.,* glutathione, catalase) and exogenous (*e.g.,* vitamins, polyphenols) sources, neutralising free radicals, chelating metals, and supporting detoxification pathways. Herbs of the *Lamiaceae* family, including sage, mint, thyme, and rosemary, are particularly rich in polyphenols with strong antioxidant activity. This study investigated the polyphenol content and antioxidant capacity of ethanolic and aqueous macerates using the Folin–Ciocalteu method and DPPH assay. The results showed that ethanol was more effective for extracting rosemary, while water exhibited higher activity for sage, mint, and thyme. Optimal extraction ratios were identified as 1 : 20 for ethanol and 1 : 100 for water, yielding total polyphenol contents ranging from 11–14 mg GAE g^{-1} for rosemary and 24–27 mg GAE g^{-1} for mint and thyme. Temperature (50 °C) and extraction time (up to 120 min) significantly affected yield. Although rosemary contained moderate polyphenol levels, it demonstrated strong antioxidant potential, with a DPPH scavenging activity of 27.8 %, while mint and thyme showed the highest antioxidant capacity, reaching 42–47.7 %. Overall, the findings highlight the potential of *Lamiaceae* herbs as sustainable sources of natural antioxidants for food, cosmetics, and pharmaceutical applications.

Keywords

Antioxidant activity, polyphenols, Lamiaceae herbs, ethanolic and aqueous extracts, DPPH assay, sustainable natural sources

1 Introduction

Antioxidants play a crucial role in protecting the human body from oxidative stress. This occurs when the body's own antioxidant defences are overwhelmed by the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS). Chronic diseases such as cancer, heart disease, diabetes, and neurological disorders can develop as a result of damage to biomolecules such as DNA, proteins, and lipids by these reactive species.[1]

The mechanism of action of antioxidants is the neutralisation of free radicals by electron donation or hydrogen atom transfer, which ends radical chain reactions. Antioxidants can also chelate transition metal ions such as Fe^{2+} and Cu^{2+} and thus prevent Fenton reactions that generate hydroxyl radicals.[2] In addition, some antioxidants modulate gene expression and the activity of enzymes involved in detoxification and repair processes.[3,4] Endogenous antioxidants such as glutathione, catalase and superoxide dismutase are supported by exogenous antioxidants such as vitamins C and E, carotenoids and polyphenols from plant sources.[5]

Polyphenols are a diverse group of secondary metabolites with strong antioxidant potential. They are categorised into flavonoids, phenolic acids, tannins, lignans, stilbenes and coumarins.[6,7] These substances have a protective function in biological systems against damage caused by oxidative stress because they scavenge free radicals, chelate metal ions, and inhibit oxidative enzymes. The *Lamiaceae* family, which includes culinary and medicinal herbs such as *Salvia officinalis* (sage), *Mentha x piperita* (mint), *Rosmarinus officinalis* (rosemary) and *Thymus vulgaris* (thyme), is rich in these compounds.[8–11] The polyphenols in *Lamiaceae* can be broadly categorised into phenolic acids, among which rosmarinic acid is notably predominant in several *Lamiaceae* species, especially in *Rosmarinus officinalis*, *Salvia officinalis*, and *Melissa officinalis*.[12] The antioxidant activity is closely related to the presence, concentration, and structural diversity of polyphenolic compounds.[13,14] The structure-activity relationship (SAR) of these compounds is of crucial importance; their antioxidant capacity is influenced by the amount and location of hydroxyl groups, the degree of conjugation, and glycosylation patterns. Rosmarinic acid, for example, is a stronger antioxidant than simpler phenolic acids such as p-coumaric acid due to its many hydroxyl groups and the ester bond between caffeic acid and 3,4-dihydroxyphenyl lactic acid.[15,16]

The total polyphenol content is most commonly determined using the Folin–Ciocalteu method, a colourimetric test in which polyphenols reduce the Folin–Ciocalteu reagent (a mixture of phosphomolybdate and phosphotungstate) under alkaline conditions. This reduction results in the formation of a blue complex, which is measured spectrophotometrically at 760 to 765 nm. The results are expressed as gallic acid equivalents (mg GAE *per* g of plant material).[17]

Although this method is widely used, it is not highly specific, as other reducing substances, such as sugar and ascorbic acid, may interfere with the reaction. Nevertheless, it provides a reliable estimate of the overall reducing power of plant extracts.

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On the other hand, antioxidant activity is measured using a variety of techniques, often based on different reaction mechanisms. The DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging test measures the ability of antioxidants to reduce the stable DPPH radical, which causes a colour shift from violet to yellow, spectrophotometrically at 517 nm.[18] The ABTS (2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) test is based on the decolourisation of the ABTS radical cation by antioxidants. The absorbance is measured at 734 nm. It is suitable for both lipophilic and hydrophilic antioxidants[19]. The FRAP (ferric reducing antioxidant power) method evaluates the reduction of the Fe^{3+}-TPTZ (2,4,6-tripyridyl-s-triazine) complex to the ferric form at low pH, measuring an intense blue colouration at 593 nm.[20] Using fluorescein as a probe, ORAC (oxygen radical absorbance capacity) quantifies the antioxidant inhibition of peroxyl radical-induced oxidation triggered by AAPH (2,2′-azobis-amidinopropane dihydrochloride). It is suitable for biological samples and represents the mechanisms of hydrogen atom transfer.[21]

The natural origin, safety profile, and biological activity of *Lamiaceae* polyphenols make them promising for use in herbal medicine, dietary supplements, and food preservation. Understanding the individual polyphenol species and combinations that exhibit antioxidant activity can help in selecting the best species and standardising extracts for specific applications.

