JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY 2012, 63, 5, 531-535
www.jpp.krakow.pl

D. KITIC[1], S. BRANKOVIC[2], M. RADENKOVIC[2], K. SAVIKIN[3], G. ZDUNIC[3], B. KOCIC[4], R. VELICKOVIC RADOVANOVIC[1]

# HYPOTENSIVE, VASORELAXANT AND CARDIODEPRESSANT ACTIVITIES OF THE ETHANOL EXTRACT OF *SIDERITIS RAESERI* SPP. *RAESERI* BOISS & HELDR

[1]Department of Pharmacy, Faculty of Medicine, University of Nis, Nis, Serbia; [2]Department of Physiology, Faculty of Medicine, University of Nis, Nis, Serbia; [3]Institute for Medicinal Plants Research, Belgrade, Serbia; [4]Institute of Epidemiology, Faculty of Medicine, University of Nis, Nis, Serbia

*Sideritis raeseri* spp. *raeseri* Boiss & Heldr is a native plant from the Mediterranean region that is used due to its medicinal and culinary properties. The aim of this study was to evaluate the effects of ethanol extract of *S. raeseri* on the blood pressure, vascular and cardiac contractions. Arterial blood pressure was registered directly from the carotid artery in the anaesthetized rabbits. Aortic rings and the spontaneously beating atria were mounted in tissue bath. An intravenous injection of extract of *S. raeseri* (0.025-7.5 mg/kg) caused a dose dependent decrease of the arterial pressure and heart rate, with $EC_{50}$ value of 24.31±3.87 mg/kg and 88.14±7.51 mg/kg, respectively. In aortic preparations precontracted with KCl (80 mM), the extract of *S. raeseri* (0.005-1.5 mg/ml) elicited a vasodilatator action ($EC_{50}$ 0.11±0.008 mg/ml). In spontaneously beating rat atria, the extract of *S. raeseri* (0.005-1.5 mg/ml) produced decrease of chronotropic and inotropic activity (with $EC_{50}$ value of 0.63±0.03 mg/ml and 0.40±0.08 mg/ml). Administration of verapamil induced inhibition of force and rate of the atrial contraction. These results demonstrate that the ethanol extract of *Sideritis raeseri* spp. *raeseri* Boiss & Heldr can produce hypotension, vasodilatation, negative chronotropic and inotropic effects.

Key words: *Sideritis raeseri, hypotension, vasorelaxation, cardiodepressant, blood pressure, isolated aorta, phenols, flavonoids*

## INTRODUCTION

Medicinal plants have been used widely for a large number of conditions throughout the centuries. In recent years increased demand for medicinal herbs as natural, effective, and safe remedies has led to the identification of plenty of natural products with promising therapeutic potential against a variety of disorders, among the others for preventing or lowering high blood pressure. Despite the enormous advances in the research, development and use of natural products as therapeutic agent, a detailed understanding of their actions is lacking (1, 2). On the other hand, evaluation of their pharmacological effects and the clarification ot the pathways of action could be still used as a logical research strategy for searching new drugs (3, 4).

The genus *Sideritis L*., a member of the family Lamiaceae, subfamily Lamioideae comprises at least 150 species (5). Plants from the genus *Sideritis*, widely are distributed in Mediterranean region, the Balkans, the Iberian Peninsula and Macaronesia, but can also be found from Germany to Morocco, and from the Bahamas to western China. *Sideritis* species, known as ironwort, mountain tea and shepherd's tea, have been universally used from ancient times for their medicinal and culinary properties. In the last two decades traditional use was proved through a series of experiments, confirming their anti-inflammatory, anti-rheumatic (6), gastroprotective properties (7), and their use in the treatment of gastrointestinal ailments, bronchitis, flu, and as diuretic has been validated (8). Phytochemical reports have shown that the main constituents of the genus *Sideritis* are various terpenoids, sterols, coumarins, flavonoid aglycones and glycosides (9). Many *Sideritis* species and their constituents, mostly phenols, have been also reported to have different biological properties: anti-inflammatory (10), carminative, antinociceptive (11), antitussive, stomachic, antimicrobial (12), anticataract, immunomodulating (13), anti-HIV replication, antifeedant, antiulcer (14), analgesic (15), hypoglycemic (16), vasoprotective (17) and antioxidant (18).

