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BIOLOGICAL VALUE AND ESSENTIAL OIL COMPOSITION OF TWO *Monarda* SPECIES FLOWERS

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ABSTRACT

In this study the quantity of the main nutritional and bioactive compounds, as well as the antioxidant activity (DPPH, FRAP and ABTS methods) and essential oil composition of *Monarda fistulosa* L. and *Monarda citriodora* subsp. *austromontana* Cerv. ex Lag. 'Bees' Favourite' flowers were investigated. The field experiment was carried out for three years (2014–2016) and the laboratory analyses in the years 2015–2016. The obtained results showed that *M. citriodora* flowers were characterized by a dry matter (22.42%), total ash (1.86% FW), crude fibre (4.89% FW), sucrose (0.31% FW), total flavonoids (0.795% DW) and antioxidant activity in FRAP test (7.96 mg TE g^{-1} FW), while *M. fistulosa* showed the highest values of total sugars/titratable acidity ratio (4.05), antioxidant activity in DPPH test (7.35 mg TE g^{-1} FW) and contents of reducing sugars (1.46% FW), total chlorophyll (401.10 µg g^{-1} FW), chlorophyll a (271.74 µg g^{-1} FW) and b (92.07 µg g^{-1} FW), L-ascorbic acid (48.99 mg 100 g^{-1} FW) and total polyphenols (7.64 mg GAE g^{-1} FW). The essential oils were obtained by hydrodistillation and analyzed by gas chromatography-mass spectrometry (GC-MS). The main compounds of *M. fistulosa* oil were carvacrol (28.16 and 23.66% in 2015, and 2016, respectively), p-cymene (20.10 and 17.94%), thymoquinone (12.60 and 16.30%) and γ-terpinene (8.72 and 4.07%), whereas linalool (34.86 and 26.96%), thymol (17.83 and 37.34%), β-thujene (6.12 and 2.00%) and camphene (5.80 and 5.64%) were the major constituents of *M. citriodora* Cerv. ex Lag. ssp. *austromontana* 'Bees' Favourite' oil.

Key words: *M. fistulosa*, *M. citriodora*, bergamot, linalool, thymol, antioxidant activity

INTRODUCTION

The genus *Monarda*, comprised of 15–17 species with numerous subspecies, belongs to the Lamiaceae family. *Monarda* is commonly known as bee balm, horsemint, Oswego tea or wild bergamot [Davidson 2007, Mattarelli et al. 2017]. It is an erect, herbaceous, aromatic, annual or perennial plant native to North America [Mattarelli et al. 2017].

M. fistulosa L. (wild bergamot) belongs to the subgenus *Monarda* [Davidson 2007]. It is an erect perennial [Davies and Mazza 1992]. The plants are very floriferous and produce lilac-purple color flowers, which have a very strong flavour. The flowers are grouped in terminal whorls, the calyx is tubular and the corolla is two lipped, 2–3 cm long [Ciurușniuc and Robu 2012]. *M. citriodora* Cerv. ex Lag. (lemon bergamot) belongs to the subgenus *Cheilyctis* and is comprised of several either annual or perennial varieties [Davidson 2007]. It is a herb with fragrant, curved and tubular flowers in purple to pink [Zhan-guo et al. 2011]. Both species are grown for medicinal, aromatic and ornamental purpos-

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es [Ciurușniuc and Robu 2012]. *Monarda* essential oil is used as an insect repellent and in perfumery [Meena et al. 2017], it is also a potential preservative against free radical-mediated deterioration of lipid-rich cosmetics, foods and pharmaceuticals. Moreover, it has got specific antimicrobial properties [Dorman and Deans 2004, Zhilyakova et al. 2009, Zhan-guo et al. 2011, Mattarelli et al. 2017]. Its main constituents are: linalool, thymol, geraniol, carvacrol and 1,8-cineole, and other terpenes [Davidson 2007, Mattarelli et al. 2017, Salehi et al. 2018]. Moreover, *Monarda* flowers are also the source of anthocyanins (pelargonidin 3,5 diglucoside acylated with coumaric and malonic acids) as well as other flavonoids (flavone and apigenin 7-O-glucosides, 5-hydroxyflavone, and a dihydroxyflavone 8-C-glucoside) [Davies and Mazza 1992]. Flavonoids and other classes of phenolic compounds are very effective antioxidants. Epidemiologic studies show that an increased consumption of flavonoid-contained foods reduces the risk of cardiovascular diseases and certain types of cancer [Kaisoon et al. 2011].

