Биолошки факултет
Број захтева: 15/558-1
Датум: 11.12.2015.

УНИВЕРЗИТЕТ У БЕОГРАДУ
ВЕЋУ НАУЧНИХ ОБЛАСТИ ПРИРОДНИХ НАУКА

З А Х Т Е В

за давање сагласности на реферат о урађеној докторској дисертацији за кандидата магистра наука који брани дисертацију према ранијим прописима

Молимо да, сходно члану 47. ст. 5. тач. 4. Статута Универзитета у Београду ("Гласник Универзитета", број 162/11-пречишћени текст, 167/12, 172/13 и 178/14), дате сагласност на реферат о урађеној докторској дисертацији:

КАНДИДАТ**:** **Mp Najat Beleed Al Sheef**

пријавио је докторску дисертацију под називом:

„Микроморфолошка и цитолошка анализа трихома и биолошки ефекти екстраката *Salvia aegyptiaca* L., *S. fruticosa* Mill. и *S. lanigera* Poir. (Lamiaceae) из Либије”

(„Micromorphological and cytological analysis of trichomes and biological effects of extracts of *Salvia aegyptiaca* L., *S. fruticosa* Mill. and *S. lanigera* Poir. (Lamiaceae) from Libya“)

из научне области: Биолошке науке.

Универзитет је дана 25.09.2014. године. својим актом под бр. 02 број: 61206-3622/2-14 дао сагласност на предлог теме докторске дисертације која је гласила:

„Микроморфолошка и цитолошка анализа трихома и биолошки ефекти екстраката *Salvia aegyptiaca* L., *S. fruticosa* Mill. и *S. lanigera* Poir. (Lamiaceae) из Либије”

(„Micromorphological and cytological analysis of trichomes and biological effects of extracts of *Salvia aegyptiaca* L., *S. fruticosa* Mill. and *S. lanigera* Poir. (Lamiaceae) from Libya“)

Комисија за оцену и одбрану докторске дисертације образована је на седници одржаној 09.10.2015. год, одлуком Факултета под бр. *15/482-09.10.2015.* год. у саставу:

	Име и презиме члана комисије	звање	научна област	Установа у којој је запослен
1.	др Соња Дулетић-Лаушевић	Ванредни професор	Морфологија, фитохемија и систематика биљака	Универзитет у Београду-Биолошки факултет
2.	др Петар Марин	Редовни професор	Морфологија, фитохемија и систематика биљака	Универзитет у Београду-Биолошки факултет
3.	др Душица Јаношевић	Ванредни професор	Физиологија и молекуларна биологија биљака	Универзитет у Београду-Биолошки факултет
4	др Ана Ћамић	Доцент	Морфологија, фитохемија и систематика биљака	Универзитет у Београду-Биолошки факултет
5.	др Снежана Будимир	Научни саветник	Физилогија биљака	Универзитет у Београду-ИБИСС

Напомена: уколико је члан Комисије у пензији навести датум пензионисања.

Наставно-научно веће факултета прихватило је реферат Комисије за оцену и одбрану докторске дисертације на седници одржаној 11. децембра 2015. године.

Декан Биолошког факултета

Проф. др Жељко Томановић

Прилог: 1. Реферат комисије са предлогом.
2. Акт Наставно-научног већа факултета о усвајању реферата
3. Примедбе дате у току стављања реферата на увид у јавности, уколико је таквих примедби било.
4. Електронска верзија.
5. Одлука о признавању дипломе 06-613-317/4-10
6. Arch Biol Sci_Confirmation of Acceptance_Janosevic et al
7. RAD ARHIV 2013
8. Salvia species water extracts JABFQ

УНИВЕРЗИТЕТ У БЕОГРАДУ
БИОЛОШКИ ФАКУЛТЕТ

Студентски трг 16
11000 БЕОГРАД
Република СРБИЈА
Тел: +381 11 2186 635
Факс: +381 11 2638 500
Е-пошта: dekanat@bio.bg.ac.rs

15/558-11.12.2015.

На основу члана 128. Закона о високом образовању и члана 59. став 1. тачка 1. Статута Универзитета у Београду-Биолошког факултета, Наставно-научно веће Факултета, на III редовној седници одржаној 11.12.2015. године, донело је

О Д Л У К У

Прихвата се Извештај Комисије за преглед, оцену и одбрану докторске дисертације кандидата:

Mp Najat Beleed Al Sheef, под називом:

„Микроморфолошка и цитолошка анализа трихома и биолошки ефекти екстраката *Salvia aegyptiaca* L., *S. fruticosa* Mill. и *S. lanigera* Poir. (Lamiaceae) из Либије"

(„Micromorphological and cytological analysis of trichomes and biological effects of extracts of *Salvia aegyptiaca* L., *S. fruticosa* Mill. and *S. lanigera* Poir. (Lamiaceae) from Libya")

Универзитет је дана 25.09.2014. године. својим актом под бр. 02 број: 61206-3622/2-14 дао сагласност на предлог теме докторске дисертације кандидата.

Радови и конгресна саопштења из докторске дисертације:

Б1. Радови у часописима међународног значаја

1. 1. Al Sheef, N.B., Duletić-Laušević, S., Janošević D., Budimir, S., Marin, M., Alimpić, A., Giwelli, A.A.M., Marin, P.D. (2013): Micromorphology and ultrastructure of trichomes of Libyan *Salvia fruticosa* Mill. Arch. Biol. Sci. Belgrade, 65(1): 239-248. **M23**

2. Janošević, D., Budimir, S., Alimpić, A., Marin, P.D., **Al Sheef, N.B.,** Giwelli, A.A.M., Duletić-Laušević, S. (2016): Micromorphology and histochemistry of leaf trichomes of *Salvia aegyptiaca* (Lamiaceae). Arch. Biol. Sci. Belgrade, *in press.* **M23**

Декан Биолошког факултета

Проф. др Жељко Томановић

Доставити:
- Универзитету у Београду,
- докторанту,
- Стручној служби Факултета.

НАСТАВНО–НАУЧНОМ ВЕЋУ БИОЛОШКОГ ФАКУЛТЕТА
УНИВЕРЗИТЕТА У БЕОГРАДУ

На I редовној седници Наставно-научног већа Биолошког факултета Универзитета у Београду, одржаној 09.10.2015. године, прихваћен је извештај ментора др Соње Дулетић-Лаушевић о урађеној докторској дисертацији мр **Najat Beleed Al Sheef** под насловом **"Микроморфолошка и цитолошка анализа трихома и биолошки ефекти екстраката *Salvia aegyptiaca* L., *S. fruticosa* Mill. и *S. lanigera* Poir. (Lamiaceae) из Либије" („Micromorphological and cytological analysis of trichomes and biological effects of extracts of *Salvia aegyptiaca* L., *S. fruticosa* Mill. and *S. lanigera* Poir. (Lamiaceae) from Libya")** и одређена је Комисија за преглед и оцену докторске дисертације у саставу: др Соња Дулетић-Лаушевић, ванредни професор Биолошког факултета Универзитета у Београду, др Петар Д. Марин, редовни професор Биолошког факултета Универзитета у Београду, др Душица Јаношевић, ванредни професор Биолошког факултета Универзитета у Београду, др Ана Џамић, доцент Биолошког факултета Универзитета у Београду и др Снежана Будимир, научни саветник Института за биолошка истраживања "Синиша Станковић" Универзитета у Београду.

Комисија је прегледала урађену докторску дисертацију кандидаткиње и Већу подноси следећи

И З В Е Ш Т А Ј

ОПШТИ ПОДАЦИ О ДОКТОРСКОЈ ДИСЕРТАЦИЈИ

Докторска дисертација мр **Najat Beleed Al Sheef** под насловом **"Микроморфолошка и цитолошка анализа трихома и биолошки ефекти екстраката *Salvia aegyptiaca* L., *S. fruticosa* Mill. и *S. lanigera* Poir. (Lamiaceae) из Либије"**

(„Micromorphological and cytological analysis of trichomes and biological effects of extracts of *Salvia aegyptiaca* L., *S. fruticosa* Mill. and *S. lanigera* Poir. (Lamiaceae) from Libya“), је написана према Упутствима за обликовање докторске дисертације Универзитета у Београду. Дисертација обухвата уобичајена поглавља, у оквиру којих су на одговарајућим местима приказане табеле и илустрације. На крају је наведена листа литературних навода који су цитирани у оквиру дисертације. Дисертација је написана на 164 нумерисане стране. Садржи 20 табела, 66 слика и 386 референци.

АНАЛИЗА ДОКТОРСКЕ ДИСЕРТАЦИЈЕ

У докторској дисертацији кандидаткиња **Najat Beleed Al Sheef** је приказала резултате истраживања микроморфологије, цитологије и хистохемије гландуларних трихома, као и хемијског састава и биолошке активности екстраката три врсте roda *Salvia* (жалфије), које су сакупљене на локалитетима у Либији.

