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COMPARATIVE CHARACTERIZATION OF BASIL, MINT AND SAGE EXTRACTS

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ABSTRACT. Aromatic plants are widely used in food preparation for aroma and fragrance, but they are also a good source of amino acids and fatty acids. Three indigenous species, Basil (*Ocimum basilicum*), mint (*Mentha piperita*) and sage (*Salvia officinalis*) which are traditionally used in medicine and food, were characterized and compared in terms of volatile extracts, amino acids, fatty acids contents and antioxidant activities. The gas chromatographic–mass spectrometry (GC–MS) method is a suitable technique for the characterization of the compounds of the herbs extracts. Antioxidant activity was measured using extracts ability to scavenge DPPH radicals. Linalool was found the major compound in basil, menthol in mint and α-thujone in sage. The dominant amino acids identified in the plants extracts were glutamic acid and aspartic acid in basil and mint, while proline was found in high concentration in sage and mint. The total free fatty acids (TFA) were highest in mint, followed by sage and basil. The essential omega 3 alpha-linolenic acid (ALA) was identified in all three extracts. All samples exhibited antioxidant activity, sage extract having the highest antioxidant activity.

Keywords: *Ocimum basilicum, Mentha piperita, Salvia officinalis, volatile compounds, amino acids, fatty acids.*

INTRODUCTION

Basil (*Ocimum basilicum*), mint (*Mentha piperita*) and sage (*Salvia officinalis*), belonging to the Lamiaceae family, are medicinal plants and culinary herbs due to their delicate aroma and fragrance. Basil, one of the most important

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and wildly used spices, has been shown to have antioxidant, antibacterial, and anti-diarrheal activities [1]. Mint (*Mentha piperita*) has anti-inflammatory, cytotoxic and antioxidant activities [2]. Sage (*Salvia officinalis*) has a savory, peppery flavor and is used as condiment. Sage's studies in healthy humans have demonstrated improved memory, attention/executive function, alertness and mood, improved cognitive functioning and behavioral function [3-5]. It can be used also in treating digestive disorders such as poor digestion and bloating.

Herbs are often used in our food as condiments, tea or in pharmaceutical extracts. Most of these herbs are investigated for their nutritional and pharmaceutical properties. The optimization of micronutrients in our food is very important. Amino acids play an important role in human nutrition. The level of essential amino acids dictates food nutritive value. The free amino acids have an important effect in food flavor, influence its palatability, and contribute to the formation of amines and volatile compounds. Omega-3 fatty acids supplementations in humans have beneficial effects on subjective global assessment score and metabolic profiles. Amino acids, fatty acids and qualitative chemical composition of herbs may be influenced by environmental and geographical conditions.

The aim of investigations was to determine the differences between three herbs purchased from Romania with respect of the essential amino acids and fatty acids present in this herbs often used as tea or condiments. Also their volatile compounds and antioxidant activity were compared.

For the characterization of the compounds in herbs extracts, gas chromatography-mass spectrometry (GC-MS) analysis is a suitable technique. Amino acids and fatty acids were first derivatizated to obtain trifluoroacetyl ester derivatives in the case of amino acids [6-11] and fatty acids methyl esters (FAMEs) for free fatty acids [12-17]. Antioxidant activities of extracts were also compared.

RESULTS AND DISCUSSION

In the present work we have evaluated and compared the chemical composition of volatiles, amino acids and fatty acids contents in three herbs species: basil, mint and sage. For compounds characterization of herbs extracts, different approaches involving extraction methods, purification by ion exchange technique in the case of amino acids, derivatization steps and gas chromatography-mass spectrometry (GC-MS) analysis were applied.

For the amino acids and fatty acids analysis, the methods were validated injecting standard solutions of amino acids and fatty acids respectively. Samples are following the same derivatization procedure as standards. Good values for linearity, precision, accuracy and limit of detection were obtained [6].