Monarda leaves and flowers, due to their sensory properties, are also widely used as a flavouring and garnishing agent in salads, pasta and soft drinks. Tea made from leaves can treat colds, coughs, fevers and respiratory problems [Meena et al. 2017]. *Monarda* flowers are very often used for decoration on entrees, cakes and many other dishes. They can also be used to make floral ice cubes. The flowers can be used fresh or preserved in jams, jellies, oils and vinegars. Moreover, like the leaves the flowers can be dried and used to prepare teas or other beverages [Brown 2011].

Although there are some results of the leaf and flower essential oil composition of *M. fistulosa* and *M. citriodora*, there is little information on their nutritional and biological value, and a lack of information about *Monarda citriodora* subsp. *austromontana* Cerv. ex Lag. Therefore, the objective of this study was to estimate the content of the main chemical constituents and antioxidant activity, as well as the essential oil composition of the flowers of these two *Monarda* species.

MATERIAL AND METHODS

Plant material

The field experiment was carried out in the years 2014–2016 at 'The Edible Flower Collection' of the Department of Horticulture of the West Pomeranian University of Technology in Szczecin. The research material consisted of edible flowers derived from two *Monarda* species: *Monarda fistulosa* L. (bilabiate, lilac-purple flowers with green bracts) and *Monarda citriodora* subsp. *austromontana* Cerv. ex Lag. 'Bees' Favourite' (lavender-pink whorled bilabiate flower heads). The field experiment was set in a randomised block design with four replications on typical pararendzinas soils, with pH in H_2O of 6.8, and the following nutrient content: N-NO_3 – 27, P – 80, K – 163, Ca – 2932, Mg – 153, Cl – 12 mg dm^{-3}. The single plot area was 1.44 m^2 (60 × 60 cm, 4 plants per plot). The seedlings of *Monarda* species were produced in the greenhouse. Seeds were sown on the 22th April 2014. The seedlings were transplanted into the open field on the 18th August 2014. The flowers were collected in the following two years (2015 and 2016). The field was prepared according to the proper agrotechnique procedure for the tested plant species [Newerli-Guz 2016]. Mineral fertilisation was quantified according to the results of the chemical analysis of the soil. Nitrogen (50 kg N ha^{-1}), phosphorus (50 kg P_2O_5 ha^{-1}) and potassium (80 kg K_2O ha^{-1}) fertilisers were applied during the field preparation, in all the years of the study. During the growing season crop management was carried out. It included mainly irrigation, weeding and soil cultivation [Grzeszczuk et al. 2018]. The flower harvest was done at full-bloom stage: *M. fistulosa* – in the middle of July, *M. citriodora* subsp. *austromontana* Cerv. ex Lag. 'Bees' Favourite' – at the beginning of August. The aggregate sample collected from the four replications was from 150 to 200 g, depending on the plant species.

Laboratory analysis

The chemical analyses of raw plant material included the determination of the content of dry matter (drying at 105ºC to constant weight), total ash (incineration of samples in 500ºC), crude fibre [Klepacka 1996], total sugars, reducing sugars and sucrose (by the method of Luff-Schoorl), and titratable acidity [ISO 750, 1998]. The sugar to acid ratio (total sugars / titratable acidity) was calculated, too. The experiment was also concerned about the content of total chlorophylls, chlorophyll a and b [Lichtenthaler and

Wellburn 1983], vitamin C as L-ascorbic acid (by the method of Tillmans), total carotenoids [Lichtenthaler and Wellburn 1983] and total flavonoids [Farmakopea Polska VI 2002]. All the determinations were carried out in three replicates.

