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OIL CONTENT OF SUNFLOWER GENOTYPES IN YEARS 2020 AND 2021 IN FUNDULEA LOCATION

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Abstract

At NARDI Fundulea, sunflower breeding program is focused for developing sunflower hybrids with high seed oil content, with resistance at main pathogens such as Plasmopara halstedii, Sclerotinia sclerotiorum, Phomopsis helianthi and with resistance at sulfonylurea herbicides (SU) and to imidazolinone (CL Plus) herbicides.

Temperature and precipitation influence seed oil content of sunflower genotypes in booth years 2020 and 2021, in Fundulea location. The amount of precipitation during the sunflower vegetation period from April to September, in both years 2020 (248.6 mm) and 2021 (273.2 mm), was lower than the 60-year average amount (351.8mm).

Average seed oil content, in year 2020, was included in the values of 48.63% at sunflower experimental hybrid H54CLP in Clearfield Plus system and 51.79% at sunflower experimental hybrid H87SU in Express Sun system and in year 2021, was included in the values of 41.69% at H54CLP and 50.56% at H87SU. Agricultural year (2020, 2021), sunflower hybrid and their interaction, influence significant positive the oil content of seed.

In year 2020 in Fundulea location, pathogen Plasmopara halstedii has an attack degree between 3% at H76SU, H50CLP and 17% at H54CLP and in year 2021, between 6% at H76SU, H50CLP and 23% at H54CLP.

Key words**: **seed oil content, system Clearfield Plus, system Express Sun, sunflower hybrids

INTRODUCTION

Sunflower (*Helianthus annuus L.)* is an important culture in Romania mainly for oil production and more than one million hectares are cultivated (www.madr.ro). An ideal sunflower genotype must have high seed yield (up to 4t\ha), high oil seed content (up to 50%), must be resistant at new races of pathogen *Plasmopara halstedii* (sunflower downy mildew), resistant at new races of parasite *Orobanche cumana* (broomrape), resistant at birds attack, resistant at *Tanymecus dilaticollis* attack, resistant to lodging, resistant to drought, resistant at herbicide in system Clearfield Plus/Express Sun. At NARDI Fundulea in sunflower breeding program we are focused in obtaining sunflower hybrids for oil production with resistance at herbicide, resistance to drought and with resistance at pathogens (*Plasmopara halstedii Farl. Berl. & de Toni*, *Sclerotinia sclerotiorum, Botrytis cinerea, Diaporthe helianthi f.c. Phomopsis helianthi, Leptosphaeria lindquistii f.c. Phoma macdonaldi, Puccinia helianthi, Alternaria helianthi*) and parasite (*Orobanche cumana Wallr*). Sunflower downy mildew produced by the pathogen *Plasmopara halstedii* has a negative impact on sunflower production causing

losses of up to 100% (Elameen et al, 2022).

The seed yield of sunflower hybrids is highly influenced by the environment, and the oil content is not greatly affected by it (Ahmed et al, 2020). Pekcan et al (2021) affirm that seed yield of sunflower hybrids, affect positively oil content under drought stress conditions and Ernst et al (2016) affirm that oil content in seeds has a negative correlation to seed yield under application of plant growth regulators (PGR). Sunflower resist longer dry periods, is tolerant to high temperatures and land suitability of sunflower decreases with over 90% by year 2070 (Mrazova et al, 2017).

MATERIALS AND METHODS

Experimental sunflower hybrids semi late, belonging to NARDI Fundulea, was sowing in two years 2020 and 2021, in non-irrigate field, in Fundulea location, in three repetition in randomized block, 7m long on four rows. We make observation regarding seed oil content, resistance at pathogens and seed yield production (t\ha at 9% humidity). Three semi late experimental sunflower hybrids CLP, H50CLP, H52CLP and H54CLP, in system Clearfield Plus was treated in the development stage of four-six true leaves with 2l\ha herbicide Pulsar Plus (active substance imazamox 25g \l) and three semi late experimental sunflower hybrids SU, H76 SU, H87 SU and H90 SU, in system Express Sun was treated with 50g\ha herbicide Express 50 SG (active substance tribenuron metil: 500 g/kg). In year 2020, experimental sunflower hybrids, was sowing in 7.04.2020 and erbicide treatment was on 11.05.2020 and in year 2021, was sowing in 15.04.2021 and erbicide treatment was on 30.05.2021. Seed oil content was measuring with RI Analysis, Oxford Instruments MQC+.