The quantitative method gave a good linearity regression curve, obtained with standards with known concentration of each amino acid, in the range 0-100μg×mL^{-1} and by adding the same quantity of internal standard (50μg×mL^{-1}). The internal standard, ^{15}N-glycine, (99 atom % ^{15}N) and glycine required correction by deconvolution and matrix calculation. Fractional isotopic abundances for natural glycine and isotopomer were obtained experimentally [8,9] (Table 1).

Table 1. The matrix design (left) and the pseudoinverse matrix (right) used for glycine calculation [6]

Glycine	[M]	[M+1]	Glycine	[M]	[M+1]
n.a.	0.95	0.05	n.a.	1.05	-0.05
^{15}N	0.01	0.99	^{15}N	-0.01	1.01

Glycine was calculated by matrix and regression curve calculation. Precision and accuracy for glycine, measured for standards of 20 and 30 μg× mL^{-1} (n=7), showed very good results, lower than 6% and respectively 11%.

Method validation, using amino acid standards following the extraction and derivatization procedure (n=3), gave good results. The regression curves for each aminoacid standard were studied between of 0 and 150 mg×mL^{-1}, and the same quantity of internal standard was added. Good linearity results for amino acids were found, the regression coefficient over 0.99 for Ala, Gly,Thr, Ser, Leu, Ile, Pro, Orn and higher than 0.97 for the other standards. The precision and accuracy were lower than 20%, for standards of 60 and 80 mg×mL^{-1}, respectively. The limits of detection (LODs) ranged from 10^{-3} for alanine, glycine, ornithine, and leucine to 10^{-2} mg mL^{-1}.

The volatile extracts of basil gave as major compounds: linalool (26.13%), estragole (21.16%), caryophyllene (10.13%) and limonene (8.18%). Sage gave as major compounds: α-thujone (25.08%), camphor (20.46 %), eucalyptol (13.85 %) and β-thujone (13.37 %) (Fig.1). In mint, the major compounds were menthol (37.7%), isomenthone (15.97%), eucalyptol (5.44%) and menthofuran (4.8%) (Fig. 2).

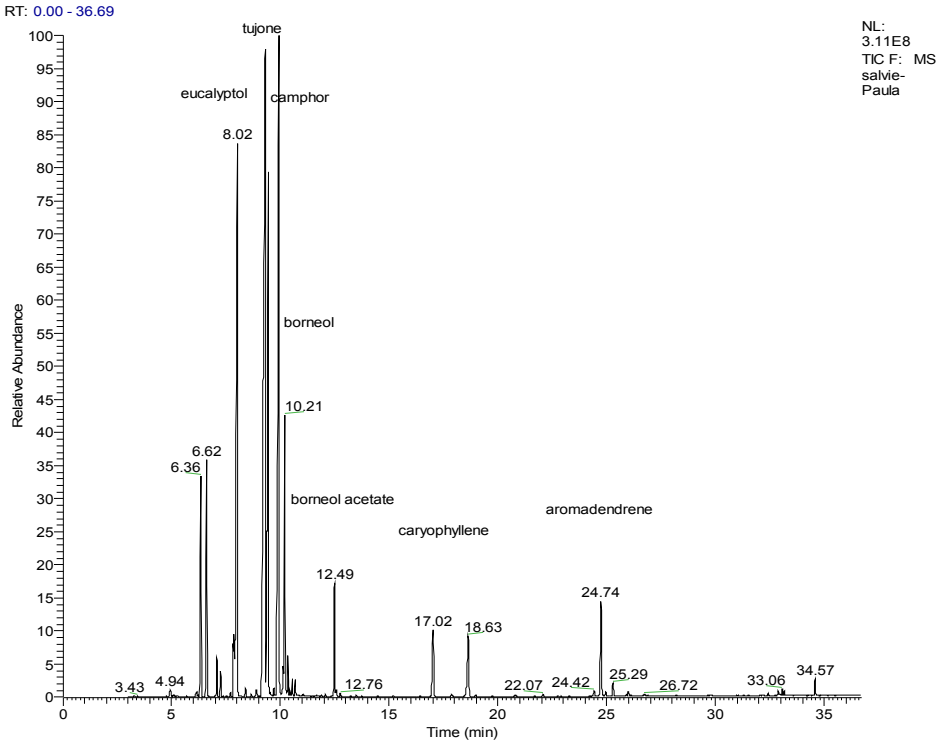

Figure 1. Identification of sage volatiles separated on a Rtx-5MS capillary column

