Genetics and Plant Physiology – 2015, Volume 5(1), pp. 48–60
Special Issue – Project BG051PO001-3.3.06-0025 "Support for training and development of young competitive experts in the field of physiology, phytochemistry, genomics, proteomics and biodiversity of eukaryotic organisms"

Available online at http://www.ifrg-bg.com

MICROPROPAGATION AND EVALUATION OF FLAVONOID CONTENT AND ANTIOXIDANT ACTIVITY OF *SALVIA OFFICINALIS* L.

Petrova M.[1], M. Nikolova[2], L. Dimitrova[1], E. Zayova[1]

[1]Institute of Plant Physiology and Genetics, Acad. G. Bonchev Street, Bldg. 21, 1113 Sofia, Bulgaria

[2]Institute of Biodiversity and Ecosystem Research, Gagarin Street 2, 1113 Sofia, Bulgaria

Received: *17 October 2014* Accepted: *23 January 2015*

Summary: *Salvia officinalis* L. (garden sage) is a very popular aromatic plant widely used in pharmaceutical and food industries as well as in cosmetics. Leaf extracts of the herb possess antibacterial, antifungal, antiviral, anti-inflammatory, antitumor and hypoglycemic activity. The medicinal efficiency of sage is due to the essential oils, flavonoids and other polyphenolic compounds contained in the plant. The aim of this study was to develop an efficient method for micropropagation of *S. officinalis* as well as to evaluate flavonoid content and antioxidant capacity in leaves of the obtained shoots. Nodal segments from *in vitro* seedlings were used to establish a micropropagation system. The effect of four different cytokinins - BAP, thidiazuron, zeatin or 2-iP combined with the auxin IAA was examined. The maximum shoot proliferation was obtained when explants were cultured on Murashige and Skoog medium supplemented with 0.5 mg/l BAP and 0.1 mg/l IAA. Then the shoots were successfully rooted and adapted in *ex vitro* conditions. Flavonoid composition and antiradical properties of leaves of *in vitro* developed *S. officinalis* were determined. Six flavonoid aglycones were identified by TLC. Apigenin and its derivatives apigenin 7-methyl ether, scutellarein 6-methyl ether and scutellarein 6,7-dimethyl ether as well as luteolin and its derivative 6-hydroxyluteolin 6,7,3‘-trimethyl ether were identified. The antioxidant potential of the methanolic extract was assayed by scavenging of DPPH radicals and presented as IC_{50} values (μg/ml). The metanolic extract of *in vitro* developed *S. officinalis* showed a significant free radical scavenging activity(IC_{50}= 22.18 μg/ml).

Citation: Petrova M., M. Nikolova, L. Dimitrova, E. Zayova, 2015. Micropropagation and evaluation of flavonoid content and antioxidant activity of *Salvia officinalis* L. *Genetics and Plant Physiology*, 5(1): 48–60.

Keywords: DPPH; garden sage; shoot proliferation; surface flavonoids.

Abbreviations: BAP – 6-benzylaminopurine; DPPH – 2,2-diphenyl-1-picrylhydrazyl; GA_3 – gibberellic acid; IAA – indole-3-acetic acid; 2-iP – 6-(γ,γ dymethylallylamino)-purine; IBA – indole-3-butyric acid; MS – Murashige and Skoog nutrient medium; NAA – α-naphthalene acetic acid; TDZ – thidiazuron; TLC – Thin-layer chromatography.

*Corresponding author: marry_petrova@yahoo.com

INTRODUCTION

Salvia officinalis L. (garden sage) is a perennial subshrub from the family Lamiaceae. This plant originates from the Mediterranean region and is widely used in pharmaceutical and food industries as well as in cosmetics (Khan et al., 2010; Garcia et al., 2013). The leaf extract of the plant possesses antibacterial, antifungal, antiviral, anti-inflammatory, antitumor and hypoglycemic activity (Baricevic and Bartol, 2000; Longaray Delamare et al., 2007; Keshavarz et al., 2011). The pharmacology action of sage is a result of the presence of essential oil, di- and triterpenes, phenolic acids, flavonoids and tannins (Azimova and Glushenkova, 2012; Kontogianni et al., 2013). There are several studies concerning micropropagation of *S. officinalis* using different explants: shoot tips (Grzegorczyk and Wysokinska, 2008), nodal segments (Santos- Gomes et al., 2002; Gostin, 2008), axillary and apical buds (Wielgus et al., 2011). However, some of the serious limitations in the above mentioned protocols were low propagation frequency, inconsistent and less number of shoots and occurrence of hyperhydricity. Callus cultures, cell suspension, immobilized cell and hairy root cultures from *S. officinalis* were also established (Bolta et al., 2000; Falk et al., 2007; Tawfik and Mohamed, 2007; Duran et al., 2013). *In vitro* cultures can serve as a model system for studying the accumulation of antioxidative compounds under controlled conditions (Santos-Gomes et al., 2002). Total polyphenol content and antioxidant activity of *S. officinalis in vitro* cultures have been reported in several publications (Grzegorczyk et al., 2005, 2006, 2007; Wielgus et al., 2010). Nevertheless, the accumulation of these compounds in micropropagated shoots has not been sufficiently studied.