We performed this study to examine the *in vivo* effects of *Sideritis raeseri* spp. *raeseri* Boiss & Heldr ethanol extract on the blood pressure and *in vitro* effects on the contractile responses of isolated aorta and atria, based on the knowledge that the extract of *S. raeseri* can induced spasmolytic effect. We have reported that the ethanol extract of *S. raeseri* inhibit the spontaneous ileum contractions and contractions induced by acetylcholine, histamine and barium ions (19).

This study is thus designed to ascertain if the ethanolic extract of *S. raeseri* has influence on the blood pressure and heart rate anaesthetized rabbits and on the contractile responses of isolated rat aortic preparations and isolated rat atria.

## MATERIALS AND METHODS

### *Reagents*

Heparine sodium salt (Hemofarm, Serbia) and urethane (Pliva, Croatia) were used. Verapamil was obtained from the Sigma Chemical Company, St. Louis, MO, USA. All drugs were dissolved in distilled water.

### *Vegetal material*

The aerial parts of cultivated *S. raeseri* spp. *raeseri* were collected in the phase of full flowering, from the experimental field at the Institute for Medicinal Plants Research in Pancevo, Serbia.

### *Preparation of plant extract*

Upper 20 cm of the plants were harvested and open-air dried in the shadow. Air-dried and powdered aerial parts were extracted with 96% ethanol in Soxhlet apparatus. The extracts were filtered and evaporated in vacuum to dryness. For experimental purposes the plant extract was first dissolved in ethanol (20% m/m), than diluted with distilled water to the appropriate concentration. Ethanol, at the same concentrations, had no effect on blood pressure and contractility of isolated vessels and atria in the control experiments.

### *Animals and treatment*

In this study, rabbits (around 1 kg) and Wistar albino rats (200-250 g) were used obtained from the Animal Research Center of Medical Faculty, University of Nis, Serbia. The animals were housed in stainless steel cages under standard laboratory conditions. These animals were maintained at 20-24°C with a 12 h light-dark cycle at least 1 week before the experiment. All animals had free access to food and water. All experimental procedures with animals were in compliance with the European Council Directive of November 24, 1986 (86/609/EEC).

### *Blood pressure measurement in anaesthetized rabbits*

The rabbits were anesthetized intravenously with urethane (750 mg/kg). The animals were implanted with carotid arterial catheter for blood pressure recording. The arterial catheter was connected to a blood pressure transducer (P-1000-A) coupled with a Narcophysiograph (NARCO Bio system, Houston, USA) for measurement arterial pressure.

The blood pressure and heart rate were recorded before and after the administration of the plant extract. Arterial pressure was allowed to return to the resting level between injections. Changes in blood pressure were recorded as the difference between the steady state values before and the peak readings after the injection. Animals were treated with *S. raeseri* ethanol extract, which was administered in a rising concentrations (0.025-7.5 mg/kg) at intervals of 15-20 min.

### *Isolation of the rat aorta and recording of contractions*

The thoracic aorta ring preparations from rats were used. Aortic rings were mounted in 10 ml tissue bath containing a Krebs solution at 37°C and aerated with carbogen. The composition of the Krebs solution was (mM): NaCl 118.2, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $KH_2PO_4$ 1.3, $NaHCO_3$ 25.0, glucose 11.7.

Before the experiments, an equilibrium period of 60 min was given. High $K^+$ (80 mM) doses were used to induce sustained contractions. The extract of *S. raeseri* (0.005-1.5 mg/ml) was than added to the organ bath, and the relaxation was evaluated as percentage of the induced vasoconstriction. In the second experimental series, the aortic rings were precontracted with high $K^+$, and verapamil was then added (0.015-1.5 μg/ml).

Tension changes in the tissue were recorded using a transducer (TSZ-04-E, Experimetria Ltd, Budapest, Hungary) and analyzed with a SPEL Advanced ISOSYS Data Acquisition System (Experimetria Ltd, Budapest, Hungary).