У првом, Уводном делу, дати су општи подаци о о лековитим биљкама, посебно из фамилије уснатица (Lamiaceae). Дат је преглед литературе о микроморфолошким, цитолошким испитивањима, као и биолошким активностима врста roda *Salvia*. Кроз неколико поднаслова даје се преглед литературних података о антиоксидативним, цитотоксичним, антимикробним и антинеуродегенеративним ефектима етарских уља и екстраката добијених из представника фамилије уснатица, а затим се детаљно наводе активности биљака из рода *Salvia*, као и њихове микроморфолошке и хистохемијске карактеристике. Затим се описују врсте жалфије одабране за истраживање у докторској дисертацији и даје преглед литературних података о претходним истраживањима биолошких дејстава њихових етарских уља и екстраката. Микроморфолошка, цитолошка и хистохемијска истраживања до сада нису рађена на овим врстама.

После овог одељка формулисани су научни циљеви дисертације који су обухватили: микроморфолошку, цитолошку и хистохемијску анализу лисних трихома (длачица) одабраних врста; хемијску анализу дихлорметанских, етил ацетатних, ацетонских, етанолних, метанолних и водених екстраката испитиваних врста; истраживање антиоксидативног, антимикробног, цитотоксичног и антинеуродегенеративног дејства екстраката са циљем утврђивања потенцијалног апликативног значаја.

Поглавље Материјал и методе организовано је у више потпоглавља. Дат је опис поступка сакупљања и обраде биљног материјала; описане су методе светлосне и електронске микроскопије; описана припрема неколико екстраката и њихова анализа течном хроматографијом под високим притиском (HPLC); одређивање садржаја фенола и флавоноида; одређивање антиоксидативне активности са четири методе коришћењем UV спектрофотометрије; одређивање цитотоксичне активности применом MTT теста, антибактеријске и антифунгалне активности коришћењем микродилуционе методе *in vitro*; инхибиције ензима повезаних са неуродегенеративним променама.

У поглављу Резултати и дискусија, добијени резултати су груписани у одговарајуће целине у оквиру посебних поднаслова, у којима се представљају добијени подаци за сваку врсту, обухватајући микроскопске анализе, антиоксидативну, цитотоксичну, антимикробну и антинеуродегенеративну активност екстраката. Резултати су, поред текстуалног дела, приказани у облику адекватних илустрација и табела. У сваком потпоглављу, добијени резултати су тумачени и дискутовани у поређењу са научним сазнањима у овим областима, кроз анализу резултата добијених у оквиру дисертације и досадашњих резултата добијених код других врста рода *Salvia* из различитих региона, за испитиване карактеристике и активности. Табеле и припадајући текстови су презентовани на начин који омогућује лако праћење тока и редоследа истраживања.

У првом потпоглављу приказани су резултати и дискусија који се односе на врсту *Salvia aegyptiaca.* Међу гландуларним трихомама, разликују се пелтатне и капитатне трихоме са кратким и дугим дршкама, док хистохемијска нализа показује комплексан састав секрета ових трихома. Добијен је висок садржај фенола и флавоноида у етанолном екстракту у поређењу са осталим врстама. Етанолни екстракт ове врсте је показао јаку антиоксидативну активност у три примењена теста.

У другом потпоглављу приказани су резултати и дискусија који се односе на врсту *Salvia fruticosa.* Међу жлезданим, разликују се пелтатне и капитатне трихоме сврстане у пет подтипова, као и дигитиформне трихоме. Висок садржај фенола и флавоноида је добијен у дихлорметанском екстракту. HPLC анализа је показала присуство кафеичне и рузмаринске киселине у метанолном и воденом екстракту. Етанолни екстракт је показао највећу антиоксидативну активност у свим примењеним тестовима. Цитотоксични и антифунгални ефекат је оцењен као слаб, док је антибактеријски ефекат етанолног

екстракта јачи на Грам-позитивне бактерије. Етанолни и водени екстракти су показали јаку инхибицију тирозиназе на тестираним концентрацијама, јачу од стандарда којичне киселине.

У трећем потпоглављу приказани су резултати и дискусија који се односе на врсту *Salvia lanigera.* Од гландуларних, нађене су пелтатне и капитатне трихоме са кратком дршком. Висок садржај фенола је добијен у дихлорметанском екстракту, а флавоноида у етил ацетатном екстракту. HPLC анализа је показала присуство кафеичне али не и рузмаринске киселине. Водени екстракт је показао највећу антиоксидативну активност у три примењена теста, а метанолни у FRAP тесту. Цитотоксични и антифунгални ефекат је оцењен као слаб, док је антибактеријски ефекат етанолног екстракта јачи на Грам-позитивне бактерије. Етанолни и водени екстракти су показали јаку инхибицију тирозиназе на тестираним концентрацијама, јачу од стандарда којичне киселине.

Свако од потпоглавља је адекватно дискутовано, кроз поређење са литературним подацима о микроскопским и хемијским карактеристикама и биоактивностима добијеним за друге врсте жалфија.

Треба посебно нагласити да су микроморфолошка, цитолошка и хистохемијска истраживања први пут рађена на овим врстама. У литератури не постоје подаци за биолошке активности екстраката ових врста које расту у Либији. Испитивања биолошке активности су показала добре резултате антиоксидативне активности појединих екстраката, као и у инхибицији ензима повезаних са неуродегенерацијом, посебно тирозиназе. Неки екстракти би стога могли имати потенцијалну примену у индустрији хране или фармацеутској индустрији, после даљих хемијских анализа и провере у *in vivo* експериментима, с обзиром на значај употребе природних продуката за исхрану и лечење.

На крају су сумирани резултати добијени током израде докторске дисертације и изводени закључци о микроморфолошким, цитолошким и хистохемијским карактеристикама, као и биолошким активностима екстраката проучаваних врста из Либије.

Поглавље Референце садржи 386 библиографских јединица. Литературни извори су адекватно и на одговарајућим местима цитирани у тексту докторске дисертације.

КРАТКА БИОГРАФИЈА

Najat Beleed Al Sheef је рођена 10. 06. 1975. у Лабиару, Либија. Основне студије завршила је у Либији 1998. на Al-Mergeb University, Faculty of Arts and Science, Dept. Biology, Botany section, Khoms. Магистарски рад у области таксономије биљака је одбранила 2005. године на истом Универзитету, где је и запослена од 1999. године.

БИБЛИОГРАФИЈА

РАДОВИ ИЗ ДОКТОРСКЕ ДИСЕРТАЦИЈЕ

Радови у часописима међународног значаја

Радови у часописима међународног значаја М23:

1. Al Sheef, N.B., Duletić-Laušević, S., Janošević D., Budimir, S., Marin, M., Alimpić, A., Giwelli, A.A.M., Marin, P.D. (2013): Micromorphology and ultrastructure of trichomes of Libyan *Salvia fruticosa* Mill. Arch. Biol. Sci. Belgrade, 65(1): 239-248.

2. Janošević, D., Budimir, S., Alimpić, A., Marin, P.D., **Al Sheef, N.B.,** Giwelli, A.A.M., Duletić-Laušević, S. (2016): Micromorphology and histochemistry of leaf trichomes of *Salvia aegyptiaca* (Lamiaceae). Arch. Biol. Sci. Belgrade, *in press.*

Напомена: поред горе наведених радова, део резултата антиоксидативног и цитотоксичног дејства је приказан у раду: Alimpić, A., Kotur, N., Stanković, B., Marin, P.D., Matevski, V., **Al Sheef, N.B**., Duletić-Laušević, S. (2015): The *in vitro* antioxidative and cytotoxic effects of selected *Salvia* species water extracts. Journal of Applied Botany and Food Quality, 88: 115-119.

Радови саопштени на скуповима међународног значаја штампани у изводу М34:

1. Janošević, D., Budimir, S., Duletić-Laušević, S., Alimpić, A., Marin, P.D., **Al Sheef, N.B**., Giweli, A.A. (2013). Micromorphology and histochemistry of *Salvia aegyptiaca* L. leaf trichomes. 1st International Conference on Plant Biology. 20th Symposium of Serbian Plant Physiology Society. Subotica, Serbia. June 4-7. Book of abrstracts: 89.

2. Duletić-Laušević, S., Alimpić, A., Knežević, A., Stajić, M., **Al Sheef, N**., Giweli, A., Mileski, K., Marin, P. (2014). Antioxidant and antifungal activities of ethanol extract of Libyan *Salvia lanigera*. 8th CMAPSEEC. Durres, Albania. May 19-22. Book of abstracts: 122.

3. Duletić-Laušević, S., Alimpić, A., Stević, T., Ćurčić, M., Šavikin, K., **Al Sheef, N.B**., Marković, S. (2015). Evaluation of antioxidant, antibacterial and cytotoxic activity of libyan *Salvia fruticosa* ethanol extract. 2nd International conference on Plant Biology, 21st Symposium of the Serbian Plant Physiology Society, COST ACTION FA1106 QUALITYFRUIT Workshop. Petnica, Serbia. June 17-20. Book of abstracts: 74.