RESULTS AND DISCUSSIONS

Precipitations registered in Fundulea, in year 2020 from months March to August was under multi annual average of 60 years (tab. 1 and fig. 1*)* and because of that was a dry year and year 2021 was the same, excepting month June with 135mm who was the biggest precipitation from all months of year. In year 2020, in period of vegetation of sunflower, from month April up to September, was registered 248.6 mm and in year 2021, was registered 273.2 mm. The soil water reserve from January to April was in 2020 only 62.4 mm, in 2021 was 183.2 mm and multi annual average of 60 years was 149.6 mm.

Average monthly temperature in April, in 2021 was 9.7^{o}C and because of that the emergence of sunflower plants was delayed (tab. 2 and fig.2). Average monthly temperature in May, in 2020 and 2021 was 17^{o}C and 17.2^{o}C and was favorable for the development of the *Plasmopara halstedii* pathogen.

Table 1. Precipitation (mm) registered in Fundulea location, in years 2020 and 2021

Month	Year		Multi annual average of 60 years
	2020 Precipitations (mm)	2021 Precipitations (mm)	
January	2	77	35.1
February	16.6	16.2	32
March	29.8	59	37.4
April	14	31	45.1
May	58	57.6	62.5
June	68.4	135	74.9
July	34.2	21.2	71.1
August	5.4	24.4	49.7
September	68.6	4	48.5
October	28.6	56.4	42.3
November	20	33.8	42
December	77.6	37.6	43.7

Table 2. Temperature (°C) in Fundulea location in years 2020 and 2021

Month	Year		Multi annual average of 60 years Temperature (°C)
	2020 Temperature (°C)	2021 Temperature (°C)	
January	0.9	1.6	-2.4
February	5.2	3.2	-0.4
March	8.3	5.1	4.9
April	12.3	9.7	11.3
May	17	17.2	17
June	21.7	21.1	20.8
July	25.1	25.3	22.7
August	25.5	24.2	22.3
September	20.8	17.3	17.5
October	14.7	10.2	11.3
November	6.1	7.7	5.4
December	3.9	2.6	0

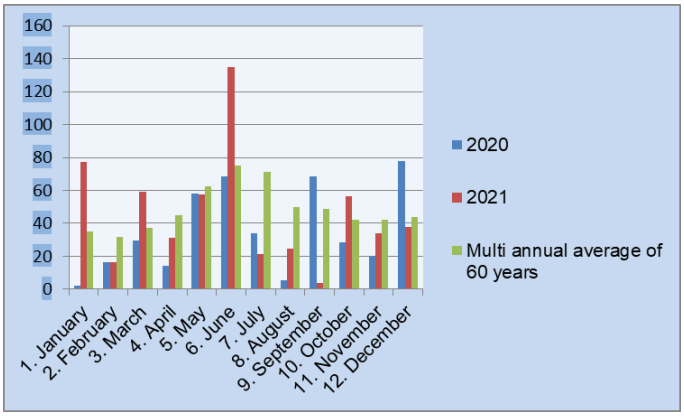

Figure 1. Precipitations (mm) registered in years 2020 and 2021, in Fundulea location