### *Isolation of the rat atria and recording of contractions*

Rat atria were dissected out cleaned off fatty tissue. The spontaneously beating atria were suspended in 10 ml tissue baths, containing Krebs solution for isolated atria, maintained at 36±1°C and continuously aerated with a carbogen. The composition of Krebs solution was (mM): NaCl 137, KCl 2.81, $CaCl_2$ 1.8, $MgCl_2$ 0.1, $NaH_2PO_4$ 0.417, $NaHCO_3$ 11.9, glucose 11.1. The force and rate of isolated atria were recorded using a transducer (TSZ-04-E, Experimetria Ltd, Budapest, Hungary) and analyzed with a SPEL Advanced ISOSYS Data Acquisition System (Experimetria Ltd, Budapest, Hungary).

In the first experimental series, after an equilibrium period of 30 min, the extract of *S. raeseri* (0.005-1.5 mg/ml) was added cumulatively. In the second experimental series, rat atria were incubated with verapamil (0.3-3 μM). The effect on force and rate of contractions was determined as percent of the pretreated control.

### *Statistical analysis*

The results were expressed as mean ±standard deviation of six determinations. Statistical evaluation was performed using the Student`s *t*-test. A probability value of $p<0.05$ was considered to be significant. The mean effective doses $EC_{50}$ that is the concentration which elicited 50% of maximal response, was established by regression analysis.

## RESULTS

### *Effects of the extract on blood pressure*

In anaesthetized rabbits the baseline mean blood pressure did not vary, and the value of the pressure was 97.84±3.14 mmHg. Intravenous administration of the ethanol extract of *S. raeseri* (0.025-7.5 mg/kg) immediately caused dose-dependent decreases in systolic, diastolic and mean arterial blood pressure. The plant extract at doses of 7.5 mg/kg induced a significant fall in the blood pressure of 35.02±5.28% ($p<0.01$), with $EC_{50}$ value of 24.31±3.87 mg/kg (*Fig. 1*). After the hypotensive peak, the blood pressure increased progressively and reached the basal value in about 2-3 min. Intravenous treatment with extracts of *S. raeseri* at doses of 7.5 mg/kg induced a significant decrease in the heart rate of anesthetized rabbits of 18.59±5.28% ($p<0.05$), with $EC_{50}$ value of 88.14±7.51 mg/kg (*Fig. 1*).

### *Effects of the extract on isolated aorta*

In isolated rat aorta preparations cumulative addition of the ethanol extract of *S. raeseri* (0.005-1.5 mg/ml) caused the relaxation of the sustained contractions induced by KCl in a concentration-dependent manner. The plant extract at the concentration of 1.5 mg/ml caused an inhibitory effect of 79.13±6.85%, with $EC_{50}$ 0.11±0.008 mg/ml (*Fig. 2*). Verapamil was used as a positive control which induced vasorelaxation in the aortic rings constricted with KCl, with $EC_{50}$ value of 0.135±0.03 μg/ml.