The aim of this study was to develop an efficient method for micropropagation of *S. officinalis* as well as to evaluate flavonoid content and antioxidant capacity in leaves of the obtained shoots.

MATERIALS AND METHODS

1. Protocol for propagation of *S. officinalis* under *in vitro* conditions

The protocol for *in vitro* propagation of *S. officinalis* (stages, nutrient media and cultural conditions) is presented briefly in Table 1.

1.1. Micropropagation of shoots

The surface sterilized seeds were germinated on MS nutrient medium supplemented with 2 mg/l GA_3 and 0.04 mg/l NAA. Nodal segments from *in vitro* seedlings (one month-old) were cultivated on eight variants of nutrient media based on that of Murashige and Skoog (1962) containing one of the following cytokinins: BAP, zeatin, thidiazuron or 2iP at concentrations of 0.5 or 1 mg/l combined with the auxin IAA at a concentration of 0.1 mg/l. The sucrose and agar contents were constant (3% and 0.7%, respectively). All media were adjusted to pH 5.6 with 1N NaOH before autoclaving. The nutrient media were sterilized and autoclaved at 120 ºC for 20 min at 1atm. The multiplication frequency, average number of shoots induced per explant and mean height of shoots were recorded after five weeks of culture.

Table 1. Protocol for propagation of *Salvia officinalis* under *in vitro* conditions.

Stage	Used explant	Nutrient medium	Cultural conditions
1. *In vitro* seed germination	Seeds	Full strength MS nutrient medium suppl. with 2% sucrose, 0.7 % agar, 2 mg/ l GA_3 and 0.04 mg/l NAA	3 weeks at 22 ± 2°C temperature, 16/8 h photoperiod, 40 μM m^{-2} s^{-1} light intensity and 70% humidity
2. Micropropagation	Nodal segments from *in vitro* seedlings	Full strength MS nutrient medium suppl. with 3% sucrose, 0.7 % agar, 0.5 mg/l BAP and 0.1 mg/l IAA	5 weeks at 22 ± 2°C temperature, 16/8 h photoperiod, 40 μM m^{-2} s^{-1} light intensity and 70% humidity
3. *In vitro* rooting	Shoots carrying a node	Half strength MS medium suppl. with 2% sucrose, 0.7 % agar, 20 mg/l yeast extract, 10 mg/l ascorbic acid and 1 mg/l IBA	4 weeks at 22 ± 2°C temperature, 16/8 h photoperiod, 40 μM m^{-2} s^{-1} light intensity and 70% humidity.
4. *Ex vitro* adaptation	Plants	Soil:peat:perlite:sand (2:1:1:1)	2 weeks at 24±1°C temperature, 16/8 h photoperiod, 40 μM m^{-2} s^{-1} light intensity and 90% humidity and 4 weeks under the same conditions, but at 70% humidity

1.2. Root formation and *ex vitro* adaptation of plants

To induce root growth, the shoots were transferred to half strength MS (½ MS) medium supplemented with 2% sucrose, 0.7 % agar, 20 mg/l yeast extract and either IAA, IBA or NAA (Table 2). Some of the media contained 10 mg/l ascorbic acid. The number of shoots that formed roots, the number of roots per explant and root length were recorded after four weeks. The plants with newly formed roots were planted into small plastic pots (6 cm diameter) with different substrate mixtures. Three types of mixtures were used: Mix1 – peat:perlite (3:1, v/v); Mix2 – soil:peat:perlite (2:1:1, v/v/v) and Mix3 – soil:peat:perlite:sand (2:1:1:1, v/v/v/v). The pots were placed in a crate with a transparent plastic cover which was removed after two weeks, thus allowing gradual adaptation to the lower air humidity in room conditions. The survival percentage of plants was assessed after six weeks.

2. Antioxidant capacity

2.1. Preparation of extracts

Acetone exudates. Acetone exudates were prepared from whole air-dried aerial plant parts by briefly (2-3 min) rinsing the material with acetone at room temperature

Table 2. Composition of nutrient media for *in vitro* rooting of *Salvia officinalis.*

Nutrient medium	Auxin [mg/l]			Yeast extract [mg/l]	Ascorbic acid [mg/l]
	IBA	IAA	NAA		
½ MSR1	0.1			20	
½ MSR2		0.1		20	
½ MSR3			0.1	20	
½ MSR4	0.25			20	10
½ MSR5	0.5			20	10
½ MSR6	1			20	10

to dissolve the lipophilic components accumulated on the surface. The obtained acetone filtrate was then dried using a rotary-evaporator to give a crude extract which was suspended in MeOH and then subjected to thin layer chromatographic (TLC) analysis.