МИШЉЕЊЕ И ПРЕДЛОГ КОМИСИЈЕ

Докторска дисертација мр **Najat Beleed Al Sheef** под насловом: **“Микроморфолошка и цитолошка анализа трихома и биолошки ефекти екстраката *Salvia aegyptiaca* L., *S. fruticosa* Mill. и *S. lanigera* Poir. (Lamiaceae) из Либије” („Micromorphological and cytological analysis of trichomes and biological effects of extracts of *Salvia aegyptiaca* L., *S. fruticosa* Mill. and *S. lanigera* Poir. (Lamiaceae) from Libya“)** представља савремено урађену студију у области микроморфологије и биолошке активности врста рода *Salvia* са територије Либије. По свом обиму, садржају, оригиналности резултата, начину њиховог представљања и интерпретацији, уз осврт на обимну и релевантну литературу, поднети текст има све одлике докторске дисертације. Кандидаткиња је на адекватан начин представила истраживачку област у којој је радила и резултате до којих је дошла. На основу резултата сопствених истраживања и прегледа обимне литературе, показала је да је оспособљена да у овој области креира и изводи експерименте на другим потенцијално економски значајним врстама биљака. Добијени резултати указују на добру перспективу истраживања са ових аспеката, с обзиром на чињеницу да постоји велики број научно недовољно истражених врста које се користе у либијској, као и у афричкој традиционалној медицини.

Комисија сматра да докторска дисертација мр **Najat Beleed Al Sheef** по свом приступу и интерпретираним резултатима, а нарочито узимајући у обзир могућу примену резултата истраживања, представља значајан доринос у познавању микроморфолошких и цитолошких одлика, као и биолошких активности одабраних врста жалфије из Либије.

Добијени резултати у оквиру докторске дисертације објављени су у радовима у међународним часописима.

На основу свега изложеног, комисија са задовољством предлаже Наставно-научном већу Биолошког факултета Универзитета у Београду да прихвати Извештај и одобри јавну одбрану ове докторске дисертације.

Београд, 30.10.2015. године Комисија:

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_____

УНИВЕРЗИТЕТ У БЕОГРАДУ

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Београд, 01.07.2010. године
Број: 06-613-317/4-10
ВМ

На основу члана 104. став 9. Закона о високом образовању ("Службени гласник РС", бр. 76/05, 100/07-аутентично тумачење и 97/08), члана 11. Правилника о признавању страних високошколских исправа ("Гласник Универзитета у Београду", бр. 129/06 и 145/08) и Одлуке Комисије Универзитета за признавање страних високошколских исправа број: 06-613-317/3-10 од 26. маја 2010. године, доносим

Р Е Ш Е Њ Е

ПРИЗНАЈЕ СЕ диплома **Универзитета Al Mergeb, Al-Khoms, Либија,** бр. 185 од 08.03.2006. године, на коме је **Najat Beleed Al Sheef** стекла образовање, као диплома магистарских студија са звањем **магистар биолошких наука**.

О б р а з л о ж е њ е

Универзитету у Београду и Биолошком факултету обратила се Najat Beleed Al Sheef рођена 10.06.1975. године у Alabjar, Либија, захтевом за признавање дипломе Универзитета Al Mergeb, Al-Khoms, Либија, на коме је именована стекла диплому магистра из области биологије, смер ботаника.

Стручни органи Факултета размотрили су све списе предмета и предложили Комисији Универзитета доношење одлуке, којом се предметна диплома признаје као диплома магистарских студија са звањем магистар биолошких наука, што је Комисија Универзитета прихватила.

Са изложеног, решено је као у изреци овог решења.

ПОУКА О ПРАВНОМ ЛЕКУ:
Ово решење је коначно у управном поступку, па се против њега може покренути управни спор код Управног суда, у року од 30 дана од дана пријема решења.

Р Е К Т О Р

Проф. др Бранко Ковачевић

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The manuscript entitled:

MICROMORPHOLOGY AND HISTOCHEMISTRY OF LEAF TRICHOMES OF *SALVIA AEGYPTIACA* (LAMIACEAE)

Author(s): Dušica Janošević, Snežana Budimir, Ana Alimpić, Petar Marin, Najat Al Sheef, Abdulmhid Giweli, and Sonja Duletić-Laušević*

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has been accepted for publication in the **Archives of Biological Sciences (**ISSN: 0354-4664; eISSN: 1821-4339) [http://serbiosoc.org.rs/arch/index.htm], 68:(2) 2016.

Prior to publication, the paper will be uploaded to the **OnLine First** page [http://www.doiserbia.nb.rs/issue.aspx?issueid=2348], which is linked to the **Archives of Biological Sciences** site.

October 20, 2015

Dr. Goran Poznanović
Editor-in-Chief
Archives of Biological Sciences

Arch. Biol. Sci., Belgrade, 65 (1), 239-248, 2013 DOI:10.2298/ABS1301239S

MICROMORPHOLOGY AND ULTRASTRUCTURE OF TRICHOMES OF LIBYAN *SALVIA FRUTICOSA* MILL.

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Abstract - Micromorphological and ultrastructural analyses of the leaf trichomes of *Salvia fruticosa* Mill. were performed by light and electron microscopy. The leaves bear numerous non-glandular unbranched trichomes, and peltate, capitate and digitiform glandular trichomes. Very elongated flagelliform non-glandular trichomes densely covered the leaf surfaces, with especially abundance on the leaf margins. Peltate trichomes consist of a basal epidermal cell, a very short stalk cell and a large round head of eight secretory cells arranged in a circle. Capitate trichomes can be divided into two main types, short-stalked and long-stalked, and further into five subtypes according to the number of stalk cells, morphology and number of glandular head cells. Digitiform trichomes consist of one basal cell, one or two stalk cells and one apical secretory cell, which are of similar diameter and approximately equal length.

Key words: *Salvia fruticosa*, Lamiaceae, trichome types, micromorphology, ultrastructure

INTRODUCTION

Salvia L. (sage), the largest genus of the Lamiaceae family, encompasses about 900 species distributed worldwide (Standley and Williams, 1973). *Salvia* species have been used in traditional medicine all around the world since the ancient times. Their essential oils and extracts have shown antimicrobial, antioxidant, antidiabetic, antitumor, antiplasmodial and anti-inflammatory activities (Sivropolou et al., 1997; Dorman and Deans, 2000; Ulubelen, 2003; Tepe et al., 2004; Kamatou et al., 2008; Şenol et al., 2010; Chan et al., 2011). Many *Salvia* species are used as herbal tea and in food, cosmetics, perfumery and the pharmaceutical industry, and some species are grown in gardens as ornamental plants (Kahraman et al., 2010).

In the *Lamiaceae* family, the morphology, distribution and frequency of glandular hairs are used as discriminative characters at various intrafamilial levels. There are a number of studies on the foliar micromorphology of the genus (Serrato-Valenti et al., 1997; Corsi and Bottega, 1999; Kaya et al., 2003; Kamatou et al., 2007; Özkan, 2008; Kahraman et al., 2009; Baran et al., 2010; Celep et al., 2011). The leaves of numerous plants, including *Salvia* species, are densely covered with glandular and non-glandular trichomes, which originate from epidermal cells (Werker, 2000). Developmental and structural studies of trichomes can shed light on the nature of the secreted material and their functional significance. Plant species bearing glandular trichomes generally produce relatively large amounts of bioactive compounds, which include highly concentrated phyto-

chemicals possessing biological activity and potential applications in the pharmaceutical or food industries (Duke, 1994; Burt, 2004; Giuliani and Maleci Bini, 2008, Miguel, 2010). The essential oil produced by glandular trichomes is one of the characteristic features for the Lamiaceae family (Werker et al., 1985).

Salvia fruticosa Mill. (Greek sage) is an aromatic perennial herb or sub-shrub. This species is native in the eastern Mediterranean basin, distributed from Italy, Sicily and Cyrenaica, through the southern Balkan Peninsula to western Syria (Hedge, 1982, Greuter et al., 1986). It is one of the most economically important *Salvia* species, valued for its beauty, medicinal properties, culinary usage, along with its sweet nectar and pollen, and has had an especially long tradition in application in Greece. *S. fruticosa* originating from Greece, Turkey, Lebanon and Jordan, was studied from different aspects, such as the chemical composition or genetic variation of essential oils, antimicrobial, cytotoxic, antiviral, antifungal, antioxidant effects of essential oils and extracts (Bayrak et Augul, 1987; Müller-Riebau et al., 1997; Sivropolou et al., 1997; Karousou et al., 1998; Skoula 1999; Abou-Jawdah et al., 2002; Pitarokili et al., 2003; Arikat, 2004; Savelev et al., 2004; Tawaha et al., 2007; Papageorgiou et al., 2008; Şenol et al., 2010).

Considering the importance of *Salvia fruticosa* L. as a medicinal plant, due to the essential oils produced in the glandular trichomes, and lack of data about the leaf ultrastructure and micromorphology, a comprehensive study of the trichomes distributed on adult leaves was done.

MATERIALS AND METHODS

Plant material

Aerial parts of the plants were collected during the flowering period from natural populations in Biadda, which is located on the Green Mountain. in eastern Libya, in March 2010. Voucher samples are stored in the Herbarium of the Institute of Botany and Botanical Garden "Jevremovac", Faculty of Biology, University of Belgrade.