Polyphenol-rich extracts from *Lamiaceae* herbs exhibit strong antioxidant activity and have broad potential applications in the food, pharmaceutical, and cosmetics industries. In food systems, they can be used as natural antioxidants to enhance oxidative stability and extend shelf life.[22] In pharmaceutical and nutraceutical products, compounds such as rosmarinic acid, carnosic acid, thymol, and carvacrol contribute to antioxidant, anti-inflammatory, and antimicrobial effects.[23] In cosmetics, these extracts are valuable for protecting the skin against oxidative stress and premature aging, supporting their use in natural and sustainable formulations.[24]

In addition, variations in polyphenol profiles and antioxidant activity arising from differences in harvest time, extraction technique, and geographical origin can significantly affect the results, highlighting the need for standardisation of experimental procedures.

The aim of this research was to investigate the influence of solvent type, temperature, and extraction time on the yield of total polyphenols and the antioxidant capacity of extracts from plants of the *Lamiaceae* family, and to construct 3D response surface models to optimise the extraction process and identify interactions between the independent variables.

2 Experimental

The above-ground parts of sage (*Salvia officinalis* L.), rosemary (*Rosmarinus officinalis* L.), thyme (*Thymus vulgaris* L.), and mint (*Mentha* × *piperita* L.), collected in the south-western and south-eastern regions of the Republic of North Macedonia, were used as working material for the purposes of this study. Thyme and mint were collected in Strumica, while rosemary and sage were collected in Bitola.

The herbal drugs were dried in the shade at room temperature (≈ 22 °C) for 10–14 days until constant weight was achieved. After drying, the plant material from the purchased packages was ground to a fine powder using a laboratory mill to ensure sample homogeneity. The powdered material was thoroughly mixed, placed in plastic containers, and stored in a dark, dry place at room temperature until further analysis.

2.1 Preparation of the green extracts (macerates, hydrolates, and total extracts)

Green extracts (macerates) were prepared in water and ethanol, comparing the efficiency of different extraction times (30, 60, and 120 min) and different temperatures (30, 40, and 50 °C). The optimal mass of the plant material and the volume of solvent for the preparation of the macerates were also determined.

To prepare the extracts, 1 g of plant material was weighed into a 100 ml beaker and the appropriate amount of water or ethanol (20, 30, and 40 ml) was added. The mixture was heated to the appropriate temperature (30, 40, and 50 °C), and the total polyphenols and antioxidant activity were determined after a precisely defined time (30, 60, and 120 min).

2.2 Determination of total polyphenol content using the Folin-Ciocalteu method

The concentration of total polyphenols in the prepared macerates was determined using the Folin-Ciocalteu method. The calibration curve was prepared by measuring a volume of 200 μl of the standard solution of known concentration with a micropipette and transferring it to a test tube to which 2 ml of Folin-Ciocalteu reagent 1 : 10 and 1.8 ml of Na_2CO_3 7.5 % (w/v) were added. The samples were kept in the dark for 20 min and the absorbance was measured at 750 nm using a UV-VIS spectrometer. The concentration of the samples was determined in the same way.[25] Each sample was analysed in duplicate.

2.3 Determination of antioxidant activity using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method

The antioxidant activity of the prepared macerates was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method.[26]

The DPPH reagent was prepared as an ethanolic solution with a final concentration of approximately 0.8 mM. The solution was allowed to stabilise for 120 min until a constant absorbance of about 0.9 at 517 nm was achieved.

The antioxidant activity of the macerates was determined by pipetting 3 ml of the previously prepared DPPH solu-

tion into a test tube and adding 100 µl of the macerate. The test tubes were kept in the dark for 30 min and then the absorbance was measured at 517 nm. The blank was prepared by measuring 3 ml of the previously prepared DPPH solution and adding 100 µl of methanol. All DPPH measurements were performed in duplicate.

The degree of antioxidant activity, %, was determined according to the Eq. (1).

$$\text{antioxidant activity} = \left[\frac{A_c - A_s}{A_c}\right] \cdot 100 \qquad (1)$$

where A_c is absorbance of blank sample, and A_s is absorbance of test sample

2.4 Isolation of the essential oils by steam distillation

The essential oils were isolated by steam distillation.[27] 1 g of the plant material was placed in a 100 ml round bottom flask and 50 ml of water was added. A distillation apparatus was set up in which a 50 ml Erlenmeyer flask served as a template. The distillate was collected until the distillate was cloudy; at least 35–40 ml of distillate was collected. Once distillation was complete, the plant material was dried and macerates were prepared using water as an extractant. The total polyphenol content was determined using the Folin-Ciocalteu method, and the antioxidant activity using the DPPH method according to the previously described procedures.

2.5 Statistical analysis of the results

The Statgraphic Centurion XV.I software package was used to analyse the influence of the independent variables of temperature and time on the concentration of total polyphenols and antioxidant activity, and to design 3D reaction surfaces.

A multistage factorial design was used for all analyses. Time (30, 60, 120 min), temperature (30, 40, 50 °C), and extraction solvent (water and 96 % ethanol) were used as independent variables in the experimental design.

3 Results and discussion

3.1 Optimisation of extraction conditions for total polyphenol determination

The total concentration of polyphenols in alcoholic and aqueous extracts was determined using the Folin-Ciocalteu method. The concentration of total polyphenols is expressed as mg of gallic acid in 1 g of drug using a calibration curve constructed from standard solutions of gallic acid in the range 10–400 mg l^{-1}. Gallic acid was used as a standard in this study as it is present in all plants studied. All results for total polyphenol content are expressed *per* 1 g of dry plant material.

The correlation coefficient obtained was 0.9995, indicating excellent linearity of the method in the concentration range tested. At gallic acid concentrations above 400 mg l^{-1}, a deviation from linearity was observed, resulting in absorbance values that were no longer directly proportional to concentration. Therefore, when preparing the macerates, care should be taken to ensure that the concentration of total polyphenols obtained is within the range of the constructed calibration curve. The absorbance values obtained by the Folin–Ciocalteu assay were used as a preliminary tool to evaluate the suitability of different drug-to-solvent ratios and to optimise extraction conditions prior to final quantification. One of the objectives of this research was to optimise the preparation of alcoholic and aqueous extracts with respect to the drug mass and the volume of the extraction solvent. For this purpose, 1 g of the drug was mixed with 20, 30, and 40 ml of water, as well as with the same volumes of ethanol. In addition, the extraction time was also investigated (Table 1).