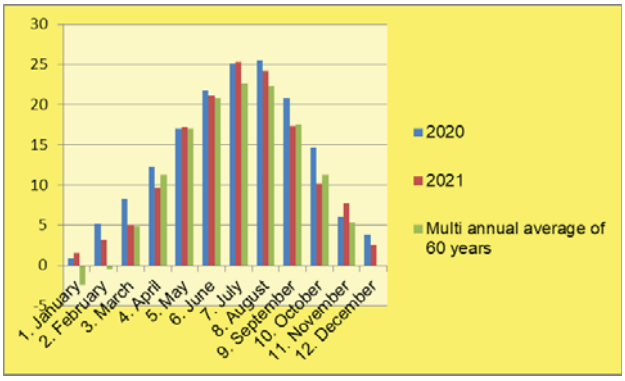

Figure 2. Temperature (°C) registered in years 2020 and 2021, in Fundulea location

Average of hectolitre weight (kg\hl) in year 2020 was between 51.1kg\hl at experimental sunflower hybrid H52CLP in system Clearfield Plus and 63.6kg\hl at check, commercial sunflower hybrid FD15E27 in system Express Sun and has a bigger value then in year 2021, when was between 42.8kg\hl at experimental sunflower hybrid H87SU in system Express Sun and 49.6kg\hl at experimental sunflower hybrid H90SU in system Express Sun (tab.3).

Table 3. Hectolitre weight (kg\hl) of experimental sunflower hybrids in Fundulea location, in years 2020 and 2021

Sunflower genotype	Hectolitre weight (Average of three repetition)	
	2020	2021
H76 SU	52.42 kg\hl	43.8 kg\hl

H87 SU	52.03 kg\hl	42.8 kg\hl
H90 SU	54.63 kg\hl	49.6 kg\hl
H50CLP	51.26 kg\hl	46.1 kg\hl
H52CLP	51.1 kg\hl	43.3 kg\hl
H54CLP	51.34 kg\hl	49.1 kg\hl
FD15E27-Check	63.6 kg\hl	45.17 kg\hl

Average of seed yield (kg\ha) in year 2020 was between 1861kg\ha at experimental sunflower hybrid H54CLP in system Clearfield Plus and 2675kg\ha at experimental sunflower hybrid H50CLP in system Clearfield Plus and in year 2021, was between 3103kg\ha at check, commercial sunflower hybrid FD15E27 in system Express Sun and 3488kg\ha at experimental sunflower hybrid H87SU in system Express Sun (tab.4).

Table 4. Seed yield of experimental sunflower hybrids in Fundulea location, in years 2020 and 2021

Sunflower genotype	Seed yield (Average of three repetition)	
	2020	2021
H76 SU	2170kg\ha	3115kg\ha
H87 SU	2050kg\hl	3488kg\ha
H90 SU	2129kg\hl	3418kg\ha
H50CLP	2675kg\ha	3360kg\ha
H52CLP	2069kg\ha	3228kg\ha
H54CLP	1861kg\ha	3118kg\ha
FD15E27-Check	2538g\ha	3103kg\ha

In Fundulea, in year 2020 pathogen *Plasmopara halstedii*, has an attack degree between 3% at experimental sunflower hybrids H76SU in system Express Sun, H50CLP in system Clearfield Plus and 17% at H54CLP in system Clearfield Plus and in year 2021 between 6% at experimental sunflower hybrid H76SU in system Express Sun, H50CLP in system Clearfield Plus and 23% at H54CLP in system Clearfield Plus (fig.3).

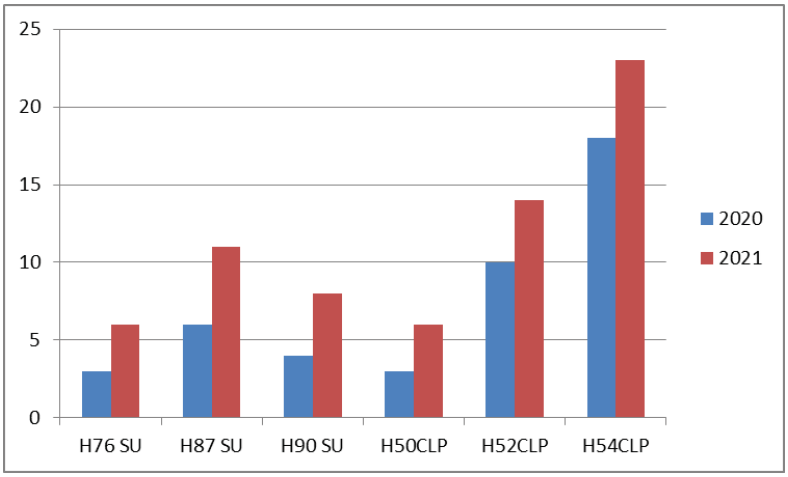

Figure 3. Attack degree of pathogen *Plasmopara halstedii* in Fundulea location in years 2020 and 2021