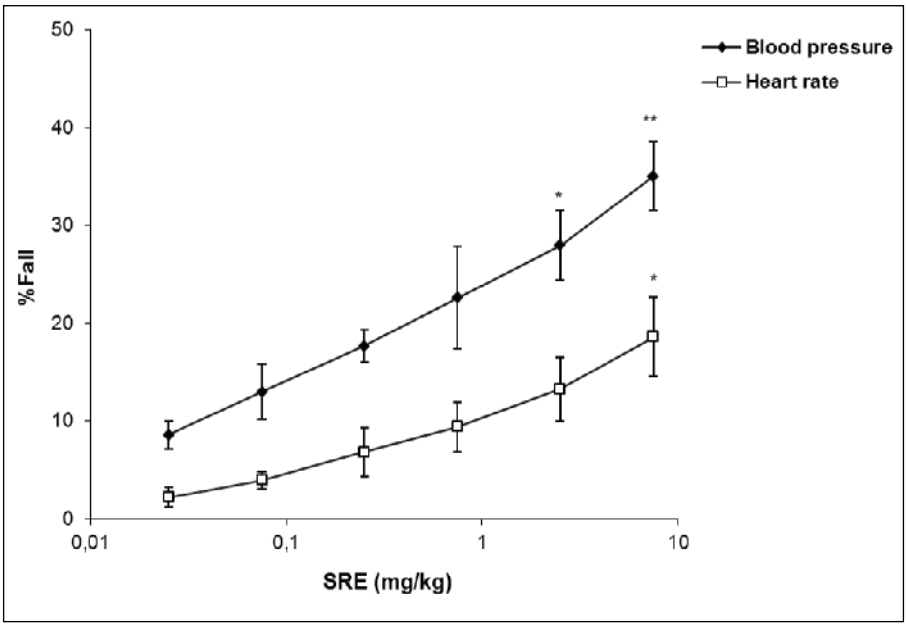


*Fig. 1.* Inhibitory response of *Sideritis raeseri* (SRE) on mean arterial blood pressure and heart rate in anaesthetized rabbits (values shown are mean ±S.E.M., n=5), *p<0.05, ** p<0.01.

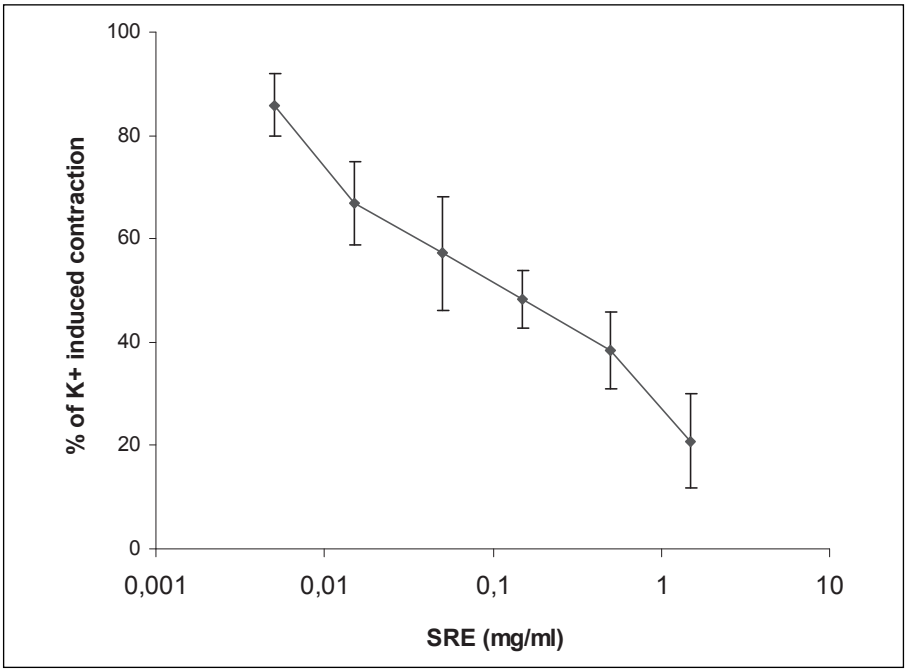


*Fig. 2.* Effects of the ethanol extract of *Sideritis raeseri* (SRE) on the high $K^+$ induced contractions in isolated rat aorta preparations (values shown are mean ±S.E.M., n=5).

*Effects of the extract on isolated atria*

The administrations of the ethanol extract of *S. raeseri* (0.005-1.5 mg/ml) to the spontaneously beating atria, caused negative inotropic and chronotropic effects. In isolated rat atria the plant extract at the concentration of 1.5 mg/ml decreased the rate of contraction for 69.59±5.11% (with $EC_{50}$ value of 0.63±0.03 mg/ml) and the force for 72.95±6.45% (with $EC_{50}$ value of 0.40±0.08 mg/ml) (*Fig. 3*). Verapamil was used as positive control which induced the inhibition of rate and the force of atrial contraction (with $EC_{50}$ value of 0.54±0.03 µg/ml and 0.46±0.01 µg/ml).

## DISCUSSION

The study demonstrates that the ethanol extract of *S. raeseri* has a hypotensive effect in rabbits, a negative inotropic and chronotropic effect in isolated rat atria and a vasorelaxant effect in isolated rat aorta.