Table 1 – Influence of drug mass, solvent volume, and extraction time on the content of total polyphenols expressed by absorbance at 750 nm

Ethanol extract			
Time / min	1 g : 20 ml	1 g : 30 ml	1 g : 40 ml
30	0.191	0.100	0.068
60	0.350	0.187	0.168
120	0.404	0.212	0.184
Aqueous extract			
Time / min	1 g : 20 ml	1 g : 30 ml	1 g : 40 ml
30	1.530	1.650*	1.650*
60	1.650*	1.650*	1.650*
120	1.650*	1.650*	1.650*

* Absorbance value reached the upper limit of the method (1.650), corresponding to a total polyphenol concentration ≥ 400 mg l^{-1}; therefore, accurate quantification was not possible without additional dilution.

Table 1 presents absorbance values at 750 nm, which were used exclusively for comparative assessment of extraction efficiency and optimisation of extraction parameters, while total polyphenol content was subsequently calculated and expressed as mg l^{-1} gallic acid equivalents (GAE), as shown in Fig. 1. The results indicate that ethanol extraction yields the best results when the macerate is prepared with 1 g of the drug in 20 ml of ethanol, while larger solvent volumes result in significantly lower absorbance values. Consequently, this ratio was selected for further studies. In contrast, water extraction produces a significantly higher polyphenol content, with most samples reaching an absorbance of 1.650, indicating that the upper limit of the method (400 mg l^{-1}) has been exceeded. This outcome demonstrated that the initially tested drug-to-solvent ratios were not appropriate for aqueous extracts. Therefore, an additional ratio (1 g : 100 ml) was tested and selected, as it provided absorbance values within the linear range

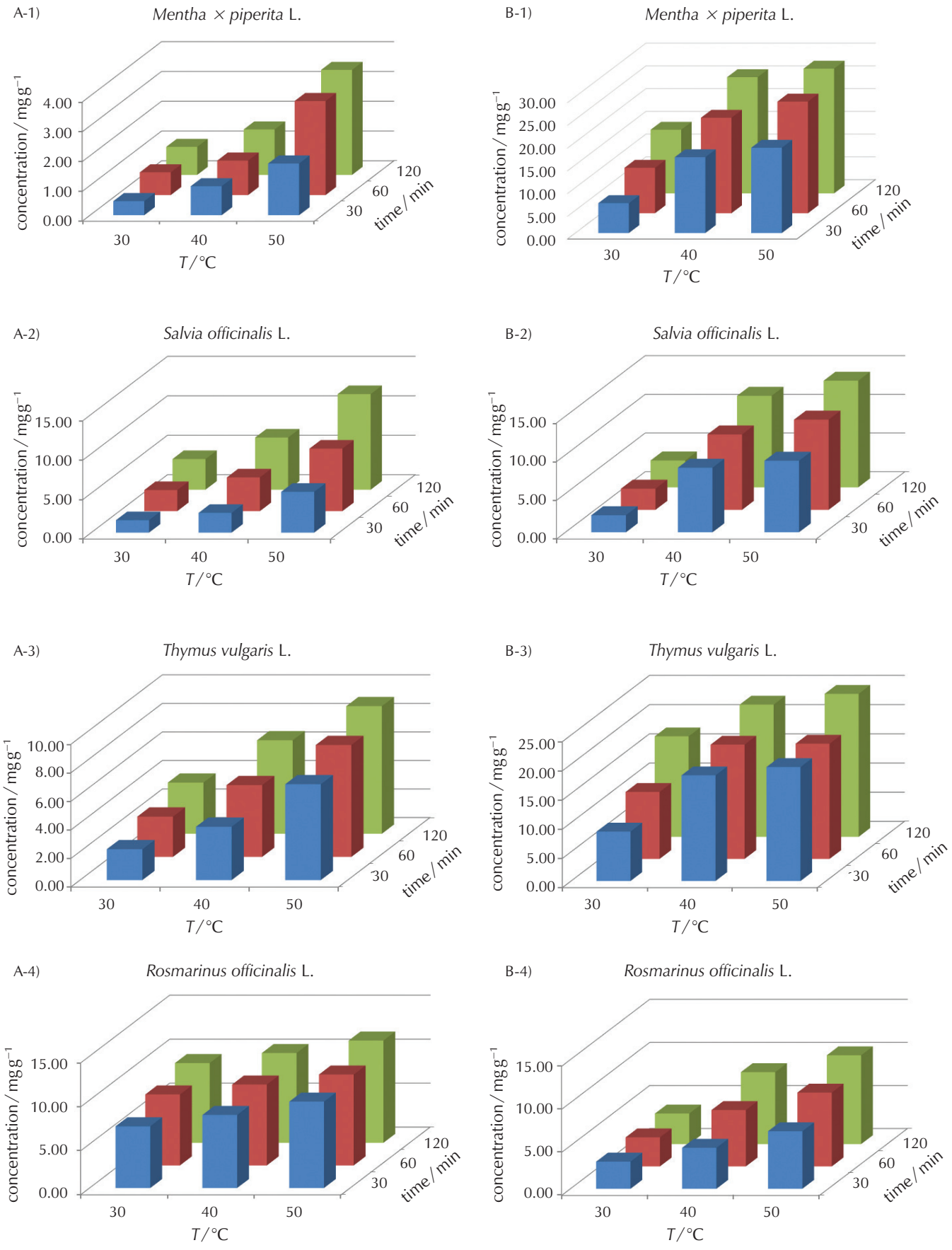

Fig. 1 – Graphical representation of total polyphenol content: A) ethanol extracts, B) aqueous extracts of *Mentha × piperita* L., *Salvia officinalis* L., *Thymus vulgaris* L., and *Rosmarinus officinalis* L. prepared at different temperatures and times

of the method. The absorbance values obtained under these conditions fall within the linear range of the method; therefore, the macerates for further studies were prepared using this ratio.