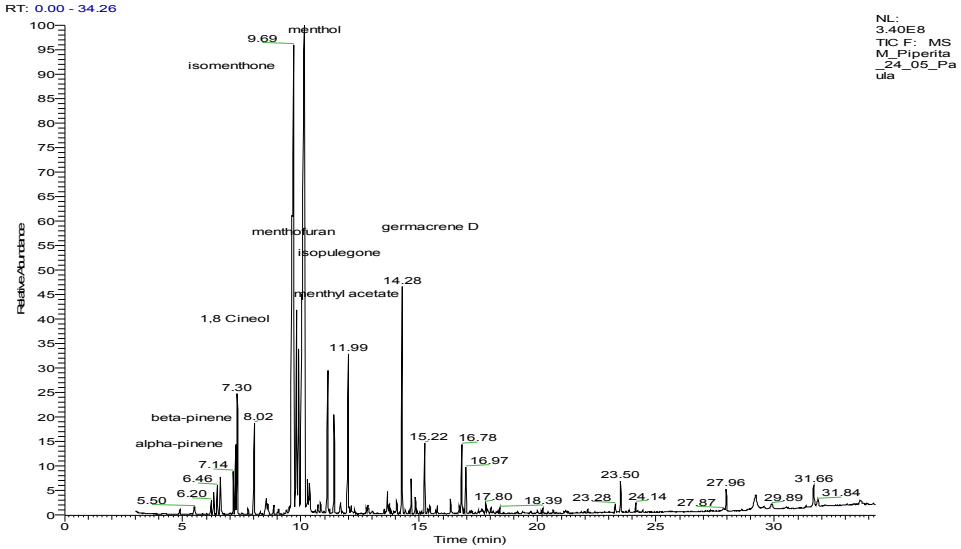

Figure 2. The chromatogram of volatiles identified in mint extract

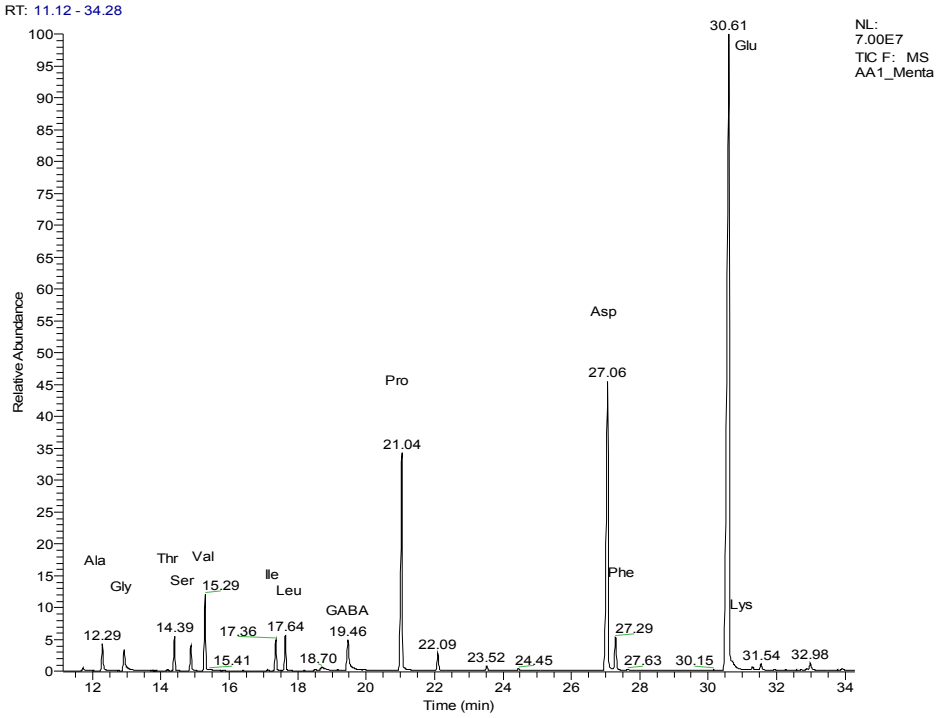

Figure 3. GC-MS separation and identification of amino acids present in mint extract

Fig. 3 presents the total ion chromatogram (TIC) of amino acids separated and identified in mint extract. NIST library was used for compounds identification. The elution order was: alanine (Ala), glycine (Gly), threonine (Thr), serine (Ser), valine (Val), leucine (Leu), isoleucine (Ile), γ-aminobutiric acid (GABA), proline (Pro), methionine (Met), aspartic acid (Asp), phenylalanine (Phe), ornitine (Orn), glutamic acid (Glu), lysine (Lys), tyrosine (Tyr).

The dominant amino acids identified were glutamic acid and aspartic acid in basil and mint and proline content was higher in sage and mint. We found that in sage, proline, glutamic acid, gama-aminobutiric acid, alanine, valine and glycine were important quantitatively. The highest free total amino acids were observed in mint and sage, over 1 mg.