The intravenous injection of the ethanol extracts of *S. raeseri* induced a dose-dependent decrease of the blood pressure and heart rate of anaesthetized rabbits. The hypotensive effect was short-term and the blood pressure reached the basal value in about 2-3 min. The blood pressure is a product of cardiac output and vascular resistance hence the extract of *S. raeseri* was studied for its possible inhibitory effects on isolated rat aorta and atria.

When tested on the high $K^+$ induced contractions of rat aorta, extract of *S. raeseri* showed a dose dependent vasorelaxant effect. The high KCl induced contraction is due to membrane depolarization, leading to the increase of the calcium influx through voltage-dependent channels (20).

The results demonstrated that the plant extract induced a negative chronotropic and inotropic effects on the spontaneously contracting cardiac tissues of atria. The cardio-inhibitory effect of the extract of *S. raeseri* was concentration dependent and

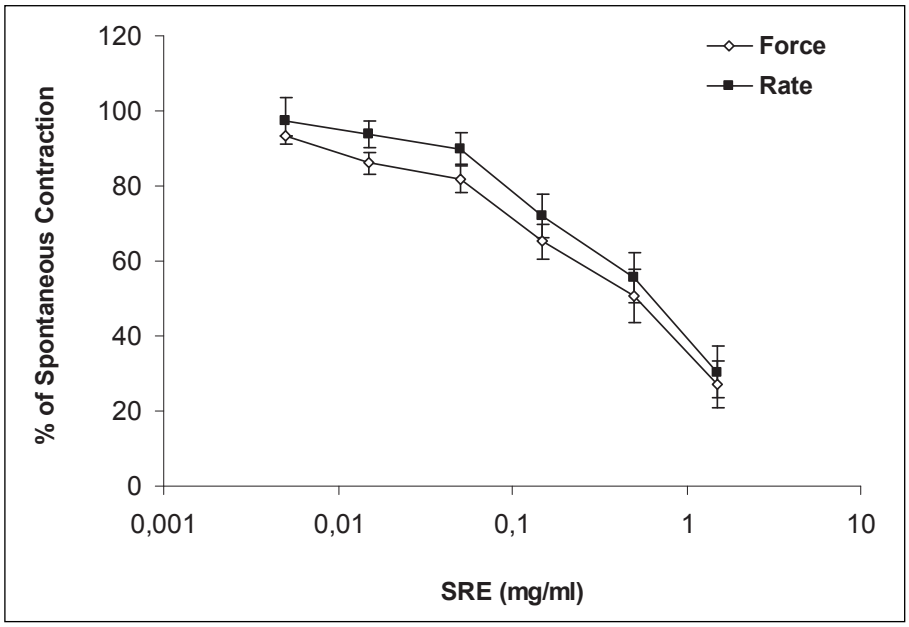


*Fig. 3.* Effect of the ethanol extract of *Sideritis raeseri* (SRE) on the force and the rate of rat atrial contraction (values shown are mean ±S.E.M., n=5).

reversible after washing, suggesting that the inhibition was not due to the damage of the myocardial cells by the extract.

The phytochemical analysis on the samples of the genus *Sideritis* revealed the presence of flavones and terpenoids (21, 22). The relatively high content of individual polyhydroxy flavones such as the 7-*O*-glycosides of 8-OH-flavones (isoscutellarein, chryseriol) and apigenin determined in the extracts of *S. raeseri* (6). The oil of *S. raeseri* has been found to be a rich source of sesquiterpenes (23). Flavones and terpenoids might be the active phytochemical constituents responsible for the biological activity of the plants. Godoy *et al.* (10) showed that flavonoids and terpenoids, obtained from plants of genus Sideritis, possessed anti-inflammatory activity.

Literature data report that flavones and terpenoids, in other plants, to exhibit activities in cardiovascular system. Presence of such compounds in *S. raeseri* might possibly contribute in the hypotensive and cardio-inhibitory effects of plant extract. It was known that the aglycones apigenin exhibited endothelium-dependent vasodilatatory activities in isolated rat aorta (24). The sesquiterpene extracted from the medicinal plant *Petasites formosanus* in anesthetized rats produced a dose-dependent hypotensive effect (25).