3.2 Total polyphenols of green extracts of *Mentha × piperita* L., *Salvia officinalis* L., *Thymus vulgaris* L., and *Rosmarinus officinalis* L. prepared at different temperatures and times

A series of experiments was carried out to investigate the influence of extractant temperature and extraction time on total polyphenols. Using water and ethanol as extractants, extraction was carried out at 30, 40, and 50 °C for 30, 60, and 120 min.

Water, being highly polar, efficiently dissolves polar compounds such as many polyphenols in mint, sage, and thyme, by binding to their hydroxyl groups (−OH).[28] Ethanol, less polar than water, is more suitable for extracting moderately polar antioxidants from rosemary, such as flavonoids and phenolic acids (rosmarinic and carnosic acid), due to its ability to dissolve both hydrophilic and lipophilic substances.[29] Many polyphenols in mint, thyme, and sage are glycosylated, increasing their solubility in water. Water also forms stronger hydrogen bonds with these polyphenols than ethanol, enhancing extraction efficiency, whereas rosemary's moderately polar compounds are better extracted with ethanol.[30]

The optimisation of temperature and time revealed that both factors exert a similar effect on total polyphenol concentration in all tested plants. Higher temperatures (50 °C) and longer extraction times (120 min) resulted in increased polyphenol contents in both aqueous and ethanolic extracts (Fig. 1). This effect can be attributed to enhanced solubility, faster diffusion, increased molecular kinetic energy, greater cell wall permeability, and the inactivation of polyphenol-degrading enzymes. Total polyphenol concentrations ranged from 0.47 to 14.94 mg GAE g^{-1}, with the highest yield obtained using water at 50 °C for 120 min. Among the plants tested, thyme and mint showed similar polyphenol contents (24–27 mg GAE g^{-1}), while sage (aqueous extract) and rosemary (ethanol extract) were slightly lower (11–14 mg GAE g^{-1}). These results indicate that the studied plants have a rich polyphenolic composition, though variations may occur due to plant species, growing conditions, and differences in experimental methodologies.

To demonstrate the influence of the tested parameters—temperature, time, and extraction agent—on total polyphenol concentration, as well as to optimise the modelled process, 3D response surface plots were constructed (Figs. 2 and 3).

The 3D response surfaces for the maceration process with ethanol and water in the studied *Lamiaceae* family plants showed the changes in the response as a function of the independent variables, namely temperature and time. These plots allow visual identification of the regions where the response is maximised, which are marked in red. The constructed 3D plots can also be used to identify interactions between the independent variables under investigation.

The constructed 3D models are characterised by high accuracy, as evidenced by the correlation coefficients ranging from 0.9645 to 0.9988.

The results obtained in this study are comparable with those reported in the literature. According to the studies of *Wojdyło et al.*[31], similar ranges of total polyphenol concentrations were observed in the respective plants. These results were obtained using the Folin-Ciocalteu method for the quantification of polyphenols in various plant extracts, and are also consistent with the results of the findings reported by *Stagos et al.*[32] and *Singleton et al.*[33] In the literature, considerably higher concentrations of total polyphenols have been reported for various extracts obtained from plants of the *Lamiaceae* family.[34–36] These higher values

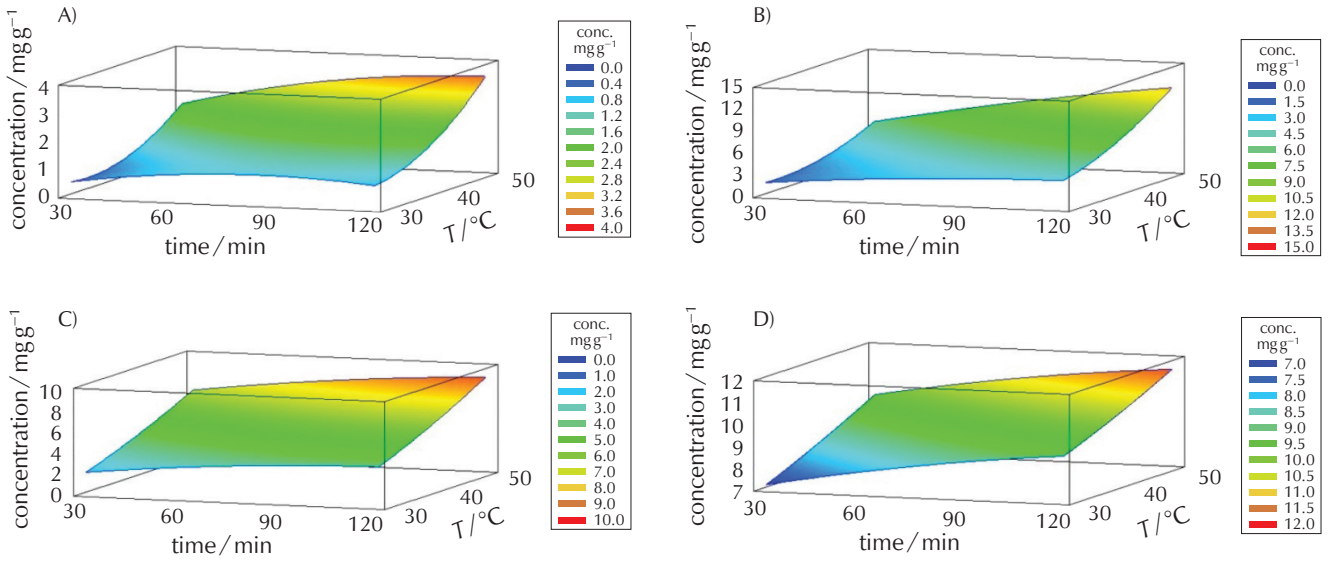

Fig. 2 – 3D reaction surface for the ethanol maceration process of A) mint, B) sage, C) thyme, and D) rosemary