Methanol extract. Air-dried powdered aerial plant parts were extracted by maceration with 80% methanol at room temperature for 24 h two times. After evaporation of the solvent the crude extract was subjected to subsequent analysis.

2.2. TLC analysis

The acetone exudates were screened for surface flavonoids by TLC analysis. Toluene-dioxan-acetic acid (95:25:4, v/v/v) was applied for the development of the aglycones mixture on silica gel plates Kiselgel 60 F_{254} (10x20 cm, 0.2 mm layer). Toluene-methylethylketone-methanol (60:25:15, v/v/v) was used for DC-Alufolien Polyamid 11 F_{254} plates (10x20 cm, 0.15 mm layer). Chromatograms were viewed under UV light before and after spraying with ‘‘Natural product reagent A”, 1% solution of diphenylboric acid 2-aminoethyl ester complex in methanol. The identification of the compounds was done by co-chromatography with authentic markers obtained from Prof. Eckhard Wollenweber.

2.3. Determination of DPPH radical scavenging activity

The effect of the methanolic extract on DPPH radicals was estimated according to Stanojević et al. (2009) with minor modifications. Different concentrations of the plant extract (10, 20, 50, 100 and 200 μg/ml), in methanol were added at an equal volume (2.5 ml) to methanol solution of DPPH (0.3 mM, 1 ml). After 30 min at room temperature, the Ab values were measured at 517 nm on a spectrophotometer (Jenway 6320D) and converted into percentage antioxidant activity using the following equation:

$$\text{DPPH antiradical scavenging capacity (\%)} = [1- (Ab_{sample} - Ab_{blank}) / Ab_{control}] \cdot 100$$

Methanol (1.0 ml) plus plant extract solution (2.5 ml) was used as a blank, while DPPH solution plus methanol was used as a control. The IC_{50} values were calculated by Software Prizm 3.00. All experiments were carried out in triplicate.

3. Data analysis

Twenty explants were used for each treatment and the experiment was repeated twice. Data were subjected to one-way ANOVA analysis of variance for comparison of means using a statistical software package (Statigraphics Plus, version 5.1 for Windows). Data are presented as means ± standard error.

RESULTS AND DISCUSSION

Micropropagation of shoots

Nodal segments of *in vitro* cultured plants obtained by seed germination were successfully applied for micropropagation of *S. officinalis*. The explants grown on Murashige and Skoog medium without growth regulators (control) did not produce adventitious shoots. Then, the explants were placed on different modified MS media for shoot formation. The plant growth regulators and their concentrations were shown to be a crucial factor for multiplication of common sage (Tawfik and Mohamed, 2007; Gostin, 2008). The efficacy of four cytokinins: BAP, thidiazuron, zeatin or 2-iP combined with the auxin IAA for shoot induction was assessed. The explants cultured on MS medium supplemented with various cytokinins at different concentrations showed variation in the multiplication percentage and number of formed shoots (Table 3, Fig. 1). Among the examined nutrient media, those contained BAP (0.5 or 1 mg/l) and IAA (0.1 mg/l) resulted in the highest percentage of shoot multiplication and the highest number of shoots per explant compared to the other media. The optimum nutrient medium was MS supplemented with 0.5 mg/l BAP and 0.1 mg/l IAA, which yielded an avarege of 5.25 shoots per explant (Fig. 2). The interaction of BAP and low level of IAA had a significant effect on the shoot

Table 3. Effect of plant growth regulators on the micropropagation of *Salvia officinalis*.

Plant growth regulator [mg/l]					Micropropagation			
BAP	TDZ	Zeatin	2-iP	IAA	Shoot multiplication [%]	Mean number of shoots per explant	Shoot height [cm]	Hyperhydricity [%]
1				0.1	90	4.05 ± 0.34^{d}	2.50 ± 0.20^{a}	5
0.5				0.1	95	5.25 ± 0.46^{e}	2.42 ± 0.21^{a}	2
	1			0.1	80	3.75 ± 0.43^{cd}	2.57 ± 0.16^{ab}	25
	0.5			0.1	85	2.80 ± 0.27^{b}	2.82 ± 0.27^{ab}	15
		1		0.1	80	3.05 ± 0.34^{bc}	2.60 ± 0.14^{ab}	5
		0.5		0.1	75	1.85 ± 0.22^{a}	2.77 ± 0.13^{ab}	0
			1	0.1	15	1.10 ± 0.06^{a}	3.10 ± 0.26^{b}	0
			0.5	0.1	20	1.50 ± 0.22^{a}	2.87 ± 0.23^{ab}	0

Data are presented as means of 20 individuals per treatment ± standard error (SE). Different letters indicate significant differences assessed by the Fisher LSD test ($P\leq0.05$) after performing ANOVA multifactor analysis.