The hypotensive effect of other plants of Lamiaceae family has been reported. The essential oil from aerial parts of *Mentha x villosa* in rats induced endothelium-dependent hypotensive and vasorelaxing effects (26). Matsubara *et al.* (27) shown that compound from *Orthosiphon aristatus* (Lamiaceae) caused hypotensive, negative chronotropic and vasodilatory effects.

The results demonstrate that the ethanol extract of *Sideritis raeseri* spp. *raeseri* Boiss & Heldr can produce hypotension, vasodilatation, negative chronotropic and inotropic effects. Vasorelaxant, negative chronotropic and inotropic actions can be responsible for the hypotensive effect of the ethanol extract of *Sideritis raeseri*. Based on our results, *Sideritis raeseri* may be phytotherapeutically used, after full pharmacological and toxicological evaluation, as an alternative drug to synthetic hypotensive agents.

*Acknowledgements:* The authors are grateful for the financial support of the Ministry of Education, Sciences and Technological Development of the Republic of Serbia, grant No. III46013, III41018 and the U.S. National Institutes of Health, Fogarty International Center (2 D43 TW00-641) to the Institute of International Health at Michigan State University for the Training and Research in Environmental Health in the Balkans program.

Conflict of interests: None declared.

REFERENCES

1. Biala A, Finckenberg P, Korpi A, *et al.* Cardiovascular effects of the combination of levosimendanand valsartan in hypertensive dahl/rapp rats. *J Physiol Pharmacol* 2010; 62: 275-285.
2. Del Rio D, Costa LG, Lean ME, Croyer A. Polyphenols and health: when compounds are involved? *Nutr Metab Cardiovasc* 2010; 20: 1-6.
3. Hajhashemi V, Ghannadi A, Peseshkian SK. Antinociceptive and anti-inflammatory effects of Satureya hortensis L. extracts and essential oil. *J Ethnopharmacol* 2002; 82: 83-87.
4. Jakala P, Pere E, Lehtinen R, Turpeinen A, Korpela R, Vapataalo H. Cardiovascular activity of milk casein-derived tripeptides and plant sterols in spontaneously hypertensive rats. *J Physiol Pharmacol* 2009; 60: 11-20.
5. Tomas-Barberan F, Lopez-Gomex C, Villar A, Tomas-Lorente F. Inhibition of lens aldose reductase by Labiatae flavonoids. *Planta Med* 1988; 52: 239-240.
6. Janeska B, Stefova M, Alipieva K. Assay of flavonoid aglycones from the species of genus Sideritis (Lamiaceae) from Macedonia with HPLC-UV DAD. *Acta Pharm* 2007; 57: 371-377.
7. Kupeli E, Sahin B, Calis I, Yesilada E, Ezer N. Phenolic compounds of Sideritis ozturkii and their in vivo anti-inflammatory and antinociceptive activities. *J Ethnopharmacol* 2007; 112: 356-360.
8. Yesilada E, Honda G, Sezik E, Tabata M, Fujita T, Tanaka T. Traditional medicine in Turkey V. Folk medicine in inner Taurus mountains. *J Ethnopharmacol* 1995; 46: 133-152.
9. Alipieva K, Petreska J, Gil-Izquierdo A, Stefova M, Evstatieva L, Bankova V. Influence of the extraction method on the yield of flavonoids and phenolics from Sideritis spp. (Pirin Mountain tea). *Nat Prod Commun* 2010; 5: 51-54.