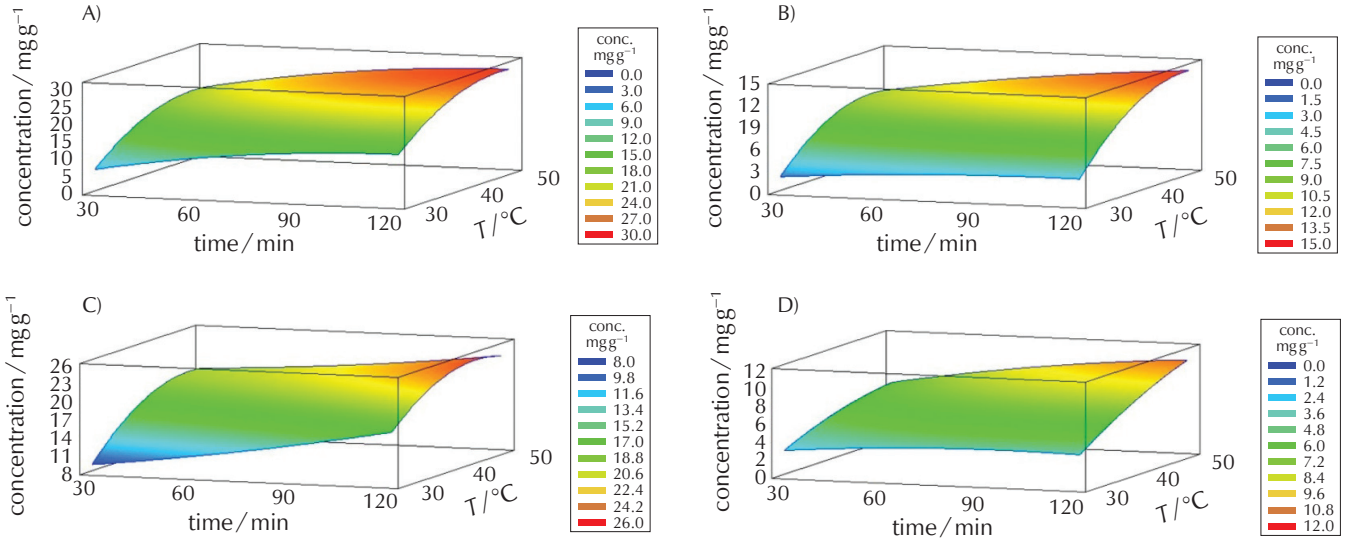

Fig. 3 – 3D reaction surface for the water maceration process of A) mint, B) sage, C) thyme, and D) rosemary

are attributable to the use of different extraction methods, such as Soxhlet extraction, microwave-assisted extraction, supercritical CO_2 extraction, and similar techniques, which generally provide much higher yields of isolated polyphenols.

The use of various organic solvents as extraction agents can also significantly influence the efficiency of polyphenol isolation. However, in the present study, satisfactory concentrations of total polyphenols were obtained using environmentally friendly, non-toxic, and non-polluting solvents. This aspect is particularly important for large-scale industrial extraction processes.

3.3 Antioxidant capacity of green extracts of *Mentha × piperita* L., *Salvia officinalis* L., *Thymus vulgaris* L., and *Rosmarinus officinalis* L. prepared at different temperatures and times

The antioxidant activity of the aqueous and ethanolic extracts of the tested herbs, prepared at different temperatures and times, was evaluated using the DPPH method. The results are expressed as the relative percentage of antioxidant capacity. The DPPH assay, widely used for assessing plant extracts, relies on a stable free radical (DPPH) that reacts with antioxidants, causing a decrease in the solution's absorbance, which is measured spectrophotometrically. The results indicate that both temperature and extraction time directly influence antioxidant activity: higher temperatures and longer extraction times lead to increased antioxidant capacity. The antioxidant activity of the macerates in this study ranged from 3 to 48 %.

Considered individually, mint extracts generally exhibited significant antioxidant activity (Fig. 4). Polyphenolic compounds in mint, such as rosmarinic acid, flavonoids, and essential oils, contribute to its antioxidant properties. The presence of menthol and menthone also contributes to the antioxidant effect by neutralising free radicals. The relative antioxidant capacity of the extract obtained under optimal conditions was 44.6 %. Although antioxidant capacity may vary, it is generally within a range indicative of medium to high antioxidant activity. In the present study, sage extracts exhibited significant antioxidant activity of 42 %, indicating effective neutralisation of free radicals (Fig. 4). This confirms that sage is a strong source of antioxidants. It can also be noted that, although the total polyphenol content was somewhat lower than in the other plants studied, sage still exhibited high antioxidant capacity. This is mainly attributed to the high content of rosmarinic acid, ursolic acid, and other phenolic compounds that enhance its antioxidant capacity. The thyme extracts obtained in this study demonstrated high antioxidant capacity when analysed using DPPH, reaching 47.7 %. These results suggest that thyme extracts possess a strong capacity to neutralise free radicals even at lower levels of total polyphenols, indicating a strong antioxidant activity. Thyme is known for its strong antioxidant properties, which are primarily associated with its high content of phenolic compounds such as thymol and carvacrol.

Unlike thyme, mint, and sage, whose aqueous extracts exhibited high antioxidant activity, rosemary showed greater antioxidant capacity when ethanol was used as the extraction solvent. This is attributed to the higher total polyphenol content in the ethanolic extracts compared to the aqueous extracts. The relative antioxidant capacity of the rosemary ethanolic extract was 27.8 %. Although this value was lower than those of the other tested plants, rosemary still demonstrated notable antioxidant potential, despite its comparatively lower total polyphenol content. Rosemary is rich in polyphenolic compounds such as rosmarinic acid, carnosic acid, and carnosol, all of which possess strong antioxidant properties.