·g^{-1} (Fig.4). Fig. 5 presents the ratio EAA/TAA (essential amino acids/total amino acids) which is higher in basil, followed by mint and sage. The highest concentration of essential amino acids were found in mint (1.07mg.g^{-1}), followed by sage (0.33 mg.g^{-1}) and basil (0.1 mg.g^{-1}) (Table 2, Fig. 6).

Analyzing the fatty acids content in these three herbs, essential omega 3 alpha-linolenic acid (ALA) were identified and the highest content was found in basil, followed by sage and mint. Also, the total free fatty acids

(TFA) content was determined in basil (72.8 mg.g^{-1}), mint (13.3 mg.g^{-1}) and sage (98.8 mg.g^{-1}). The unsaturated fatty acids (UFA) and polyunsaturated fatty acids (PUFA) were analyzed, the content been higher in sage and basil in comparison with mint. The ratio UFA/SFA was higher in basil and mint, followed by sage (Table 3, Fig. 8).

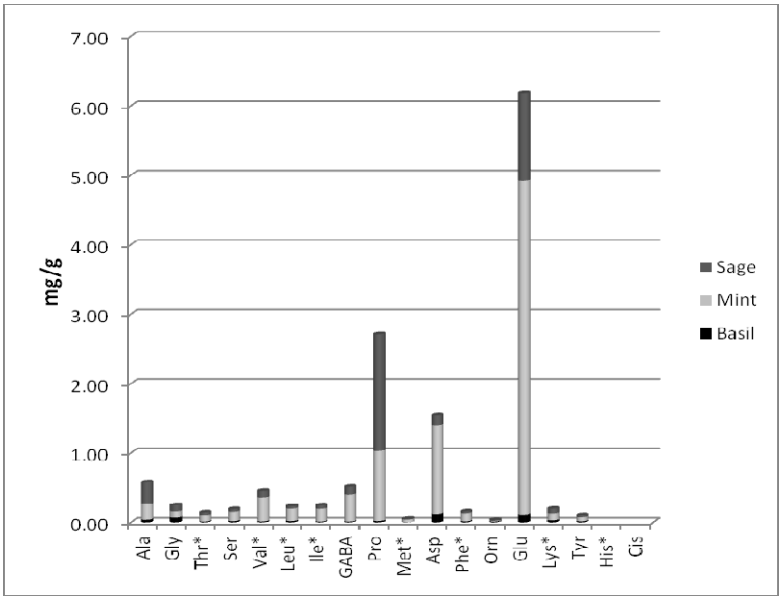

Figure 4. Free amino acids comparison in basil, sage and mint extracts (mg.g^{-1})