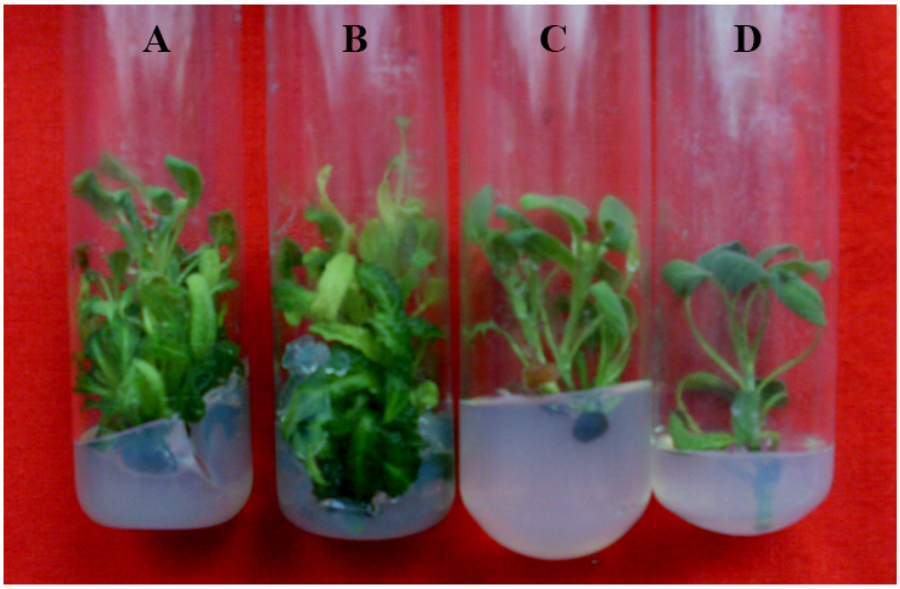

Figure 1. Micropropagation of *Salvia officinalis* L. on MS media supplemented with A) BAP (1 mg/l) and IAA (0.1 mg/l); B) TDZ (1 mg/l) and IAA (0.1 mg/l); C) zeatin (1 mg/l) and IAA (0.1 mg/l) D) 2 iP (1 mg/l) and IAA (0.1 mg/l).

number production. The effectiveness of BAP over other cytokinins in inducing multiplication of *S. officinalis* has been described also by other authors (Avato et al., 2005; Grzegorczyk and Wysokinska, 2008; Weielgus et al., 2011).

The nutrient media supplemented with thidizuron (0.5 or 1 mg/l) and IAA (0.1 mg/l) were also appropriate for micropropagation, although some shoots showed hyperhydricity. This is a common phenomenon during *in vitro* culture that has been reported in other *Salvia* micropropagation protocols (Avato et al., 2005; Weielgus et al., 2011). Different factors provoke the emergence of hyperhydricity in *in vitro* culture, one of which is the excess of cytokinins in the nutrient medium (Zuzarte et al., 2010). The shoots restored normal phenotype when transferred on MS medium without plant growth regulators or on rooting medium. The plants cultured on MS

Figure 2. Micropropagation of *Salvia officinalis* L. on MS medium supplemented with BAP (0.5 mg/l) and IAA (0.1 mg/l).

media containing BAP or thidiazuron and a low level of IAA were characterized by shorter internodes, higher number of leaves and decreased average shoot height compared to those grown in the combination of zeatin or 2-iP with IAA. The shoot proliferation was 75-80% on MS medium containing zeatin and IAA. About 2-4 new shoots were developed at the base of explants grown on MS medium supplemented with 1 mg/l zeatin and 0.1 mg/l IAA. This combination influenced positively shoot growth and development. Supplementation of the culture medium with 2-iP did not result in shoot multiplication. Only 1.1-1.5 shoots per explant were observed, but the plants were characterized with the largest leaf area, absence of hyperhydricity and increased average shoot height.

In vitro rooting and *ex vitro* adaptation

The microshoots derived from the multiplication stage were grown on six modifications of half strength MS nutrient medium containing different auxins (Table 2). Avato et al. (2005) have reported that auxin supplementation is not necessary for root formation of *S. officinlais*. In our study, the plants cultured on control medium without plant growth regulators did not induce rhizogenesis. Firstly, the influence of three types of auxins (IBA, IAA and NAA) at a concentration of 0.1 mg/l was examined (Table 4). The nutrient medium was enriched with yeast extract. The yeast extract is used as a supplement in order to promote plant growth due to its high amino acid content (Molnár et al. 2011). The mean percentage of rooting (ranging from 40 to 55%) did not differ significantly between the three tested nutrient media (½ MSR1, ½ MSR2 and ½ MSR3). IAA was the less effective auxin for root formation, while IBA and NAA showed a similar response. However, the mean number of roots per explant was highest (2.05) on the nutrient medium (½ MSR1) containing 0.1 mg/l IBA. Thus, this growth regulator was used as a component of nutrient media ½ MSR4, ½ MSR5 and ½ MSR6. Ascorbic acid was added to these nutrient media. It is known that ascorbic acid prevents the browning and hyperhydricity of explants, improves *in vitro* rooting and *ex vitro* survival of