Average seed oil content of sunflower hybrids, in Fundulea, in year 2020 was between 48.55% at commercial sunflower hybrid (check) FD15E27 in system Express Sun and 51.79% at experimental sunflower hybrid H87SU in system

Express Sun and in year 2021, between 41.69% at experimental sunflower hybrid H54CLP in system Clearfield Plus and 50.56% at experimental sunflower hybrid H87SU in system Express Sun (tab. 5 and fig. 4).

Table 5. Seed oil content of experimental sunflower hybrids in two years 2020 and 2021, in Fundulea location

Sunflower Genotype	Seed oil content (%)							
	Year 2020, Fundulea location				Year 2021, Fundulea location			
	Repetition I	Repetition II	Repetition III	Average seed oil content	Repetition I	Repetition II	Repetition III	Average seed oil content
H76 SU	50.59	50.54	50.75	50.62	49.93	49.26	49.42	49.53
H87 SU	51.91	51.54	51.93	51.79	49.03	51.43	51.24	50.56
H90 SU	51.38	51.53	51.26	51.39	47.14	51.56	49.35	49.35
H50CLP	51.59	46.17	49.03	48.93	43.13	42.92	42.43	42.82
H52CLP	51.93	51.38	50.06	51.12	44.06	43.27	45.74	44.35
H54CLP	48.61	46.72	50.58	48.63	40.53	41.25	43.31	41.69
CHECK FD15E27	53.06	45.26	47.35	48.55	41.44	46.25	45.23	44.30

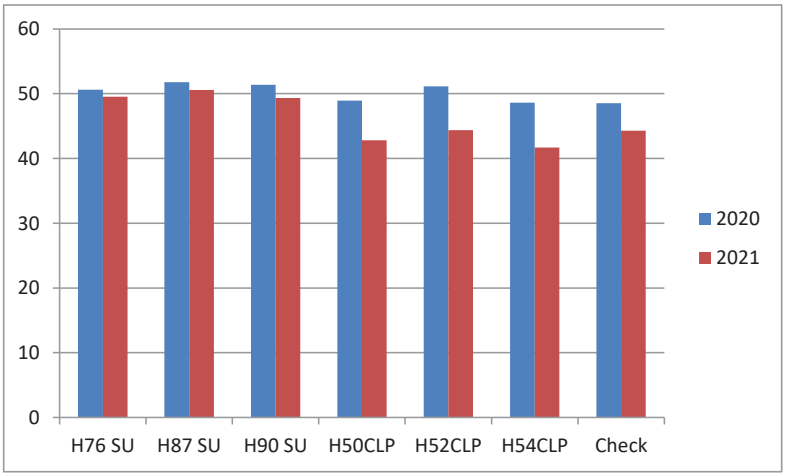

Figure 4. Seed oil content of experimental sunflower hybrid, in Fundulea location in years 2020 and 2021

After ANOVA analysis of variance for seeds oil content with result that years 2020 and 2021, sunflower hybrid and interaction between them, influence significant positive the oil content of seed (tab.6).

Table 6. ANOVA analysis of variance for seeds oil content

Source of variance	Degree of freedom	Sum of squares	Mean square	F value	Meaning
Factor A (year)	1	146.007	146.007	33.6024	***
Factor B (sunflower hybrid)	5	190.117	38.023	22.2639	***
Interaction A x B	5	61.078	12.216	7.1526	***

CONCLUSIONS

Seed oil content of experimental sunflower hybrids is significantly positively influenced by temperature and precipitation in the crop year.