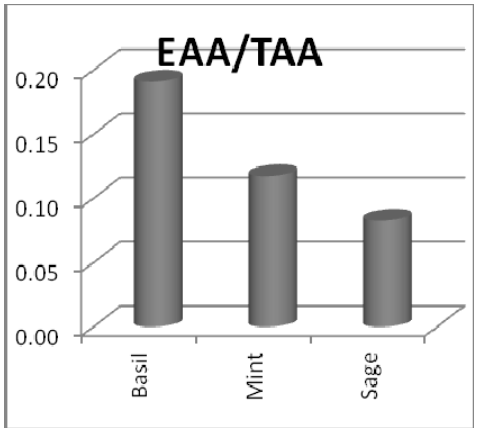

Figure 5. Free amino acids comparison in basil, sage and mint extracts (mg.g^{-1})

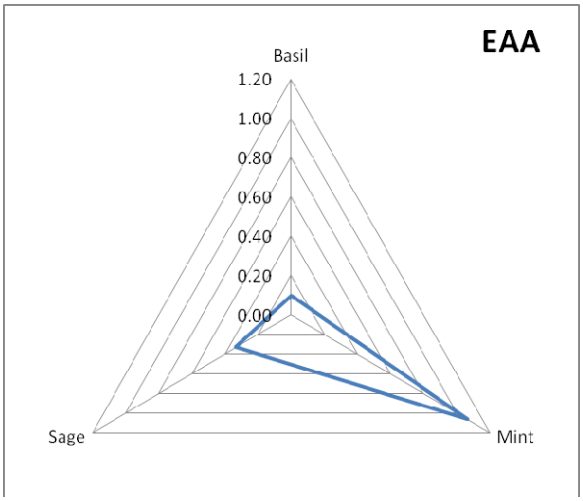

Figure 6. Comparison of essential amino acids in the three herbs extracts (mg.·g^{-1})

Table 2. Amino acids values, in mg.·g^{-1}, in the analyzed herbs

AA/Herbs	Basil	Mint	Sage
Ala	0.04	0.22	0.32
Gly	0.07	0.09	0.09
Thr*	0.01	0.09	0.04
Ser	0.02	0.14	0.04
Val*	0.01	0.34	0.10
Leu*	0.02	0.18	0.04
Ile*	0.01	0.19	0.05
GABA	0.01	0.38	0.12
Pro	0.02	1.01	1.68
Met*	0.00	0.04	0.01
Asp	0.12	1.28	0.15
Phe*	0.01	0.12	0.03
Orn	0.01	0.01	0.01
Glu	0.11	4.81	1.26
Lys*	0.03	0.10	0.07
Tyr	0.02	0.06	0.04
TAA	0.51	9.07	4.03
EAA	0.10	1.07	0.33
EAA/TAA	0.19	0.12	0.08