Table 4. Effect of nutrient media on *in vitro* rooting of *Salvia officinals.*

Nutrient media	Rooted shoots [%]	Number of roots per explant	Root length [cm]
½ MSR1	55	2.05 ± 0.49^{ab}	2.37 ± 0.32^{a}
½ MSR2	40	1.05 ± 0.28^{a}	1.56 ± 0.18^{a}
½ MSR3	50	1.80 ± 0.50^{ab}	3.72 ± 0.44^{b}
½ MSR4	60	2.40 ± 0.53^{ab}	3.67 ± 0.42^{b}
½ MSR5	65	3.50 ± 0.79^{bc}	4.18 ± 0.31^{b}
½ MSR6	75	4.75 ± 0.83^{c}	3.86 ± 0.35^{b}

Data are presented as means of 20 individuals per treatment ± standard error (SE). Different letters indicate significant differences assessed by the Fisher LSD test ($P\leq0.05$) after performing ANOVA multifactor analysis.

the plants (Tawfik and Mohamed, 2007; Ngomuo et al., 2014). The highest rooting response in our study (75%) was observed on the nutrient medium (½ MSR6) containing 1 mg/l IBA in combination with 20 mg/l yeast extract and 10 mg/l ascorbic acid, where the mean number of roots per explant reached 4.75. The plants were stable with very good foliage development and profuse rooting (Fig. 3A). The mean root length ranged from 1.56 to 4.18 cm for the examined nutrient media. All nutrient media tested accelerated shoot growth by 1-2 cm. Most authors also recommend the addition of auxins to the nutrient medium to stimulate root induction and growth of *S. officinlais* plants (Tawfik and Mohamed, 2007; Gostin, 2008; Grzegorczyk and Wysokinska et al., 2008; Wielgus et al., 2011). The highest percentage of surviving plants (75%) was observed after transferring the plants to the mixture of soil:peat:perlite:sand (2:1:1:1) compared to the other tested substrates. This mixture had a beneficial effect on plant growth (Fig. 3B). The plants transferred to the other two mixtures - peat:perlite (3:1) and soil:peat:perlite (2:1:1) showed a lower survival rate – 50 and 65%, respectively. It is known from the literature that *Salvia* is not soil demanding, but prefers well-drained, sandy or loamy soils (Sutton, 2004). Perhaps for that reason, the most effective mixture in our study, allowing successful adaptation, was the mixture, which in addition to soil, peat and perlite contained also sand in its composition.

Flavonoid content and antioxidant activity

Extracts of sage collected from natural habitats show great diversity in phenolic content and antioxidant activity due to various environmental factors (Areias et

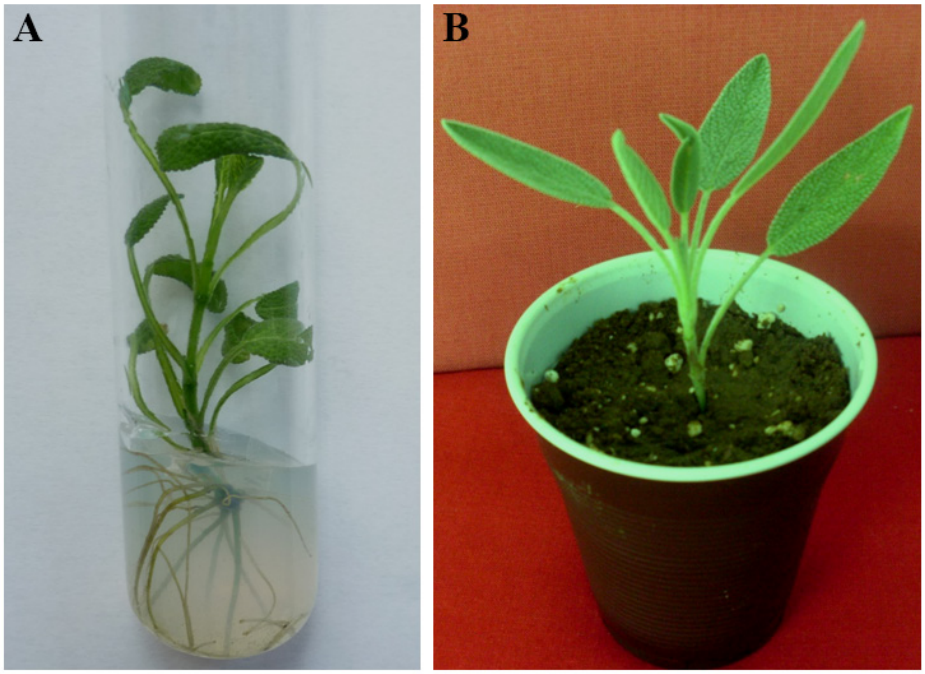

Figure 3. A) *In vitro* rooted *Salvia officinalis* plant on ½ MSR6; B) *Ex vitro* adapted *Salvia officinalis* plant on a mixture (Mix3).