Microscopical investigation

For light microscopy (LM), leaf sections of *S. fruticosa* were fixed with 3% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.2, for 24 h at 4˚C. Subsequently, the material was washed in sodium phosphate buffer 3 times over 2 h, post-fixed in 1% osmium tetroxide in same buffer, for 24 h at 4˚C. The fixed material was washed with distilled water, dehydrated in a graded ethanol series and embedded in Araldite resin CY 212 (Agar Scientific Ltd. England). Semi-thin cross sections (1-1.5 μm thick) were cut on a LKB III ultramicrotome and stained with 0.1% methylene blue in 1% borax. Sections were photographed under a Zeiss Axiovert microscope (Carl Zeiss GmbH, Göttingen, Germany).

For transmission electron microscopy (TEM), ultra-thin sections were stained with uranyl acetate and lead citrate and examined with a MORGAGNI 268 (FEI Company, Eindhoven, The Netherlands) transmission electron microscope operated at 100 kV.

For scanning electron microscopy (SEM), adult leaf segments were coated with a thin layer of gold and palladium in a BAL-TEC SCD 005 sputter coater. Both adaxial and abaxial surfaces were examined with a JEOL JSM-6390 LV at an acceleration voltage of 13 kV.

RESULTS

The densely-pubescent leaves bear numerous non-glandular and glandular trichomes on both surfaces. Non-glandular trichomes densely covered the whole leaf surface, but were more abundant on the abaxial leaf side (Fig. 1). Particular abundance was noticed on the margins. They are single, uniseriate, multicellular, pointed, and erect. Numerous trichomes are very elongated, flagelliform, variable in length, consist of five or more cells and are supported by epidermal cells. During the development of the leaf, the density of non-glandular trichomes decreases, although they remain abundant on both surfaces of mature leaves, predominating on the margin and veins of the abaxial surface.

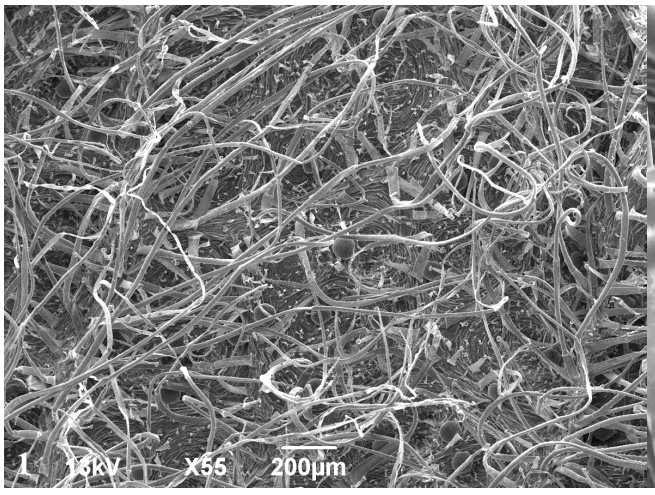


Fig. 1. SEM micrograph showing the morphology of non-glandular and glandular trichomes on the leaf surface of *S. fruticosa*.

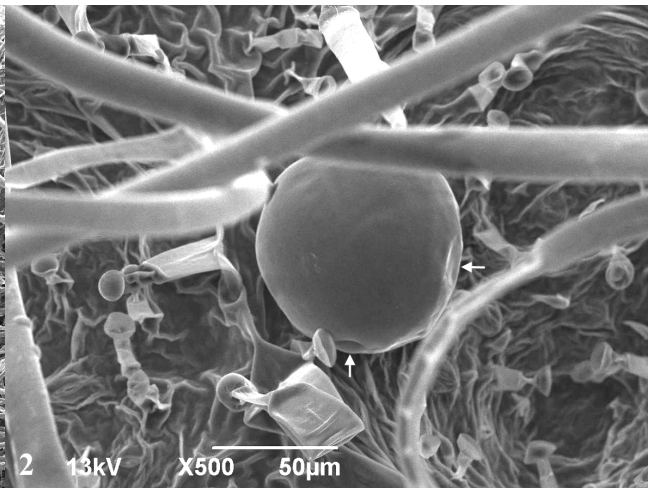
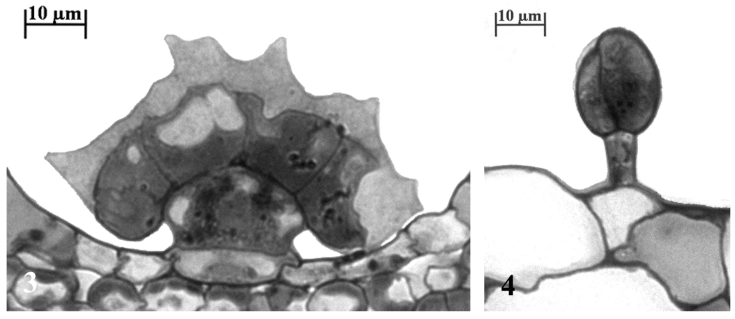


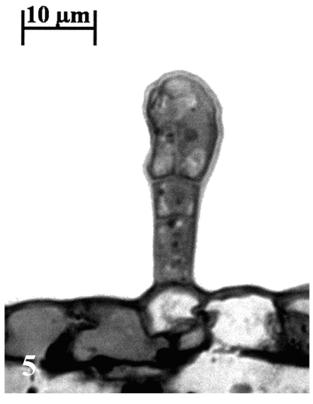
Fig. 2. SEM micrograph of peltate trichome. Note equatorial line (↑).

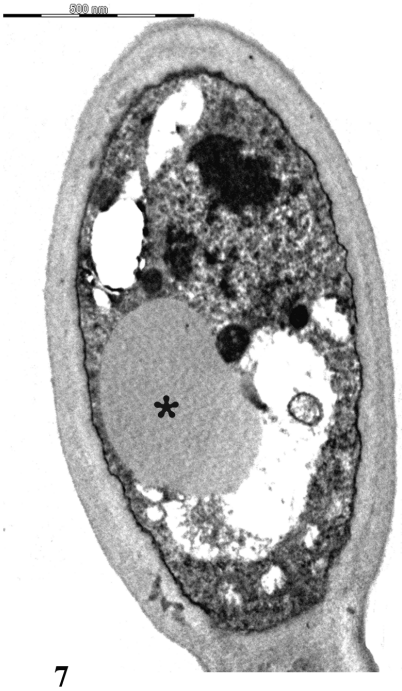
The glandular trichomes are of three main types – peltate, capitate and digitiform. Peltate trichomes have, *in vivo*, a green to brownish color and balloon shape (Fig. 2). They consist of the basal epidermal cell, a very short stalk cell and a large round head of eight secretory cells arranged in a circle (Fig. 3). The peltate glandular hairs have a smooth surface indicating the close attachment of the cuticle to the secretory upper cell walls (Fig. 2). The large space in which the secreted material accumulated developed at the time of secretion by the elevation of the cuticle together with the outermost layer of the secretory cell walls (Fig. 3). An equatorial line of weakness became a visible round head (Fig. 2); the rupture of the cuticle along this line and subsequent rise of the cuticular cap led to release of exudate.

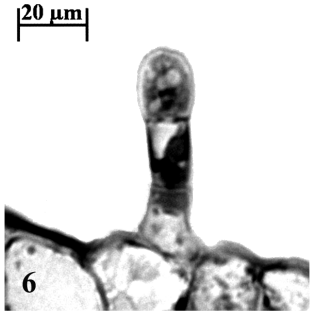
Capitate trichomes can be divided into two main types, short-stalked and long-stalked, and further into five subtypes according to the number of stalk cells, the morphology and the number of glandular head cells, and the secretion process. Short-stalked capitate trichomes subtype I possess one basal cell, one stalk cell with thick cutinized lateral walls and a bicellular ovoid glandular head (Fig. 4). Subtype II have one basal cell, two stalk cells and a globoid secretory head of two cells (Fig. 5), while subtype III capitate trichomes have one basal cell, one stalk cell and one head cell (Fig. 6). The secretory product accumulates inside the apical cells (Fig. 7). Long-stalked capitate trichomes subtype IV possess one basal cell, a long two-celled stalk and a unicellular spherical head (Fig. 8); subtype V have one basal cell, a three-celled stalk and also one cell in the spherical glandular head (Fig. 9).