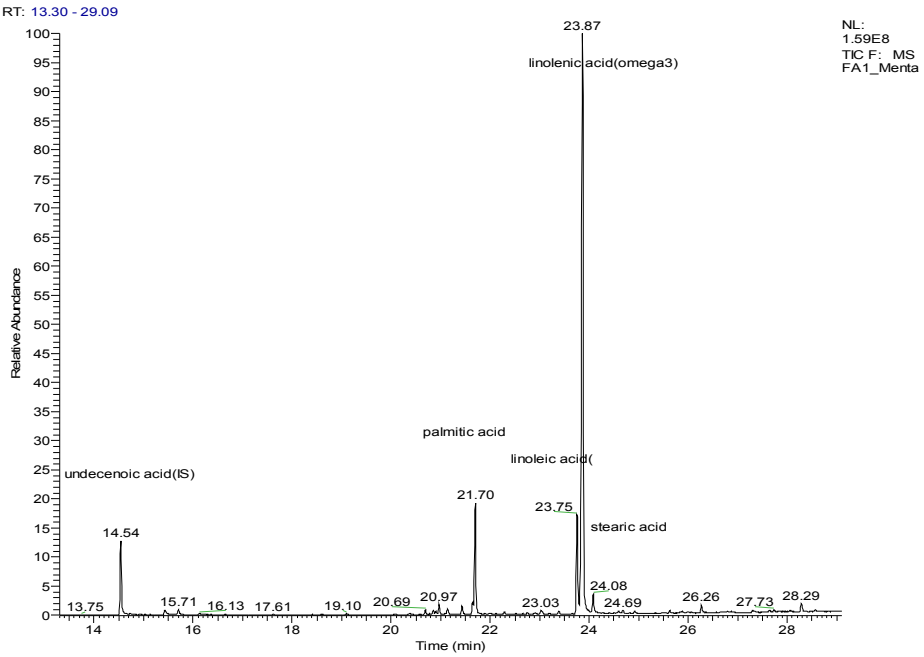

Figure 7. GC-MS separation and identification of fatty acids in mint extract

The identified fatty acids separated as FAMEs in the mint extract is presented in Fig.7. The omega-3 linolenic acid was the highest concentrated fatty acid determined in each herb extract.

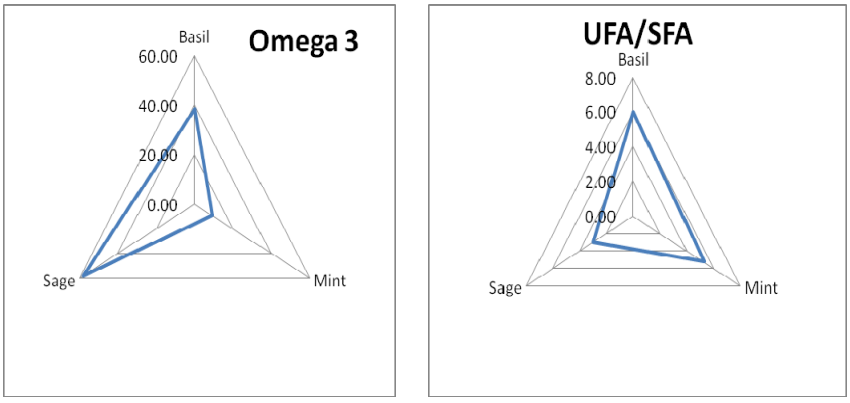

Figure 8. Linolenic acid and UFA/SFA ratio comparison in the herbs studied

Table 3. Free fatty acids(FA) values, in mg.·g^{-1}, in the analyzed herbs

FA	Basil	Mint	Sage
C16:1	0.12	0.18	0.60
C16:0	7.12	1.44	13.65
C18:2	23.89	1.44	15.36
C18:3	38.39	9.55	58.11
C18:0	2.66	0.37	3.54
C20:0	0.26	0.13	4.33
C22:0	0.13	0.13	1.81
C24:0	0.18	0.04	1.38
omega6/omega3	0.62	0.15	0.26
MUFA	0.12	0.18	0.60
UFA	62.41	11.18	74.06
SFA	10.35	2.11	24.70
PUFA	62.28	11.00	73.46
UFA/SFA	6,03	5,30	3,00
TFA	72,76	13,29	98,76

Antioxidant activity was determined using DPPH scavenging activity. All three herbs ethanolic extracts showed an antioxidant activity. Sage and mint extracts proved to have a very high antioxidant potential, while basil showed a moderate antioxidant potential. The half maximal effective concentration (EC_{50}) for each type of extract was determined. The most antioxidant extact was sage extract (EC_{50}= 8.22 μg*mL^{-1}), followed by mint extract EC_{50}=13.65 μg*mL^{-1}, and basil extract EC_{50}=112.58 μg*mL^{-1}. The procentage of DPPH scaveging activity at 50 μg*mL^{-1} of each plant extract, after 30 minutes was determined and the highest value was obtained in sage extract (92.26%) followed by mint extract (88.66%) and basil extract (24.28%).