Experimental sunflower hybrids in system Express Sun, H76 SU, H87 SU, H90 SU are more stable regarding seed oil content in both years of testing than experimental hybrids in system Clearfield Plus, H50CLP, H52CLP, H54CLP.

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PRELIMINARY CHROMATOGRAPHIC INVESTIGATION OF FOUR *STACHYS* SPP. (*LAMIACEAE*) FROM OLTENIA FLORA

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Abstract

Concerning four Stachys spp. (Lamiaceae) from the Oltenia flora, the paper highlights the polyphenolic content of aerial parts through high-performance thin-layer chromatography coupled with photodensitometry. Chlorogenic acid (CA) was identified and quantified in all 20% methanolic extracts of Stachydis herba. The highest CA amount was determined in S. recta (59.88 mg%), followed by S. sylvatica (32.90 mg%), S. officinalis (31.34 mg%), and S. germanica (25.16 mg%), respectively.

***Key words*:** *high-performance thin-layer chromatography, Lamiaceae, photodensitometry, polyphenols, Stachys spp.*

INTRODUCTION

Commonly known as hedge nettle, *Stachys* genus, *Lamiaceae* family, contains more than 300 different annual or perennial species (herbs or small shrubs) originating from Mediterranean region, Asia, Americas, and southern Africa. In the Romanian flora, 12 *Stachys* spp. are also found (Ciocârlan, 2009; Imbrea *et al.*, 2011).

The flowering aerial part of *Stachys* spp. contains important active principles, such as: essential oil (germacrene D, limonene, *β*-caryophyllene, *β*-pinene, nerolidol, linalyl acetate, linalool, caryophyllene oxide, spathulenol, *α*-cadinene, *α*-pinene, *β*-myrcene, *α*-terpineol, *β*-bourbonene, *β*-ylangene); iridoids (ajugoside, aucubin, harpagide, acetyl-harpagide, harpagoside); flavonoids (apigenin, luteolin, chrysoeriol, isoscutellarein, kaempferol, quercetin, isorhamnetin, naringenin and their glycosides); diterpenes with labdane, *neo*-clerodane, rosane and *ent*-kaurene type (deoxyandalusol, stachaegyptins, roseo-stachone, stachyrosanes); triterpenes (ursolic acid and oleanolic acid derivatives); phytosterols; phenylethanoid glycosides (acteoside, verbascoside, martynoside, leucosceptoside A, lavandulifoliosides); phenylpropanoid glycosides (coniferin, syringin); lignans (sesamin, paulownin, urolignoside); phenolic acids (chlorogenic acid, caffeic acid); fatty acids (linoleic, oleic and palmitic acids): oligosaccharides (Giuliani *et al.*, 2009; Goren *et al.*, 2011; Hajdari *et al.*, 2012; Háznagy-Radnai *et*

al., 2006 & 2012; Khanavi *et al*., 2009; Kiliç *et al.*, 2017; Lazarević *et al*., 2013; Sarikurkcu *et al.*, 2021; Stegăruş *et al.*, 2021; Tomou *et al*., 2020; Tundis *et al*., 2014; Venditti *et al.*, 2013).

In Turkey, *Stachys* spp. are used as wild (mountain) tea mainly against stomatitis, diabetes, rheumatism, cough and cold, or as functional foods for a healthy diet (Carović-Stanko *et al.*, 2016; Gören, 2014; Satıl & Açar, 2020; Tomou *et al*., 2020).

Stachys spp. exhibited significant pharmacological actions, such as: antimicrobial (against *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Candida albicans*), antioxidant, anti-inflammatory (useful for polycystic ovary syndrome), hepatoprotective, anti-diabetes, wound healing, antiviral, cytotoxic and antiproliferative, enzyme inhibitory (Alizadeh *et al.*, 2020; Goren *et al.*, 2011; Hajdari *et al.*, 2012; Háznagy-Radnai *et al.*, 2012; Khanavi *et al*., 2009 & 2012; Lazarević *et al*., 2010 & 2013; Paun *et al.*, 2018; Sarikurkcu *et al.*, 2021; Tomou *et al*., 2020; Tundis *et al*., 2014).