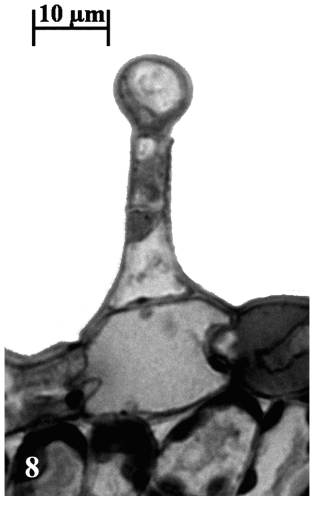
Digitiform trichomes consist of one basal cell, one or two stalk cells and one apical secretory cell. The cells are of similar diameter and approximately equal length (Fig. 10). There is no clear distinction between the head and stalk cells. The glandular apical cells have rounded tips, thin walls and are rich in cytoplasm (Fig. 11). The apical cell of a few digitiform trichomes possesses very small subcuticular spaces, but many of them have not developed a subcuticular space (Fig. 11, 12). In the secretory phase, digitiform trichomes cells contained a dense cytoplasm, numerous dark organelles, osmiophilic drops and translucent vacuoles, and the outer cell wall was covered with a thick cuticle (Fig. 11). During further development, as a result of the accumulation of secretion, the periplasmatic space gradually enlarged, leading to a drastic retraction of the plasma membrane from the cell wall (Fig. 12).











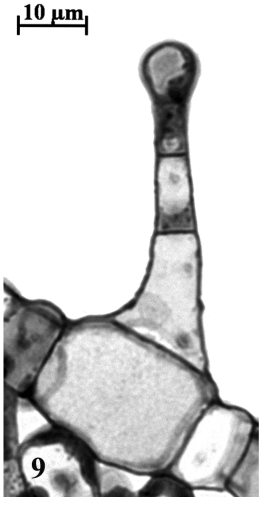


Fig. 3. LM micrograph of peltate trichome of *S. fruticosa* showing basal epidermal cell, very short stalk cell and large head of eight secretory cells.

Fig. 4. LM micrograph of short stalked capitate trichome subtype I.

Fig. 5. LM micrograph of short stalked capitate trichome subtype II.

Fig. 6. LM micrograph of short stalked capitate trichome subtype III.

Fig. 7. TEM micrograph of capitate trichome subtype III showing the secretory product –lipid droplet (*) inside the apical cell.

Fig. 8. LM micrograph of long-stalked capitate trichome subtype IV.

Fig. 9. LM micrograph of long-stalked capitate trichome subtype V.

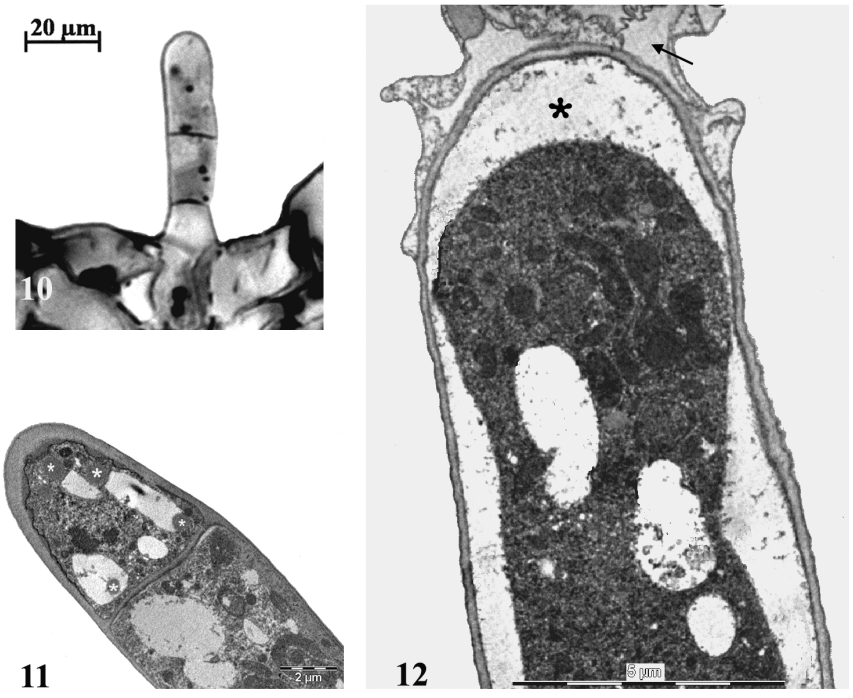


Fig. 10. LM micrograph of digitiform trichome of *S. fruticosa*.
Fig. 11. TEM micrograph of the apical cell of digitiform trichome. Note lipid droplets (*).
Fig. 12. TEM micrograph of the apical cell of digitiform trichome. Note periplasmatic space (*) and subcuticular space (↑) filled with secreted material.

DISCUSSION

The presence of non-glandular and glandular hairs is characteristic for Lamiaceae species (Werker et al., 1985). The distribution of non-glandular trichomes on the epidermis is a natural phenomenon in most angiosperms and could be associated with protection against foraging insects and airborne propagules of fungi (Fahn, 1967).

Glandular trichomes vary in morphology, structure and in number per unit area of the epidermis, among species and organs (Serrato-Valenti et al., 1997; Ascensao et al., 1999). Researchers have mostly found two main types on the leaves and flowers of different Lamiaceae species; peltate with a large secretory head, and capitate trichomes with a stalk and small head. Different variations have been observed in the studied species, in stalk length, head shape and number of cells, release of secretion products, etc.

Several *Salvia* species were studied for their glandular hair characteristics and essential oils composition. Different types of glandular trichomes were found in different species. The results obtained in this research showed that peltate, capitate and digitiform glandular hairs are present on the leaves of *S. fruticosa*.

Peltate trichomes are usually composed of a basal epidermal cell, a short, wide stalk cell and a large round head of several secretory cells, which could vary in number among *Salvia* species from four cells in *S. blepharophylla* (Bisio et al., 1999) and *S. verticillata* (Krstić et al., 2006) to six or eight cells in a single disc in *S. aurea* (Serrato-Valenti et al., 1997) and *S. officinalis* (Werker et al. 1985), or by 12-18 cells arranged in two concentric circles with four central and eight or more peripheral cells (Corsi and Bottega, 1999; Kamatou et al., 2006; Özkan, 2008; Baran et al., 2010; Kahraman et al., 2010). The present

study showed that *S. fruticosa* has peltate glandular trichomes consisting of a basal epidermal cell, a very short stalk cell with cutinized lateral walls and large round head of eight secretory cells arranged in a circle, as was observed in some of the studied *Salvia* species.

Capitate trichomes are also widespread in Lamiaceae, but they are more variable in stalk length and head shape. They generally consist of one to two stalk cell(s) and one to two cell(s) forming a rounded to pear-shaped secretory head (Werker et al. 1985). Variations in capitate trichomes were noticed, and Werker et al. (1985) for the first time classified capitate hairs as types I, II and III, according to their morphology and secretion mode. Following Werker et al. (1985), type IV capitate hairs were described in *S. officinalis* by Corsi and Bottega (1999). Several researchers divided capitate trichomes into only two types according to the dimensions of the stalk, the morphology of glandular head and the secretion process (Ascensao et al., 1999; Serrato-Valenti et al., 1997, Bisio et al., 1999). Serrato-Valenti et al. (1997) in *S. aurea* described the capitate trichome type 1 consisting of a short stalk and bicellular head, and capitate trichome type 2 consisting of 1-4 stalk cells, a narrow neck cell and globose unicellular head; similar types were found in *S. blepharophylla* (Bisio et al., 1999), *S. albicaulis, S. dolomitica* (Kamatou et al., 2007), etc. In our research we noticed two main types, short-stalked and long-stalked capitate trichomes, but further divided into five subtypes according to the number of stalk cells, the morphology and the number of the glandular secretory head cells and the secretion process. Short-stalked capitate trichomes subtype I have one stalk cell and a bicellular ovoid head, while subtype II have two stalk cells and a globoid glandular head, consisting of two cells. Subtype III of capitate trichomes have one stalk cell and one head cell. Long-stalked capitate trichomes subtypes IV and V have a unicellular spherical glandular cell, but these types of trichomes have a different number of stalk cells. Some other *Salvia* species also shown variations in the morphology of capitate trichomes, and been divided into more subtypes, such as *S. chrysophylla* with 4 subtypes (Kahraman et al., 2010), *S. officinalis* (Corsi et Bottega, 1999), or 3 types in *S. smyrnea* (Baran et al., 2010), *S. verticillata* (Krstić et al., 2006), and *S. argentea* (Baran et al., 2010). Because of the great variations in structure and size of capitate trichomes, and since they form part of the floral specialized properties for pollination, some authors emphasize their significance as a taxonomic character of the Lamiaceae (Navarro et El Oulalidi, 2000).

In the analyzed leaves of *S. fruticosa*, one specific type of glandular trichome was observed – digitiform trichomes. These trichomes are less abundant than peltate and capitate trichomes. A digitiform type of glandular trichome was reported for some Lamiaceae species, such as *Plectranthus ornatus* (Ascensao et al., 1999). These trichomes consist of three to four cells, in line, of similar diameter and approximately equal length (one basal, one to two stalk cells and one apical, head-like cell which does not develop a subcuticular space). There is no clear distinction between head and stalk cells. Talebi et al. (2012) found four types of digitiform trichomes in *Ziziphora tenuior* (one- to four-celled).