Comparing the DPPH scavenging activity of these three plants extracts with plants extracts from other geographical sources, showed that we have obtained similar results. Our sage extract (92.26%), from Târgu Mures area, proved to have comparable DPPH scavenging activity with other sage extracts from different Romanian sources (85.12%) [18], but higher then methanolic extract from Tunisia. (EC_{50}=29.33 μg*mL^{-1}) [19]. *Mentha piperita*, from Târgu Mures (EC_{50}=13.65 μg*mL^{-1}) area, exhibits a lightly higher activity then *Mentha piperita* from Northeastern Algeria (EC_{50}=17.00μg*mL^{-1}) [20], while DPPH scavenging activity of our mint ethanolic extracts (88.66%) showed higher activity than diethylether mint extract from Saudi Arabia (34.21%) [21].

The EC 50% of our Ocimum basilicum etahnolic extract (EC50=112.58 μg*mL^{-1}) showed comparable activity with other ethanolic basil extract from different Romanian sources (EC_{50}=124.95 μg*mL^{-1}) [22], while aqueous basil extract from Serbia (EC_{50}=17.93 μg*mL^{-1}) showed a powerful scavenging activity [23].

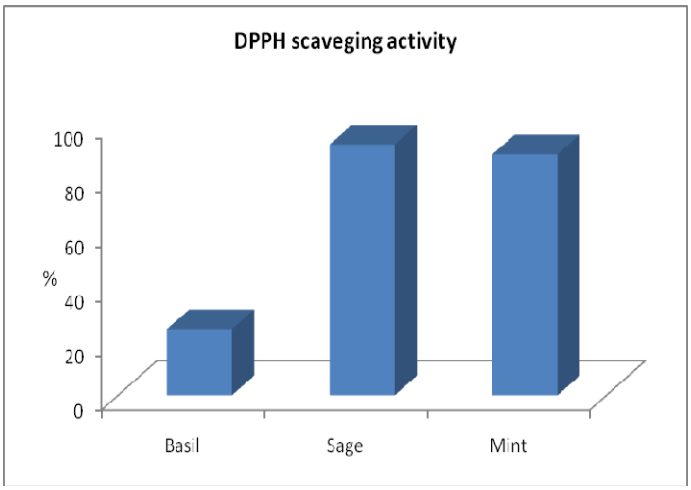

Figure 9. DPPH scavenging activity of ethanolic herbs extracts (50 μg*mL^{-1})

CONCLUSIONS

The GC-MS is a suitable technique for the characterization of the compounds of the herbs extracts. The validation parameters: linearity, correlation coefficients, precision, accuracy, in the range of interest, were good. Isotopic dilution by using a labeled internal standard increased precision and avoids the overlapping of analyzed compounds. The methods are useful for nutrient and diet control. The compounds identified in the herbs studied are characteristic for the odour or aroma of these plants.

The highest free total amino acids were observed in mint and sage (>4 mg.·g^{-1}). Proline was higher in sage and mint. The presence of omega 3 fatty acid is very important for healthy and the highest quantity was found in sage, followed by basil and mint. Sage and mint extract proved to have a high and comparable antioxidant activity, while basil extract have a moderate antioxidant activity.

EXPERIMENTAL SECTION

Materials and methods

Plants**,** basil (*Ocimum basilicum*), mint (*Mentha piperita*) and sage (*Salvia officinalis*), were purchased from Botanical Garden of Târgu Mures, Transylvania, Romania. All reactive and standards were purchased from Merck (Darmstadt, Germany).