10. Godoy A, De Las Heras B, Vivas M, Villar A: Antiinflammatory properties of a lipid fraction obtained from Sideritis javalambrensis. *Biol Pharm Bull* 2000; 23: 1193-1197.
11. Guvenc A, Okada Y, Akkol E, Duman H, Okuyama T, Calis I. Investigations of anti-inflammatory, antinociceptive, antioxidant and aldose reductase inhibitory activities of phenolic compounds from Sideritis brevibracteata. *Food Chem* 2010; 118: 686-692.
12. Kose E, Deniz I, Sarikurkcu C, Aktas O, Yavuz M. Chemical composition, antimicrobial and antioxidant activities of the essential oils of Sideritis erythrantha Boiss. and Heldr. (var. erythrantha and var. cedretorum P.H. Davis) endemic in Turkey. *Food Chem Toxicol* 2010; 48: 2960-2965.
13. Navarro A, de Las Heras B, Villar A. Immunomodulating properties of diterpene andalusol. *Planta Med* 2000; 66: 289-291.
14. Aboutabl E, Nassar M, Elsakhawy F, Maklad Y, Osman A, El-Khrisy E. Phytochemical and pharmacological studies on Sideritis taurica Stephan ex Wild. *J Ethnopharmacol* 2002; 82: 177-184.
15. Hernandez-Perez M, Rabanal RM. Evaluation of the antinflammatory and analgesic activity of Sideritis canariensis var. pannosa in mice. *J Ethnopharmacol* 2002; 81: 43-47.
16. Piozzi F, Bruno M, Rosselli S, Maggio A. The diterpenoids from the genus Sideritis. *Stud Nat Prod Chem* 2006; 33: 493-540.
17. Charami M, Lazari D, Karioti A, Skaltsa H, Hadjipavlou-Litina D, Souleles C. Antioxidant and antiinflammatory activities of Sideritis perfoliata subsp. Perfoliata (Lamiaceae). *Phytother Res* 2008; 22: 450-454.
18. Erkan N, Cetin H, Ayranci E. Antioxidant activities of Sideritis congesta Davis et Huber-Morath and Sideritis arguta Boiss et Heldr: Identification of free flavonoids and cinnamic acid derivatives. *Food Res Int* 2011; 44: 297-303.
19. Brankovic S, Kitic D, Radenkovic M, *et al.* Spasmolytic activity of the ethanol extract of Sideritis raeseri spp. raeseri Boiss. & Heldr on the isolated rat ileum contractions. *J Med Food* 2011; 14: 1-4.
20. Galicia J, Andrade R, Espana P, *et al.* Antihypertensive and vasorelaxant activities of Laelia autumnalis are mainly through calcium channel blockade. *Vascular Pharmacol* 2008; 49: 26-31.
21. Gabrieli C, Kokkalou E. A glucosylated acylflavone from Sideritis raeseri. *Phytochemistry* 1990; 29: 681-683.
22. Petreska J, Stefova M, Ferreres F, *et al.* Potential bioactive phenolics of Macedonian Sideritis species used for medicinal "Mountain Tea". *Food Chem* 2011; 125: 13-20.
23. Pljevljakusic D, Savikin K, Jankovic T, *et al.* Chemical properties of the cultivated Sideritis raeseri Boiss. & Heldr. subsp. raeseri. *Food Chem* 2011; 124: 226-233.
24. Zhang YH, Park Y, Kim T, *et al.* Endothelium-dependent vasorelaxant and antiproliferative effects of apigenin. *Gen Pharmacol* 2000; 35: 341-347.
25. Wang GJ, Shum AY, Lin YL, *et al.* Calcium channel blockade in vascular smooth muscle cells: major hypotensive mechanism of S-petasin, a hypotensive sesquiterpene from Petasites formosanus. *J Pharmacol Exp Ther* 2001; 297: 240-246.
26. Guedes DN, Silva DF, Barbosa-Filho JM, Medeiros IA. Endothelium-dependent hypotensive and vasorelaxing effects of the essential oil from aerial parts of Mentha x villosa in rats. *Phytomedicine* 2004; 11: 490-497.
27. Matsubara T, Bohgaki T, Watarai M, Suzuki H, Ohashi K, Shibuya H. Antihypertensive actions of methyl-ripariochromene A from Orthosiphon aristatus, an Indonesian traditional medicinal plant. *Biol Pharm Bull* 1999; 22: 1083-1088.

Received: July 20, 2012
Accepted: September 17, 2012

Author's address: Dr. Dusanka Kitic, Department of Pharmacy, Faculty of Medicine, University of Nis, Bulevar Zorana Djindjica 81, 18 000 Nis, Serbia.
E-mail: duska@medfak.ni.ac.rs; kitic@msu.edu