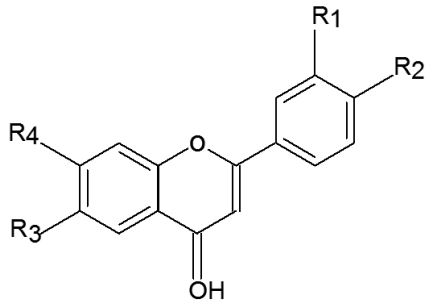

Figure 4. Structures of the identified flavonoid aglycones (1–6):
(**1**) R_1=H; R_2=OH; R_3=H; R_4=OH
apigenin
(**2**) R_1=H; R_2=OH; R_3=H; R_4=OCH_3
apigenin 7-methyl ether
(**3**) R_1=H; R_2=OH; R_3= OCH_3; R_4=OH
scutellarein 6-methyl ether (hispidulin)
(**4**) R_1=H; R_2=OH; R_3= OCH_3; R_4= OCH_3
scutellarein 6,7-dimethyl ether
(**5**) R_1=OH; R_2=OH; R_3=H; R_4=OH
luteolin
(**6**) R_1= OCH_3; R_2=OH; R_3= OCH_3; R_4= OCH_3
6-hydrohyluteolin 6,7,3'-trimethyl ether.

al., 2000). Therefore in the development of in vitro cultures is necessary to have easier control over factors as environment and nutritional and growth regulators.

In our study, plant material (leaves from *in vitro* micropropagated shoots) of *S. officinalis* was analyzed for its profile of surface flavonoid aglycones. Several flavonoids were detected by TLC, but six were identified by direct comparison with authentic compounds obtained by Prof. Eckhard Wollenweber (Fig. 4). Apigenin and its derivatives apigenin 7-methyl ether (**2**), scutellarein 6-methyl ether (**3**) and scutellarein 6,7-dimethyl ether (**4**), as well as luteolin (**5**) and its derivative 6-hydrohyluteolin 6,7,3'-trimethyl ether (**6**) were determined as components of the flavonoid profile of the studied material. Thin layer chromatographic data – Rf (rate of flow) and color of the detected flavonoid aglycones are presented in Table 5. The identified compounds were in accordance with those reported previously for wild and cultivated *S. officinalis* plants (Wollenweber and Dietz, 1981; Nikolova et al., 2006)

The antioxidant potential of the methanolic extract was assayed by scavenging of DPPH radicals. The degree of discoloration of the violet colour

Table 5. Thin layer chromatographic data on flavonoid aglycones detected in acetone exudates of *Salvia officinalis.*

Flavonoid aglycones	Thin layer chromatographic data – R_F (rate of flow) and color	
	S_1	S_2
Apigenin (1)	0.28 brown/yellow	0.08 brown/yellow
Apigenin 7-methyl ether (2)	0.48 brown/yellow	0.56 brown/yellow
Scutellarein 6-methyl ether (3)	0.31 brown/brown	0.24 brown/brown
Scutellarein 6,7-dimethyl ether (4)	0.33 brown/brown	0.61 brown/brown
Luteolin (5)	0.11 brown/yellow	0.05 brown/yellow
6-Hydrohyluteolin 6,7,3'-trimethyl ether (6)	0.57 brown/brown	0.65 brown/brown

Legend: S_1 (sorbent – silica gel; eluent – toluene:dioxan:acetic acid, 90:25:4); S_2 (sorbent polyamid; eluent - toluene:methylethylketone:methanol, 60:25:15).

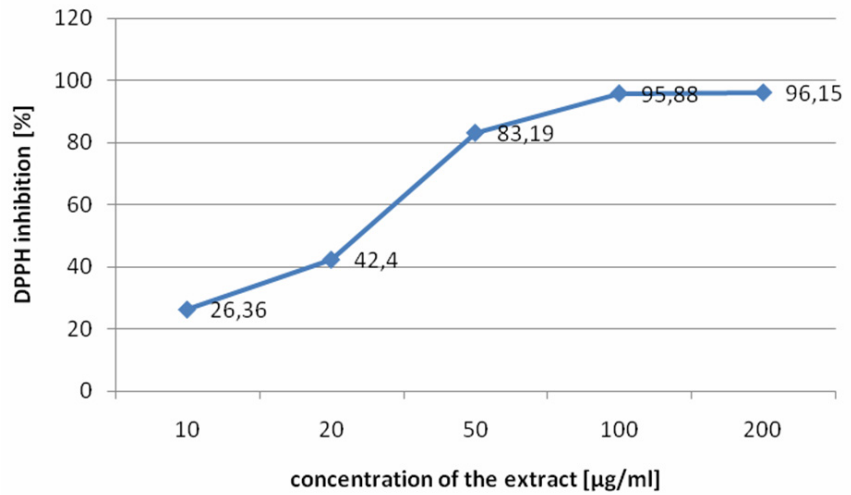

Figure 5. Free radical scavenging activity of *Salvia officinalis* extract.