Ultrastructural analyses are important in trichome research because the relations among morphology, cytology and secretion processes can be established. Ultrastructural changes during the secretory phase of glandular trichomes were characterized by proliferation of the endomembrane system. Numerous mitochondria are connected with the high metabolic activity of the cell (Dunkić et al., 2007). Smooth ER, leucoplasts without thylakoids containing osmophilic drops, are characteristic organelles involved in terpene production, while dictyosomes are responsible for polysaccharide production (Cheniclet and Carde, 1985; Dunkić et al., 2007; Huang et al., 2008).

The analysis conducted and presented showed that *S. fruticosa* is very rich from the aspect of glandular trichome diversity and quantity. It is suggested that the secretions of the glandular trichomes may be involved in the chemical defense of the plants, or may guide insects, or act as floral rewards to pollina-

tors, but the specific function of each trichome type is not known (Ascensao et al., 1999). Detailed studies of the morphology, anatomy and ultrastructure may be useful in the interpretation of their functions. The abundant non-glandular hairs are involved in mechanical defense, and protect the plant from excessive transpiration and insolation (Corsi and Bottega, 1999).

Some authors (Baran et al., 2010) assumed that features such as abundance and the diversity of glandular trichomes on plant organs, the presence or absence of neck cells, the thickness of their side walls and the stalk length, could show variation according to the xeromorphic character of the plants. The abundant and diverse glandular trichomes and long stalks of the capitate trichomes of *S. fruticosa* show the xeromorphic character of the investigated plant species which, in its native environment, grows as part of the Maquis shrubland and is very drought-resistant.

Acknowledgments - This work was supported by the Ministry of Education and Science of the Republic of Serbia (Grant No. 173029 and Grant No. 173015).

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Journal of Applied Botany and Food Quality 88, 115 - 119 (2015), DOI:10.5073/JABFQ.2015.088.016

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The *in vitro* antioxidative and cytotoxic effects of selected *Salvia* species water extracts

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(Received October 10, 2014)

Summary

The current paper presents antioxidant and cytotoxic activities and total phenolic and flavonoid content of the selected species of genus *Salvia* (Lamiaceae) growing wild in Macedonia (*S. jurisicii* Košanin, *S. amplexicaulis* Lam., *S. ringens* Sibth. & Sm.) and Libya (*S. fruticosa* Mill. and *S. lanigera* Poir.). Crude water extracts, obtained from aerial parts, were yielded from 6.50 to 14.32%. Total phenolic content was the highest in water extracts of *S. amplexicaulis* and *S. ringens* (226.30 and 189.01 mg GAE/g, respectively), while the flavonoids were the most abundant in *S. jurisicii* extract (32.36 mg QE/g). Antioxidant activities of extracts were measured using DPPH, ABTS and FRAP assays. *S. amplexicaulis* and *S. ringens* extracts showed the strongest antioxidant activity, measured using DPPH (14.21 and 23.44 μg/mL, respectively) and ABTS assays (2.91 and 2.42 mg AAE/g, respectively). In FRAP assay, *S. amplexicaulis* and *S. fruticosa* extracts exhibited strongest activity (1406.73 and 1191.51 μmol Fe(II)/g). Water extract of *S. amplexicaulis* and *S. ringens* performed the strongest cytotoxic activity against K562 cells (151.07 and 173.06 μg/mL, respectively). Based on these findings, it can be concluded that *S. amplexicaulis* and *S. ringens* water extracts could be considered as possible source of antioxidant and cytotoxic agents.

Introduction

The genus *Salvia* (Lamiaceae) represents an enormous and cosmopolitan assemblage of nearly 1000 species worldwide of which 36 were found in Europe (Hedge, 1972) and 10 in Libya (Jafri and El-Gadi, 1985). The representatives of *Salvia* genus are widely cultivated and used in flavoring and folk medicines.

Sage and rosemary from the Lamiaceae family were shown to have similar patterns of phenolic compounds and the antioxidant activity had been attributed mainly to carnosic, rosmarinic acid and their isomers. Additional classes of active compounds include terpenoids, flavonoids and other phenolic acids (Lu and Foo, 2001). Majority of these compounds, excluding terpenoids, are water-soluble and present in aqueous extract obtained using common techniques of extraction (Tiwari et al., 2011).

The genus *Salvia* was the research topic of numerous chemical, medicinal and pharmacological studies. Species of *Salvia* showed diverse biological activities of plant material and/or isolated essential oil/extracts due to the presence of large number of different compounds. The antioxidant (Couladis et al., 2003; Janicsák et al., 2010; Orhan et al., 2013; Alimpić et al., 2014), cytotoxic (Fiore et al., 2006; Kamatou et al., 2005; Janicsák et al., 2007; Abu-Dahab et al., 2012), antimicrobial (Kamatou et al., 2005; Hawas and El-Asnari, 2006), antiinflammatory (Kamatou et al., 2005; 2010), antimalarial (Kamatou et al., 2005), anticholinesterase (Şenol et al., 2010, Orhan et al., 2012; 2013), etc. effects were reported.

S. jurisicii Košanin, perennial herb inhabiting arid habitats, is an endemic species in the central part of Republic of Macedonia (Hedge, 1972). It was previously investigated for antioxidant activity (Janicsák et al., 2010).

S. amplexicaulis Lam. is a perennial plant, distributed on Balkan Peninsula (Hedge, 1972). It was investigated for vasodepressor (Ulubelen, 2003), antioxidant, and neurobiological activity (Orhan et al., 2012) of extracts.

S. ringens Sibth. & Sm. is a hardy herbaceous perennial plant, distributed in South and Eastern parts of Balkan peninsula (Hedge, 1972). Total acetone extract and some isolated compounds showed cytotoxic activity against HeLa cells (Janicsák et al., 2007), while ethanol extracts performed strong antioxidant activity (Couladis et al., 2003).

S. lanigera Poir. is perennial herb with thick woody rootstock distributed in North Africa, from northern Egypt and Arabia, to the south of Turkey and Iran (Jafri and El-Gadi, 1985). There are a few reports on phenolic composition, antimicrobial and cytotoxic activity of *S. lanigera* extracts (Hawas and El-Asnari, 2006; Shaheen et al., 2011).

S. fruticosa Mill. (*S. triloba* L.) is subshrub distributed in Canary Islands and North Africa. It is sometimes used for flavouring tea and cultivated as an ornamental plant (Jafri and El-Gadi, 1985). Jordanian *S. fruticosa* ethanol extract performed significant cytotoxic activity (Abu-Dahab et al., 2012).

The objective of this study was to investigate antioxidative and cytotoxic activity as well as phenolic/flavonoid contents in water extracts of selected Macedonian and Libyan *Salvia* species.

Material and methods

Plant material

Aerial parts of the *Salvia* species were collected in flowering period from their natural populations at localities in Macedonia and Libya and were identified by Prof. P.D. Marin and Prof. V. Matevski. Plant material was dried and kept in shadow at room temperature for further processing. Total sample for each species consisted of at least 20-50 individuals (about 500 g of dry material, depending of species). Voucher specimens were deposited in the Herbarium of the Institute of Botany and Botanical Garden "Jevremovac", Faculty of Biology, University of Belgrade. Collection data and voucher numbers of investigated *Salvia* species are given in Tab. 1.

Preparation of water extracts

Extracts were prepared of whole aerial plant parts using classic maceration procedure. Dry plant material (5 g), randomly taken

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Tab. 1: Collection data and voucher numbers of examined *Salvia* species

Salvia species	Collection data			Voucher No.
	Locality	Date	Geographical data	
S. jurisicii Košanin	Štip (Macedonia)	11.7.2011	N 41°50.324' E 22°08.289' Alt. 420 m	BEOU; 16674
S. amplexicaulis Lam.	Pletvar (Macedonia)	12.7.2011	N 41°22.169' E 21°39.180' Alt. 1010 m	BEOU; 16673
S. ringens Sibth. & Sm.	Krivolak (Macedonia)	2.7.2012	N 41°53.33' E 21°41.08' Alt. 229 m	BEOU; 16675
S. lanigera Poir.	Zintan (Libya)	22.3.2010	N 31°55.50' E 12°14.54' Alt. 694 m	BEOU; 16880
S. fruticosa Mill.	Biadda (Libya)	10.3.2010	N 32°45.59' E 21°44.30' Alt. 624 m	BEOU; 16702

from whole collected sample of each species, was grounded in small pieces (2-6 mm) and extracted by 50 ml of boiling distilled water (10% w/v). Extraction was performed during 24 h at room temperature. The mixture was exposed to ultrasound 1 h before and after 24 h-maceration. Subsequently, extracts were filtered through a paper filter (Whatman No.1) and evaporated under reduced pressure by the rotary evaporator (Buchi rotavapor R-114). The obtained crude extracts (Tab. 2) were stored in the fridge at +4 °C for further experiments.

Tab. 2: The yield, total phenolic and flavonoid content of *Salvia* water extracts. Values are presented as mean ± standard deviation.