In summary, the results for the antioxidant capacity of the tested plants indicate that they all exhibit high antioxidant

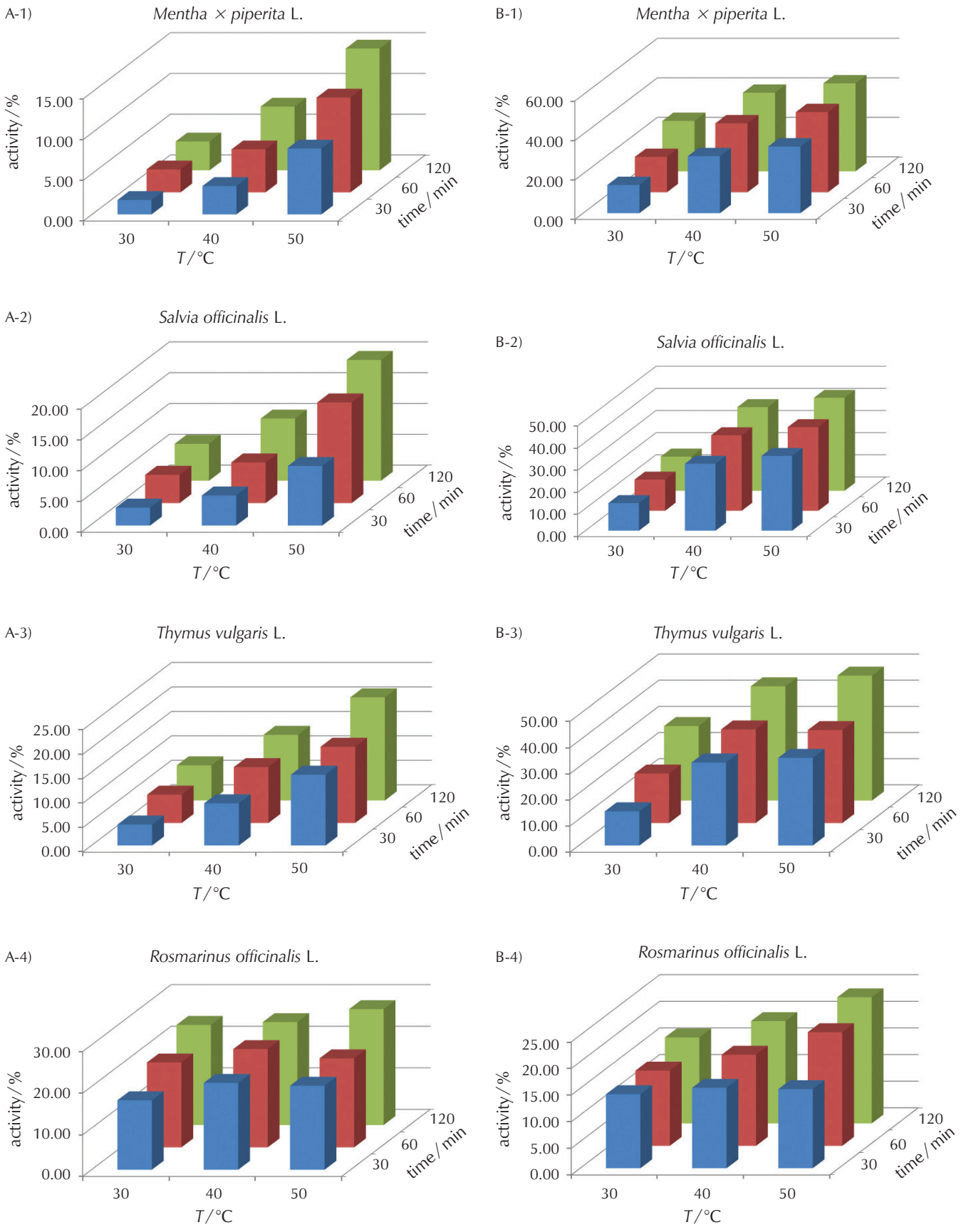

Fig. 4 – Graphical representation of the results of antioxidant activity: A) ethanolic extracts, B) aqueous extracts of *Mentha* × *piperita* L., *Salvia officinalis* L., *Thymus vulgaris* L., and *Rosmarinus officinalis* L. prepared at different temperatures and times

capacity. The antioxidant activity of the plants studied is an important aspect of their biochemical profiles and contributes to various health benefits by neutralising free radicals and reducing oxidative stress.

Using the 3D response surface plots for antioxidant activity during maceration with ethanol and water, the regions corresponding to maximum response can be visually identified. The conditions under which the highest antioxidant activity was achieved are marked in red on the 3D response surface (Figs. 5 and 6.).

The constructed 3D models are characterised by high accuracy, as indicated by correlation coefficients ranging from 0.8972 to 0.9986.

The results of this study are consistent with the literature data on the antioxidant activity of plants from the *Lamiaceae* family. It is confirmed that the studied plants have medium to high antioxidant capacity, which is consistent with the studies of *Abdel-Mawgoud et al.*[37], Kulisic *et al.*[38], *Lu et al.*[39] and *Dorman et al.*[40]

3.4 Determination of total polyphenol content and antioxidant activity of macerates obtained from dry herbal residues after isolation of essential oils by steam distillation

To assess the total polyphenol content and antioxidant activity of dry plant residues, essential oils were first isolated by steam distillation. The residues were then dried, and aqueous extracts were prepared using previously optimised methods. The results indicate that the dry residues still contained polyphenols and exhibited measurable antioxidant activity.