of DPPH as it gets reduced indicates the radical scavenging potential of the antioxidant. The results of the DPPH scavenging activity is presented as IC_{50} values (µg/ml) – the extract concentration providing a 50% inhibition of the DPPH solution. The metanolic extract of *in vitro* developed *S. officinalis* showed a significant free radical scavenging activity: IC_{50}= 22, 18 µg/ml (Fig. 5). The received value was comparable to that reported by other authors for extracts of wild plants or commercial product of *S. officinalis* (Nickavar et al., 2007; Wojdyło et al., 2007; El-Agbar et al., 2008; Hamrouni-Sellami et al., 2013).

In conclusion, the optimum conditions for *in vitro* micrpropagation of *S. officinalis* were defined. The highest mean number of shoots per explant (5.25), scored on the basis of all explants planted, indicated that the MS medium supplemented with 0.5 mg/l BAP and 0.1 mg/l IAA was the optimum nutrient medium for *in vitro* multiplication of *S. officinalis*. Half strength MS rooting medium containing 1 mg/l IBA in combination with 20 mg/l yeast extract and 10 mg/l ascorbic acid allowed the production of hardened plants successfully adapted in *ex vitro* conditions. Leaves from *in vitro* developed shoots of *S. officinalis* exhibited the same strong antioxidant activity and flavonoid composition as wild and cultivated plants of this species. The developed efficient and reproducible system for micropropagation ensured production of numerous *S. officinalis* plants which could be used as a potential source of natural antioxidants.

ACKNOWLEDGEMENTS

The Operational Programme Human Resources Development (2007–2013), the European Social Fund and the Ministry of Education and Science of Bulgaria (Grant № BG051PO001-3.3.06-0025) are thanked for financial support.

REFERENCES

Areias F, P Valentão, PB Andrade, F Ferreres, RM Seabra, 2000. Flavonoids and phenolic acids of sage: Influence of some agricultural factors. *J Agric Food Chem*, 48(12):

6081–6084.

Avato P, IM Fortunato, C Ruta, R D'Elia, 2005. Glandular hairs and essential oils in micropropagated plants of *Salvia officinalis* L. *Plant Sci*, 169(1): 29–36.

Azimova SS, AI Glushenkova, 2012. *Salvia officinalis* L. In Lipids, Lipophilic Components and Essential Oils from Plant Sources. Springer London. pp. 494–494.

Baricevic D, T Bartol. 2000. V. Pharmacology 11. The Biological/ pharmacological activity of the Salvia Genus. The Genus Salvia, 143.

Bolta Z, D Baricevic, B Bohanec, S Andrensek, 2000. A preliminary investigation of ursolic acid in cell suspension culture of *Salvia officinalis*.. *Plant Cell Tiss Org Cult*, 62: 57–63.

Duran RE, Y Coskun, AS Tulgar, M Altuncu, Y Koparal, 2013. *In vitro* callus induction and rosmarinic acid accumulation of *Salvia officinalis*. *Curr Opin Biotechnol*, 24, S118.

El-Agbar ZA, AK Shakya, NA Khalaf, M Al-Haroon, 2008. Comparative Antioxidant Activity of Some Edible Plants. *Turk J Biol*, 32: 193–196.

Falk K, J Gershenzon, R Croteau, 1990. Metabolism of Monoterpenes in Cell Cultures of Common Sage (*Salvia officinalis*), *Plant Physiol*, 93: 1559–1567.

Garcia CSC, MR Ely, RA Wasum, BCDA Zoppa, C Wolhheim, GÂ Neves, VW Angeli, KCBD Souza, 2013. Assessment of *Salvia officinalis* (L.) hydroalcoholic extract for possible use in cosmetic formulation as inhibitor of pathogens in the skin. *Revista de Ciências Farmacêuticas Básica e Aplicada*, 33(4): 509–514.

Gostin I, 2008. Effects of different Plant Hormones on *Salvia officinalis*, cultivated *in vitro*, *Int J Bot*, 4 (4): 430–436.

Grzegorczyk I, A Krolicka, H Wysokinska, 2006. Establishment of *Salvia officinalis* L. hairy root cultures for the production of rosmarinic acid. *Z Naturforsch C*, 61: 351–356.

Grzegorczyk I, H Wysokińska, 2008. Liquid shoot culture of *Salvia officinalis* L. for micropropagation and production of antioxidant compounds; effect of triacontanol. *Acta Societatis Botanicorum Poloniae*, 77(2): 99–104.