The purpose of our research was the preliminary analysis of polyphenols in the aerial parts of four *Stachys* spp. from Oltenia flora, by high-performance thin-layer chromatography (HPTLC)–densitometry.

MATERIALS AND METHODS

Plant material

The plant material was harvested during the flowering period, in May–June 2022, from the Oltenia Region, South-West of Romania. It was represented by the aerial parts of four *Stachys* spp. (*S. germanica* L., *S. officinalis* (L.) Trevis. sin. *Betonica officinalis* L., *S. recta* L., and *S. sylvatica* L.) Our study did not involve endangered or protected herbal species. The voucher specimens (StG-20220602/1, StO-20220623/1, StR-20220524/1, and StS-20220607/1, respectively) were deposited in the Herbarium of the Department of Pharmaceutical Botany, Faculty of Pharmacy, University of Medicine and Pharmacy of Craiova.

HPTLC analysis

Starting from the aerial parts of four *Stachys* spp. (*Stachydis herba*), preliminary HPTLC–densitometric analysis of polyphenols was made on CAMAG (Muttenz, Switzerland) system, according with some specific experimental conditions (Altemimi *et al.*, 2015; Bojić *et al.*, 2013; Gîrd *et al.*, 2014; Jug *et al.*, 2018): stationary phase: HPTLC silica gel 60 F_{254} (Merck, Darmstadt, Germany) 20×10 cm precoated glass plates; mobile phase: ethyl acetate–formic acid–methanol–water (15:1:0.1:1, in volumes); 10 mL of mobile phase were added in the developing twin-chamber (CAMAG) and then oversaturated for 20 minutes; sample: five 20% methanolic extracts of *Stachydis herba*; reference compounds (Merck): 0.1% methanolic solutions of caffeic acid, chlorogenic acid and rutin; migration distance: 62 mm (sample application line 8 mm, solvent front 70 mm); application of sample (2 µL) and reference solutions (2 µL, 3 µl, 4 µL): CAMAG Linomat 5 semi-automatic system – spraying gas nitrogen, syringe volume 100 µL, dosage speed 150 nL/s, pre-dosage volume 0.2 µL, bands length of 8 mm; plate drying: 5 minutes, at 25ºC (cold air dryer); plate shooting: ultraviolet (UV) light (254 nm and 365 nm); detection: CAMAG TLC Scanner 3 photodensitometer, for densitogram and *in situ* UV light (280 nm) spectra, without derivatization, deuterium–tungsten lamp, scanning speed 20 mm/s, data resolution 100 µm/step, measurement

mode absorbance; visionCATS ver. 3.1 software package (CAMAG).

DPPH *in situ* qualitative assay

In the CAMAG TLC Spray Cabinet 2, the HPTLC plates were sprayed with 0.5 mM methanolic solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH); then, the plates were dried at room temperature, in the dark, for 90 seconds, heated at 60°C, in an oven, for 30 seconds, and analyzed in white light illumination (Pozharitskaya *et al.*, 2008).

RESULTS AND DISCUSSIONS

The experimental results on the preliminary HPTLC analysis of polyphenols from the aerial parts of four *Stachys* spp. (*Stachydis herba*) were highlighted in Figures 1–9.

Through linear regression mode, with 5% range deviation, 1.68% coefficient of variation (CV), and 0.9961 correlation coefficient (*R*), the calibration curve for chlorogenic acid (Figure 9) was used for quantitative analysis: $y = 3.571\times10^{-9}x + 4.636\times10^{-3}$.

In the 20% methanolic extracts of *Stachydis herba*, the highest amount of chlorogenic acid (R_f 0.22) was quantified in *S. recta* (59.88 mg%), followed by *S. sylvatica* (32.90 mg%), *S. officinalis* (31.34 mg%), and *S. germanica* (25.16 mg%) aerial parts, respectively.