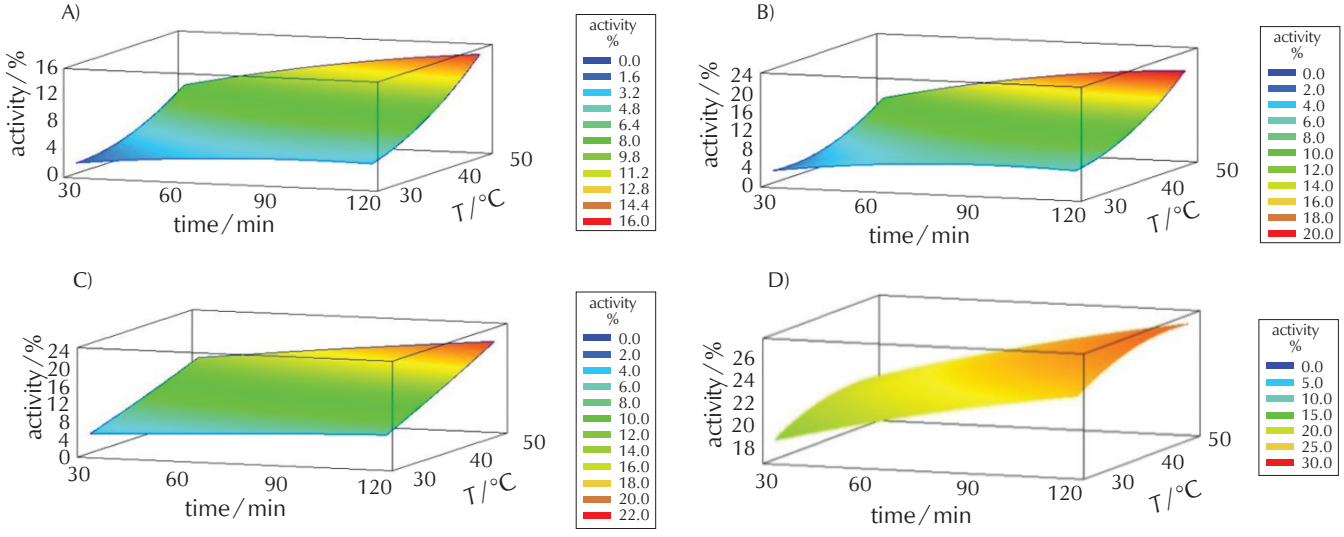

Fig. 5 – 3D reaction surface for antioxidant activity during ethanol maceration of A) mint, B) sage, C) thyme, and D) rosemary

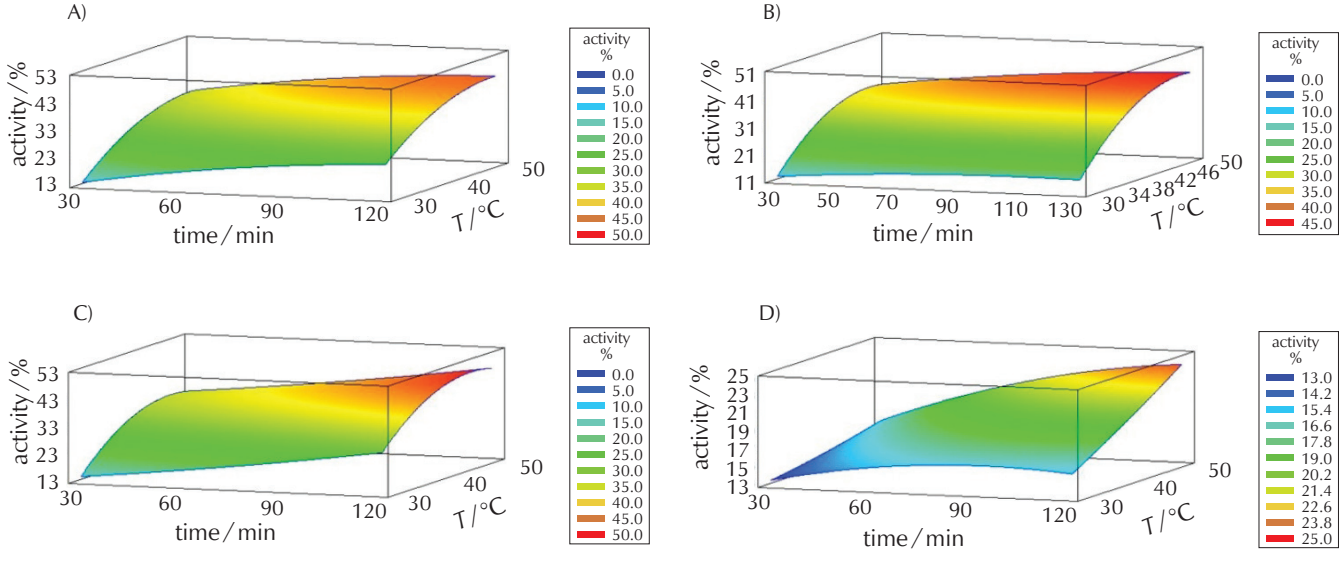

Fig. 6 – 3D response surface for antioxidant activity during water maceration of A) mint, B) sage, C) thyme, and D) rosemary

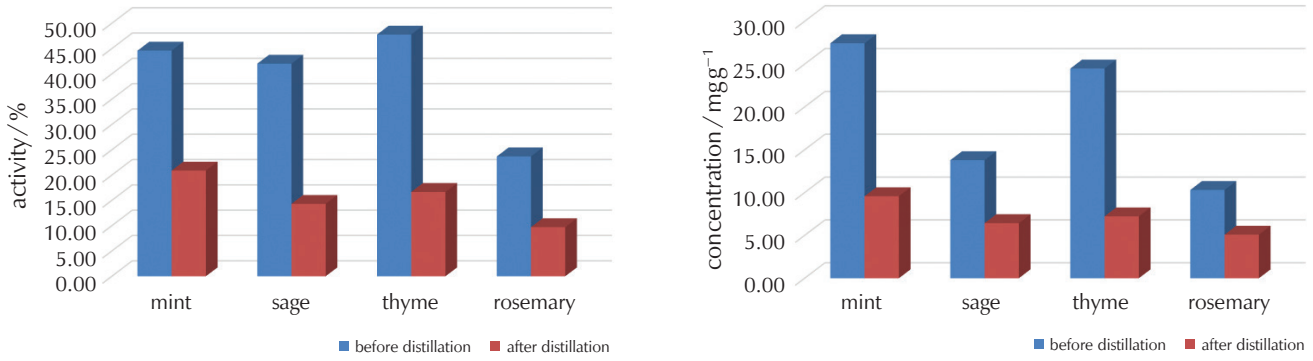

Fig. 7 – Graphical representation of total polyphenol concentration and antioxidant activity of herbal extracts from the *Lamiaceae* family, determined before and after steam distillation

Polyphenol retention varies depending on the plant species, the essential oil extraction method, and the extraction conditions. Steam distillation generally preserves more polyphenols in the residue compared to organic solvent extraction. Residues of rosemary, thyme, sage, and mint contained significant total polyphenols, ranging from 5.09 to 14.87 mg GAE g^{-1} (Fig. 7).