Water extracts	Extract yield[a]	Total phenolic content[b]	Total flavonoid content[c]
S. jurisicii	9.50	81.03 ± 0.216	32.36 ± 0.731
S. amplexicaulis	14.32	226.30 ± 1.179	17.87 ± 0.089
S. ringens	6.50	189.01 ± 1.699	22.64 ± 0.898
S. lanigera	7.32	58.47 ± 0.200	17.18 ± 0.544
S. fruticosa	7.62	67.68 ± 0.001	21.73 ± 0.163

[a] % of weight of dry plant material
[b] mg GAE/g dry extract
[c] mg QE/g dry extract

Evaluation of antioxidant activity

Antioxidant activity was evaluated using three spectrophotometric assays: DPPH, ABTS, and FRAP. Stock solutions of dry extracts were prepared in the distilled water in concentration of 1000 μg/mL (w/v).

DPPH assay

For evaluation of antioxidant activity of extracts, 2,2-dyphenyl-1-picrylhydrazyl (DPPH) free radical scavenging method (Blois, 1958) with slight modifications was used. Stock extract solution was diluted with methanolic solution of DPPH (40 μg/mL) to adjust the final volume of reaction mixture (2000 μL) of the test tube. Methanol was used as a blank, while methanol with DPPH solution was used as a control. BHA, BHT and ascorbic acid were used as positive controls (standards). Each blank, samples and standards' absorbances were measured in triplicate. Absorbance of the reaction mixture was measured after 30 min in the dark at room temperature at 517 nm using the JENWAY 6305UV/Vis spectrophotometer. The decrease of absorption of DPPH radical at 517 nm was calculated using equation:

$$\text{Inhibition of DPPH radical (\%)} = [(A_C - A_S)/A_C] * 100\%$$

where A_c is the absorbance of control (without test sample) and A_s is the absorbance of the test samples at different concentrations. IC_{50} values (μg/mL) (concentrations of the test samples and standard antioxidants providing 50% inhibition of DPPH radicals) were calculated from DPPH absorption curve at 517 nm.

ABTS assay

ABTS assay is performed according to procedure of Miller et al. (1993) with some modifications. Fresh $ABTS^+$ solution was prepared 12-16 hours before use by dissolving ABTS in 5 ml of 2.46 mmol potassium-persulfate to obtain concentration of 7 mmol/L and stored in the dark at room temperature. The $ABTS^+$ solution was dissolved by distilled water to achieve an absorbance of working solution 0.700 ± 0.020 at 734 nm. 50 μL of test samples (1 mg/mL) and/or standards (0.1 mg/mL) were mixed with 2 mL of diluted $ABTS^+$ solution and incubated for 30 min at 30 ºC. Absorbance was recorded at 734 nm using JENWAY 6305UV/Vis spectrophotometer. Distilled water was used as blank. BHA and BHT dissolved in methanol were used as standards. ABTS activity was calculated from ascorbic acid calibration curve (0-2 mg/L) and expressed as ascorbic acid equivalents per gram of dry extract (mg AAE/g).

Ferric-reducing ability of plasma (FRAP) assay

The FRAP assay was performed according to Benzie and Strain (1996) procedure with slight modifications. FRAP reagent was prepared freshly to contain sodium acetate buffer (300 mmol/L, pH 3.6), 10 mmol/L TPTZ in 40 mmol/L HCl and $FeCl_3*6H_2O$ solution (20 mmol/L), i.e. in proportion 10:1:1(v/v/v), respectively. Working FRAP solution was warmed to 37 °C prior to use. 100 μL of test sample (500 μg/mL) were added to 3 mL of working FRAP reagent and absorbance was recorded at 593 nm after 4 min using the

JENWAY 6305UV/Vis spectrophotometer. Blank was prepared to contain distilled water instead of extract. Ascorbic acid, BHA and BHT dissolved in methanol in concentration 0.1 mg/mL were used as standards. The same procedure was repeated for standard solution of $FeSO_4 * 7H_2O$ (200-1600 µmol/L) in order to construct calibration curve. FRAP values of sample was calculated from standard curve equation and expressed as µmol Fe (II)/g dry extract).

Determination of total phenolic content

The total phenolic content was measured using spectrophotometric method (Singleton and Rossi, 1965). The reaction mixture was prepared by mixing 0.2 mL of extract solution in concentration of 1 mg/mL, 1 mL of 10% Folin-Ciocalteu reagent and 0.8 mL of 7.5% Na_2CO_3. Blank was prepared to contain distillated water instead of extract. Absorbance was recorded at 740 nm after 2 h incubation at room temperature using JENWAY 6305UV/Vis spectrophotometer. Phenolic content in samples was calculated from standard curve equation and expressed as gallic acid equivalents (mg GAE/g dry extract).

Determination of flavonoid concentration

Flavonoid concentrations of samples were measured spectrophotometrically according to procedure of Park et al. (1997). The reaction mixture was prepared by mixing 1 mL of extract solution in concentration 1 mg/mL, 4.1 mL of 80% ethanol, 0.1 mL of 10% $Al(NO_3)_3$ x 9 H_2O and 0.1 mL 1M CH_3COOK. Blank was prepared to contain 96% ethanol instead of extract. After 40 min of incubation at room temperature, absorbance was measured at 415 nm using JENWAY 6305UV/Vis spectrophotometer. Concentration of flavonoids in samples (mg/ml) was calculated from standard curve equation and expressed as quercetin equivalents (mg QE/g dry extract).

Cytotoxic assay – MTT assay

To assess cytotoxic effect of sage water solutions, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed (Drakulić et al., 2012). It is a colorimetric assay which detects conversion of yellow tetrazolium salt into purple formazon. The conversion is catalyzed by cellular enzymes and its rate represents measure of cells viability. K562 cells are human immortalised myelogenous leukemia line. K562 cells are of the erythroleukemia type. The line is derived from a CML patient in blast crisis. K562 cells were maintained in essential minimal medium (MEM) supplemented with 10% FCS. Cells were treated in 96 well plates for 48 h with 50, 100, 150, 200, 300 and 400 µg/mL sage water extracts in MEM. After addition of MTT solution (0.5 mg/mL), plate was incubated for additional 3 h. Acidified isopropanol was added to dissolve tetrazolium salts. Absorbance was measured at 620 nm.

Statistical analysis

All experimental measurements were carried out in triplicate and are expressed as average of three measurements ± standard deviation. Pearson's correlation coefficients were calculated between on one hand total phenolics and flavonoids and on the other hand cytotoxic and antioxidant assays and interpreted according to Taylor (1990). Calculations and constructing of the charts were performed using the MS Office Excel, 2007.

Results and discussion

The yield of extract, total phenolic and flavonoid content

The yields were expressed as percent of dry extract of dry plant material. The yields of obtained water extract were ranged from 14.32% for *S. amplexicaulis* to 6.50% for *S. ringens* extract (Tab. 2). Considering the uniform extraction procedure applied in this study, variations in extract's yields can be attributed to differences of plant material (species). In some previous studies researchers have observed that type of plant material, choice of extraction solvent and extraction procedure affects composition, activity and possible future use of the obtained extract (Tiwari et al., 2011). Şenol et al. (2010) obtained that yield of methanolic extract of 55 *Salvia* taxa varied between 2.88 and 13.41% and our results are in agreement with these findings.

Total phenolic and flavonoid contents of water extracts were evaluated spectrophotometrically and expressed as gallic acid equivalents/g dry extract and flavonoids concentrations as quercetin equivalents/g dry extract. As can be seen in Tab. 2, the highest amount of phenolics was measured in water extracts of *S. amplexicaulis* and *S. ringens* (226.30 and 189.01 mg GAE/g, respectively), while the content of phenolics in the other extracts was lower than 100 mg GAE/g. On the contrary to phenolics, flavonoids were the most abundant in *S. jurisicii* extract (32.36 mg QE/g). When the obtained results are compared to the values for total phenolic content of *Camellia sinensis* green tea (140.11 mg GAE/g) and *Ginkgo biloba* (140.18 mg GAE/g) standardized extract (Stanković et al., 2010), it can be seen that *S. amplexicaulis* and *S. ringens* have higher values. Our results are in agreement with the literature data for ethanol extracts of fourteen Turkish *Salvia* species (57.10-218.09 mg GAE/g for total phenols and 8.29-108.78 mg QE/g for flavonoids) obtained by Orhan et al. (2013). Stagos et al. (2012) found total phenolic content of 267 and 190 mg GAE/g for methanolic and water extract of *S. fruticosa* from Greece, respectively. As many researchers reported, amount of extracted phenolics and flavonoids depends on selection of extraction solvent.

Evaluation of antioxidant activity

Oxygen sometimes can be fatal for the organisms although eukaryotic organisms cannot exist without it, which is known as oxygen paradox. A free radical is any species capable of independent existence that contains one or more unpaired electrons. Reactive oxygen species (ROS) may damage important cellular molecules such as DNA, proteins and lipids, causing cancer, cardiovascular, neurodegenerative and other diseases (Halliwell, 2006).

Antioxidant activities of *Salvia* species water extracts measured using DPPH, ABTS and FRAP assays are presented in Tab. 3. DPPH and ABTS assays were applied to examine scavenging activity of extracts, while FRAP assay measured ability of extracts to reduce Fe (III) to Fe (II) ion. Water extract of *S. amplexicaulis* showed markedly the strongest antioxidant activity in all three assays.