After the chromatographic separation, using HPTLC–DPPH assay, for *Stachydis herba* methanolic extracts, the screening of the antioxidant activity was performed *in situ*. For the HPTLC bands, the intensity of the yellow color is directly proportional with the concentration of polyphenols (chlorogenic acid) in the examined samples (Figure 3).

Starting from the polyphenolic content of *Stachys* spp., our results agree with the specialized research in the field of natural products chemistry (Khanavi *et al*., 2009; Lazarević *et al*., 2010; Stegăruş *et al.*, 2021; Tomou *et al*., 2020).

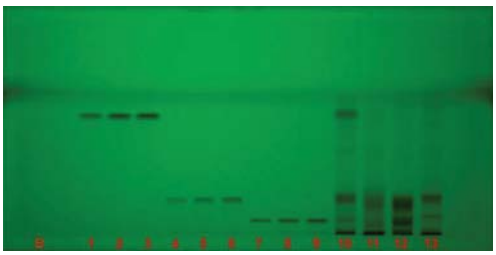

Figure 1. HPTLC chromatogram of polyphenols from *Stachydis herba* 20% methanolic extracts: UV 254 nm, without derivatization. B: Blank; Lanes 1–3: Caffeic acid, R_f 0.76; Lanes 4–6: Chlorogenic acid, R_f 0.22; Lanes 7–9: Rutin, R_f 0.08; Lanes 10–13: Samples (*S. germanica*, *S. officinalis*, *S. recta*, and *S. sylvatica*, respectively).

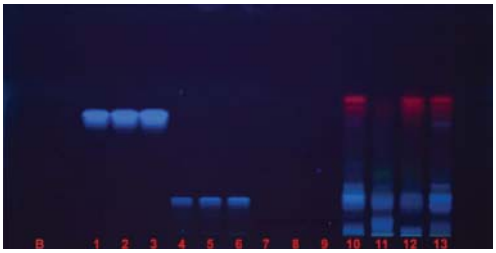

Figure 2. HPTLC chromatogram of polyphenols from *Stachydis herba* 20% methanolic extracts: UV 365 nm, without derivatization. B: Blank; Lanes 1–3: Caffeic acid, R_f 0.76; Lanes 4–6: Chlorogenic acid, R_f 0.22; Lanes 7–9: Rutin, not visualized; Lanes 10–13: Samples (*S. germanica*, *S. officinalis*, *S. recta*, and *S. sylvatica*, respectively).

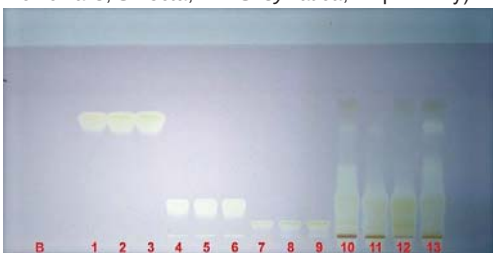

Figure 3. HPTLC chromatogram of polyphenols from *Stachydis herba* 20% methanolic extracts: white light illumination, derivatization with DPPH. B: Blank; Lanes 1–3: Caffeic acid, R_f 0.76; Lanes 4–6: Chlorogenic acid, R_f 0.22; Lanes 7–9: Rutin, R_f 0.08; Lanes 10–13: Samples (*S. germanica*, *S. officinalis*, *S. recta*, and *S. sylvatica*, respectively).

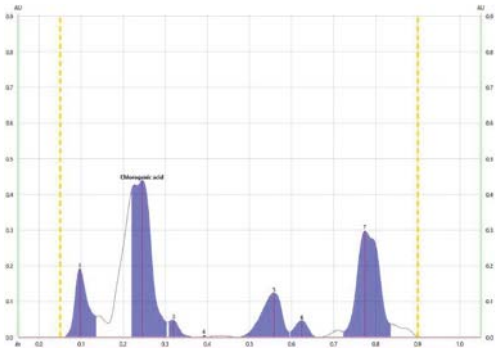

Figure 4. Densitogram of chlorogenic acid (UV 280 nm, without derivatization) separated from the *Stachys germanica* aerial parts 20% methanolic extract.