Although antioxidant activity in the residues was lower than that of the extracts prepared before oil isolation – being reduced by 40–60 % – it remained substantial, ranging from 9.72 to 38.74 %.

These findings suggest that, due to their antioxidant properties, dry residues can be utilised in nutritional products, dietary supplements, natural preservatives, functional foods,[41] and cosmetic formulations for skin care. Recycling these residues not only adds value to potential waste but also aligns with sustainable practices by minimising waste and maximising resource utilisation.[42]

4 Conclusion

The total polyphenol content and antioxidant activity of *Mentha × piperita*, *Salvia officinalis, Thymus vulgaris*, and *Rosmarinus officinalis* were analysed. Polyphenols were accurately quantified using the Folin–Ciocalteu method. Solvent selection influenced extraction efficiency: ethanol proved more effective for rosemary, whereas water enabled more efficient extraction of polyphenols from mint, sage, and thyme. Optimal extraction conditions (1 : 100 for water, 1 : 20 for ethanol), together with higher temperatures (50 °C) and longer extraction times (up to 120 min), resulted in increased polyphenol yields.

Mint and thyme showed the highest polyphenol concentrations, while rosemary exhibited strong antioxidant potential despite moderate polyphenol levels. According to the DPPH assay, compounds such as rosmarinic acid, ursolic acid, thymol, and carvacrol contributed to high free-radical scavenging activity (42–47.7 %), with rosemary showing notable antioxidant activity when extracted with ethanol.

This study confirms the usefulness of 3D response surface models for optimising extraction parameters. After steam distillation, the dry residues retained significant antioxidant content, although reduced by 40–60 %, and remain suitable for applications in food, cosmetics, and pharmaceutical industries. Overall, all tested *Lamiaceae* herbs demonstrated significant antioxidant properties. The findings of this study highlight the importance of solvent selection, process optimisation, and sustainable reuse of plant residues to achieve both economic and environmental benefits.

List of abbreviations
Popis kratica

ROS – reactive oxygen species
RNS – reactive nitrogen species
DNA – deoxyribonucleic acid
GAE – gallic acid equivalents
DPPH – 2,2-diphenyl-1-picrylhydrazyl
ABTS – 2,2′-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid
FRAP – ferric reducing antioxidant power
TPTZ – 2,4,6-tripyridyl-s-triazine
ORAC – oxygen radical absorbance capacity
AAPH – 2,2′-azobis-amidinopropane dihydrochloride
UV-VIS – ultraviolet-visible

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SAŽETAK

Procjena antioksidacijskog potencijala ekstrakata aromatičnih biljaka iz porodice *Lamiaceae*

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Antioksidansi štite ljudski organizam od oksidacijskog stresa uzrokovanog reaktivnim kisikovim i dušikovim vrstama, koje oštećuju biomolekule i doprinose razvoju kroničnih bolesti, uključujući rak, kardiovaskularne, metaboličke i neurodegenerativne bolesti. Njihovo djelovanje ostvaruje se putem endogenih (npr. glutation, katalaza) i egzogenih (npr. vitamini i polifenoli) izvora, pri čemu neutraliziraju slobodne radikale, keliraju metalne ione i podupiru detoksikacijske procese. Aromatične biljke iz porodice usnača (*Lamiaceae*), poput kadulje, metvice, majčine dušice i ružmarina, posebno su bogate polifenolnim spojevima izraženog antioksidacijskog djelovanja.

U ovom radu istraženi su sadržaj ukupnih polifenola i antioksidacijski kapacitet etanolnih i vodenih macerata primjenom Folin–Ciocalteu metode i DPPH testa. Rezultati su pokazali da je etanol učinkovitije otapalo za ekstrakciju polifenola iz ružmarina, dok je voda omogućila veći prinos bioaktivnih spojeva iz kadulje, metvice i majčine dušice. Optimalni omjeri biljne sirovine i otapala iznosili su 1 : 20 za etanol te 1 : 100 za vodu, pri čemu je sadržaj ukupnih polifenola iznosio 11 – 14 mg GAE g^{-1} za ružmarin te 24 – 27 mg GAE g^{-1} za metvicu i majčinu dušicu. Također je utvrđeno da temperatura ekstrakcije (50 °C) i vrijeme ekstrakcije (do 120 min) znatno utječu na prinos polifenola.

Iako je ružmarin sadržavao umjerenu količinu polifenola, pokazao je izražen antioksidacijski potencijal s učinkovitošću uklanjanja DPPH radikala od 27,8 %. Najveću antioksidacijsku aktivnost ostvarili su ekstrakti metvice i majčine dušice s vrijednostima DPPH aktivnosti od 42 do 47,7 %. Dobiveni rezultati potvrđuju da biljke iz porodice usnača predstavljaju održiv i vrijedan prirodni izvor antioksidansa s potencijalnom primjenom u prehrambenoj, kozmetičkoj i farmaceutskoj industriji.

Ključne riječi
Antioksidacijska aktivnost, polifenoli, biljke iz porodice usnača (Lamiaceae), etanolni i vodeni ekstrakti, DPPH test, održivi prirodni izvori

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