GC–MS apparatus

A DSQ Thermo Finnigan Proanalysis, Bucharest, Romania quadrupole mass spectrometer coupled with a Trace GC was used. Gas chromatography was performed on a 5% phenyl methylpolysiloxane Rtx-5MS capillary column, 30 m × 0.25 mm, 0.25 μm film thickness in a suitable temperature program. In the case of amino acids separation the program was: from 70 ^{o}C, 2 min, 5 ^{o}C/min to 110 ^{o}C, 10 ^{o}C/min to 290 ^{o}C, 16 ^{o}C/min to 300 ^{o}C. [1]. The temperature program for FAMEs and volatiles separation was: 50°C for 2 min rising with a rate of 8°C/min at 310°C (8 minutes). Helium was used as carrier gas at a flow rate of 1 mL/min. 1μL of each sample was injected into the GC-MS using the split mode (10:1) using a TriPlus autosampler (Proanalysis, Bucharest, Romania). The mass spectrometer was operated in EI mode at 70 eV, emission current was 100μA and mass spectra mass range 50-500 a.m.u. Transfer line temperature was set at 250^{o}C, injector at 250^{o}C and ion source at 250^{o}C. Antioxidant activity was determined using a Varian Cary 50 Spectrophotometer.

Extraction procedures

For extraction and derivatization of amino acids, 100 mg of crushed leaves were extracted with 1 ml of 6% trichloroacetic acid in a ultrasound bath for 5 min. The mixture was centrifuged for 5 min at 6000 rpm and supernatant was collected for purification. 0.5 ml of the supernatant and 50 μg [^{15}N]-glycine (internal standard) was passed through a Dowex 50W-W8 exchange resin, 4 x 40 mm column (activated). The collected solution was dried in a nitrogen flow at 60°C or by using a vacuum centrifuge at 60°C. The derivatization method included an esterification of the carboxylic function using 200 μl butanol : acetyl chloride (4:1 v/v), for 1 h at 110^{o}C, followed by an acetylation of the amine function using 100 μl trifluoroacetic anhydride, for 20 min at 80°C.

For extraction and derivatization of fatty acids, 100mg of crushed leaves was sonicated with 0.6 ml water/NaCl and 0.8 ml methanol for 1 min, then mixed with 0.8 ml chloroform and 3 min centrifuged (5800 rot/min); the lower layer was collected and extraction was repeated with 0.4 ml chloroform. The lower chloroform phase containing the extracted fatty acids was then dried in a nitrogen flow, at 60°C.

The lipids were converted to corresponding FAMEs by esterification of the carboxylic functions with 200 µL methanol: acetyl chloride 4:1 (v:v), 20 min, 80°C. The derivatives were evaporated to dryness by a nitrogen stream, at 60°C, and then dissolved in 500 µL dichloromethane. 10 µg of C11:1 was added to each sample for GC-MS quantitation.

For extraction of volatiles, 100 mg of crushed leaves were ultrasounded and extracted with 1mL ethanol at 60°C for 15 minutes. The mixture was centrifuged at 5800 rpm and the supernatant collected filtred and injected in the GC/MS and tested for antioxidant activity.

For determination of antioxidant activity, DPPH antioxidant assay was used. A solution of 40µM 2,2-diphenyl-1-picrylhydrazil (DPPH•) in ethanol was decolorize using different concentration of each plant extract. The monitoring of DPPH reduction was followed at 517nm. The percentage of DPPH scavenging activity is expressed using following formula: $DPPH_{inhibition}\% = [(A_i-A_t)/A_i]x100$. For determination of effective concentration (EC_{50}), different concentration of each plant extract 5 µg/mL,10 µg/mL, 20 µg/mL, 50 µg/mL, 100µg/mL were used. The EC_{50} was determined by plotting the $DPPH_{inhibition}\%$ against used extract concentration.

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