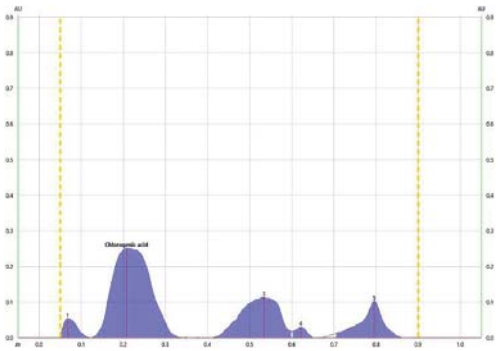

Figure 5. Densitogram of chlorogenic acid (UV 280 nm, without derivatization) separated from the *Stachys officinalis* aerial parts 20% methanolic extract.

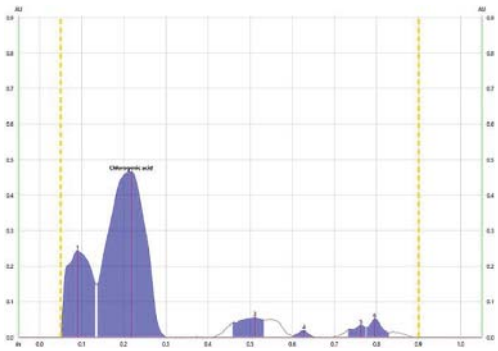

Figure 6. Densitogram of chlorogenic acid (UV 280 nm, without derivatization) separated from the *Stachys recta* aerial parts 20% methanolic extract.

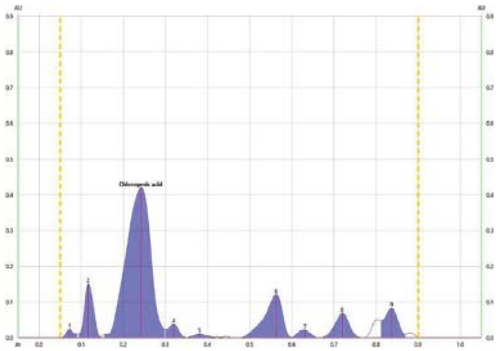

Figure 7. Densitogram of chlorogenic acid (UV 280 nm, without derivatization) separated from the *Stachys sylvatica* aerial parts 20% methanolic extract.

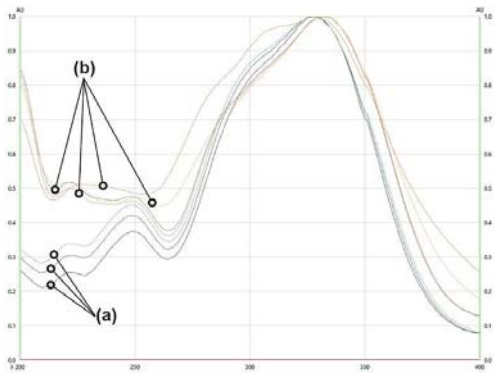

Figure 8. *In situ* UV spectra (280 nm) of chlorogenic acid reference (a) and compound separated from the analyzed samples (b).

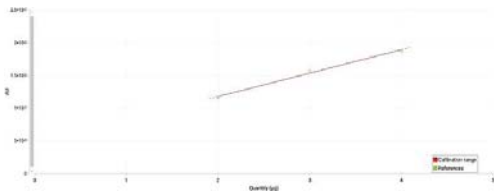

Figure 9. Chlorogenic acid reference calibration curve.

CONCLUSIONS

Preliminary chromatographic analysis of the polyphenols in the aerial parts of four *Stachys* spp. from the Oltenia flora was achieved by HPTLC coupled with photo-densitometry. Identified and quantified in

the 20% methanolic extracts of *Stachydis herba*, the amount of chlorogenic acid was fluctuating (mg%): *S. recta* (59.88) > *S. sylvatica* (32.90) > *S. officinalis* (31.34) > *S. germanica* (25.16).

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