Grzegorczyk I, I Bilichowski, E Mikiciuk-Olasik, H Wysokin´ska, 2005. *In vitro* cultures of *Salvia officinalis* L. as a source of antioxidant compounds. *Acta Societatis Botanicorum Poloniae*, 74: 17–21.

Grzegorczyk I., A Matkowski, H Wysokinska, 2007. Antioxidant activity of extracts from *in vitro* cultures of *Salvia officinalis* L., *Food Chem*, 104: 536–541.

Hamrouni-Sellami I, FZ Rahali, IB Rebey, S Bourgou, F Limam, B Marzouk, 2013.Total phenolics, flavonoids, and antioxidant activity of Sage (*Salvia officinalis* L.) plants as affected by different drying methods. *Food Bioprocess Technol*, 6: 806–817

Keshavarz M, A Bidmeshkipour, A Mostafaie, K Mansouri, HR Mohammadi-Motlagh, 2011. Anti tumor activity of *Salvia officinalis* is due to its anti-angiogenic, anti-migratory and anti-proliferative

effects. *Cell J*, 12: 477–482.

Khan IA, EA Abourashed, 2010. Leung's Encyclopedia of Common Natural Ingredients: Used in Food, Drugs and Cosmetics. (Hoboken, Ed.) (3rd ed., p. 845). N.J.: John Wiley & Sons Inc.

Kontogianni VG, G Tomic, I Nikolic, AA Nerantzaki, N Sayyad, S Stosic-Grujicic, I Stojanovic, IP Gerothanassis, AG Tzakos, 2013. Phytochemical profile of *Rosmarinus officinalis* and *Salvia officinalis* extracts and correlation to their antioxidant and anti-proliferative activity. *Food Chem*, 136(1): 120–129.

Longaray Delamare AP, IT Moschen-Pistorello, L Artico, L Atti-Serafini, S Echeverrigaray, 2007. Antibacterial activity of the essential oils of *Salvia officinalis* L. and *Salvia triloba* L. cultivated in *South Brazil Food Chem*, 100(2): 603–608.

Molnár Z, E Virág, V Ordog, 2011. Natural substances in tissue culture media of higher plants. *Acta Biologica Szegediensis*, 55(1): 123–127.

Murashige T, F Skoog, 1962. A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. *Physiol Plant*, 15(3): 473–497.

Ngomuo M, E Mneney, P Ndakidemi, 2014. Control of lethal browning by using ascorbic acid on shoot tip cultures of a local Musa spp.(Banana) cv. Mzuzu in Tanzania. *African J Biotechnol*, 13(16): 1721–1725.

Nickavar B, M Kamalinejad, H Izadpanah, 2007. *In vitro* free radical scavenging activity of five Salvia species. *Pak J Pharm Sci*, 20(4): 291–294.

Nikolova M, JG Renée, E Genova, EA Porter, 2006. Exudate flavonoids from Bulgarian species of *Salvia.* Biochemical *Systematics and Ecology,* 34(4): 360–364.

Santos-Gomes PC, RM Seabra, PB Andrade, M Fernandes-Ferreira, 2002. Phenolic antioxidant compounds produced by *in vitro* shoots of sage (*Salvia officinalis* L.). *Plant Science*, 162(6): 981–987.

Stanojević L, M Stanković, V Nikolić, L Nikolić, D Ristić, J Čanadanovic-Brunet, V Tumbas, 2009. Antioxidant activity and total phenolic and flavonoid contents of *Hieracium pilosella* L. extracts. *Sensors*, 9: 5702–5714.

Sutton, J, 2004. The Gardener's Guide to Growing Salvias. Workman Publishing Company. p. 17.

Tawfik A, F Mohamed, 2007. Regeneration of salvia (*Salvia officinalis* L.) via induction of meristematic callus. *In Vitro Cell Dev Biol Plant*, 43: 21–27.

Wielgus K, A Luwanska, M Szalata, S Mielcarek, A Gryszczynska, D Lipinski, .& R Slomski, 2011. Phytochemical estimation of Sage (*Salvia officinalis* L.) cultivated *in vitro*-flavonoids and phenolic acids. *Acta Fytotechnica et Zootechnica, Special Number Nitra*, 8–11.

Wojdyło A, J Oszmiański, R Czemerys, 2007. Antioxidant activity and phenolic compounds in 32 selected herbs. *Food Chemistry*, 105: 940–949.

Wollenweber E, V Dietz, 1981. Occurrence and distribution of free flavonoid aglycones in plants. Phytochemistry, 20: 869–932.

Zuzarte MR, AM Dinisa, C Cavaleiro, LR Salgueiro, JM Canhoto, 2010. Trichomes, essential oils and *in vitro* propagation of *Lavandula pedunculata* (Lamiaceae) *Ind Crop Prod*, 32: 580–587.