Tab. 3: DPPH, ABTS and FRAP activity of *Salvia* water extracts.

Water extracts	DPPH assay (IC50, µg/ml)	ABTS assay (mg AAE/g)	FRAP assay (µmol Fe(II)/g)
S. jurisicii	212.24	1.15 ± 0.062	290.14 ± 6.880
S. amplexicaulis	14.21	2.91 ± 0.019	1406.73 ± 8.055
S. ringens	23.44	2.42 ± 0,019	615.44 ± 6.720
S. lanigera	230.87	1.77 ± 0.085	79.13 ± 5.255
S. fruticosa	48.11	1.98 ± 0.005	1191.51 ± 8.109
Positive controls			
BHT	17.94	2.75 ± 0.021	445.34 ± 5.772
BHA	13.37	2.82 ± 0.011	583.72 ±5.255
Ascorbic acid	5.11	/	180.81 ± 8.607

DPPH activity of extracts was assessed as good (IC_{50}<30 μg/ml) for *S. amplexicaulis* and *S. ringens*, as moderate 30<IC_{50}<80 μg/ml) for *S. fruticosa* and as poor (IC_{50}>80 μg/ml) for *S. jurisicii* and *S. lanigera* according to KAMATOU et al. (2010). *S. amplexicaulis* water extract showed the best DPPH radical scavenging activity (14.21 μg/ml), higher than activity of the synthetic antioxidant BHT (17.94 μg/ml) and commercially used green tea (20.62 μg/ml) (STANKOVIĆ et al., 2010) and very close to BHA (13.37 μg/ml). Besides, water extract of *S. amplexicaulis* performed stronger activity than ethanol and methanol extracts (28.74 and 21.28 μg/ml, respectively) as previously reported by ALIMPIĆ et al. (2014). *S. fruticosa* water extract showed lower DPPH activity than those measured by STAGOS et al. (2012) for methanolic and water *S. fruticosa* extracts, 22 and 16 μg/ml, respectively. JANICSÁK et al. (2010) previously found low antioxidant activity of *S. jurisicii* aqueous-methanol extract (191.2 μg/ml), as confirmed in this study.

In ABTS assay, *S. amplexicaulis* and *S. ringens* showed the strongest activity (> 2 mg AAE/g), while other extracts were quite weaker than aforementioned (Tab. 3). Some researchers preferred to express ABTS activity by IC_{50} value (KAMATOU et al., 2010; STAGOS et al., 2012), and more frequently using standard equivalents (Trolox, ascorbic acid, etc.). In this study, the ascorbic acid calibration curve was chosen for presentation of obtained results. LI et al. (2008) measured wide range of ABTS activity of selected Chinese medicinal plants (0.97-265.43 μmol Trolox/g dry plant material). *S. fruticosa* showed activity of 13 μg/ml for methanolic and 29 μg/ml for water extract (STAGOS et al., 2012).

S. amplexicaulis followed by *S. fruticosa* water extract showed FRAP activity over 1000 μmol Fe(II)/g. In contrast, *S. lanigera* water extract performed FRAP activity lower than 100 μmol Fe(II)/g (Tab. 3). BENZIE and STRAIN (1996) suggested expressing of results using Fe(II) calibration curve. Some researches preferred to present results by IC_{50} values or as Trolox equivalents. The lacking of a unique form of presenting results makes comparison of results obtained in various studies rather complicated. LI et al. (2008) measured FRAP ability of methanol extracts of 45 Chinese medicinal plants, and results ranged as 1.23-453 μmol Fe(II)/g. Ethyl-acetate extracts of selected Turkish *Salvia* species exhibited quite low FRAP activity, whereas methanol extracts were highly active (ORHAN et al., 2012).

Cytotoxic activity of extracts

Cytotoxic activity of extracts was tested using MTT assay against K562 cell line over 48 h. Obtained results, expressed as IC_{50}, are presented in Fig. 1. All tested water extracts exhibited cytotoxic effect on K562 cells. Among examined *Salvia* species, *S. amplexicaulis* and *S. ringens* showed the strongest antioxidant activity (IC_{50} < 200 μg/ml), followed by *S. fruticosa* and *S. lanigera* (IC_{50} 200-400 μg/ml) and *S. jurisicii* (> 400 μg/ml).

Some of the species examined in this study were partially investigated before for their cytotoxic activity. Ethanol extract of *S. fruticosa* collected in Jordan showed lower IC_{50} values (17.43-38.91 μg/ml) against breast cancer cell lines measured by Sulphorhodamine B assay (ABU-DAHAB et al., 2012) than those in our study. Water extract of Libyan *S. lanigera* in this study was less active than several extracts of Egyptian *S. lanigera* (IC_{50} values from 9.83 to over 100 μg/ml), especially obtained by acetone (SHAHEEN et al., 2011). JANICSÁK et al. (2007) reported on cytotoxic activity of Bulgarian *S. ringens* extract and its isolated components. The literature data on cytotoxic activity of *S. ringens, S. jurisicii* and *S. amplexicaulis* were not available. Previous studies have demonstrated that South African and Jordanian *Salvia* species showed IC_{50} values from approximately 20 to above 100 μg/ml (KAMATOU et al., 2005) and from 90 to 400 μg/ml (FIORE et al., 2006), respectively. Our findings are in accordance with previous reports.

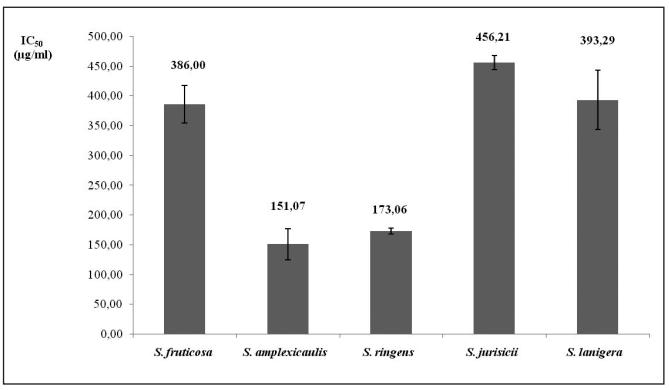

Fig. 1: Cytotoxic activity of water extracts of tested *Salvia* species tested against K562 cell line

Correlation between cytotoxic and antioxidant activities and total phenolic and flavonoid content

Pearson's correlation coefficients were calculated between total phenolics and flavonoid content of the *Salvia* water extracts and their antioxidant and cytotoxic activities (Tab. 4). In this study, interpretation of correlation coefficients according to TAYLOR (1990) was chosen. On the contrary to ABTS and FRAP activity, cytotoxic and DPPH activity of extracts were negatively correlated to phenolic and positively to flavonoid content because of presenting of results as IC_{50} values (Tab. 4). Correlation between total phenolic and flavonoid contents was negative (data not presented). It was previously reported that water soluble flavonoids (mostly anthocyanins) have no antimicrobial significance and water soluble phenolics were only important as antioxidant compounds (TIWARI et al., 2010). However, obtained results indicated that biological activities of extracts were negatively correlated to flavonoid content in extracts. Previously, BEN FARHAT et al. (2014) reported on negative correlation of some flavonoids from *Salvia officinalis* extracts and antioxidant activity evaluated by DPPH, ABTS and FRAP assays. Our findings are in agreement with various studies which showed that antioxidant activity of rosemary and sage, both belonging to the family Lamiaceae, is mostly manifested by presence of the phenolic acids (rosmarinic and carnosic acids and their derivates) and then terpenoids, flavonoids and other phenolic acids (LU and FOO, 2001; KAMATOU et al., 2010; ORHAN et al., 2012).

Tab. 4: Linear correlation coefficients (r) of cytotoxic and antioxidant activities versus total phenolic and flavonoid content of *Salvia* water extracts

	Total phenolic content	Flavonoid content
Cytotoxic activity	-0,9514[c]	0,4886[b]
DPPH activity	-0,7338[c]	0,3248[a]
ABTS activity	0,8394[c]	-0,6972[c]
FRAP activity	0,5512[b]	-0,3169[a]

According to Taylor (1990):
[a]r ≤ 0.35 weak correlation; [b]0.36 < r < 0.67 moderate correlation;
[c]0.68 < r < 1 strong correlation

Conclusions

Based on these findings, it can be concluded that some of the examined extracts could be taken into consideration as possible anti-

oxidant and cytotoxic agents. The results showed that certain species have optimal ratio of the yield, total phenolic and flavonoid content and performed antioxidant and cytotoxic activities, such as Macedonian *S. amplexicaulis*, *S. ringens* and Libyan *S. fruticosa*. Taking into account non-toxicity of the water as extraction solvent, their application in prevention and treatment of some free radical caused disorders such as cancer, cardiovascular and neurodegenerative diseases could be proposed. Future studies will provide data on qualitative composition of phenolics and other possible biologically active components of the water extracts (research in progress).

Acknowledgments
Authors are grateful to the Ministry of Education, Science and Technological Development of Serbia for financial support (Project No. 173029 and 41004).

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