Determination of total polyphenol content and antioxidant activities

Preparation of plant extracts. The preparation of plant extracts for the determination of the total polyphenol content and antioxidant activities was performed using the method proposed by Wojdyło et al. [2007] with some modifications described in the previous paper [Andrys et al. 2017]. The sample of 1 g homogenized raw plant material was treated with 80% aqueous methanol (MeOH) to 100 ml volume. The mixtures were ultrasonicated for 30 min (2 × 15 min) and then left for 24 h at room temperature (~20°C). The obtained extracts were filtered over Whatman No. 1 filter paper. Then, the filtrates were centrifuged at 1500 rpm for 10 min. All the extractions were prepared in triplicate. The extracts were kept at 4°C and used for the analyses within 24 h.

Total polyphenol content. Total polyphenol content was analysed spectrophotometrically using the Folin-Ciocalteu colorimetric method as described by Wojdyło et al. [2007]. Plant extract (100 µl), 0.2 ml of the Folin-Ciocalteu reagent, 2 ml of deionized water and 1 ml of 20% sodium carbonate were mixed and incubated for 1 hour at room temperature in darkness. Then, the absorbance was measured at 760 nm. Gallic acid (GAE) was used to calculate the standard curve, and the results were expressed as GAE milligrams per g of fresh weight (FW).

Determination of DPPH radical scavenging capacity. Antioxidant activity of *Monarda* flowers on DPPH (2,2-diphenyl-1-picrylhydrazyl) radical was determined according to the procedure of Kumaran and Karunakaran [2007] and Wojdyło et al. [2007]. DPPH (0.3 mM) was dissolved in pure ethanol (99.8%). Plant extract (0.6 ml) was added to 1.8 ml of pure ethanol (EtOH) and 0.6 ml of DPPH solution. The samples were incubated at room temperature for 10 min in the dark. The reduction of the DPPH radical was determined spectrophotometrically by measuring the absorbance at 517 nm. For the calibration of the standard curve trolox (TE, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) was used and the antioxidant activity of the sample was expressed as mg of trolox equivalent per g of fresh weight sample (mg TE/g FW).

Determination of ferric reducing antioxidant power (FRAP). The antioxidant activity of the samples was also determined using the ferric reducing ability of plasma FRAP assay by Wojdyło et al. [2007]. FRAP reagent was prepared freshly by mixing an acetate buffer (300 mM, pH 3.6), a solution of 10 mM TPTZ (2,4,6-tris(2-pyridyl)-s-triazine) in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ (iron(III) chloride hexahydrate) at 10 : 1 : 1 (v/v/v) and warmed at 37°C before being used. For the determination of antioxidant activity, 2.7 ml of the FRAP reagent and 0.3 ml of the sample solution were mixed. The absorbance was measured spectrophotometrically at 593 nm after 4 min. The standard curve was prepared using different concentrations of trolox. The results were expressed in mg TE per g FW.

Determination of free radical-scavenging ability using a stable ABTS radical cation. The free radical-scavenging activity was determined by ABTS radical cation decolorization procedures described by Re et al. [1999], Wojdyło et al. [2007] and Chew et al. [2011] with some modifications described in the previous work [Andrys et al. 2017]. ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt was dissolved in deionized water to a 7 mM concentration. ABTS radical cation ($ABTS^{\bullet+}$) was generated by mixing ABTS stock solution with 2.45 mM potassium peroxodisulfate. The reagent was kept in the dark at room temperature for 16 h before using. Subsequently, the $ABTS^{\bullet+}$ solution was diluted with PBS (phosphate buffered saline, pH 7.4) until its absorbance was equilibrated to 0.7 (±0.02) at 734 nm before usage. The reaction was initiated by adding 3 ml of diluted $ABTS^{\bullet+}$ solution (A734 = 0.7 ±0.02) to 300 µl of methanolic plant extracts. The absorbance was measured at 734 nm, exactly 6 min after the mixing. Trolox was used for the calibration of the standard curve and the results were expressed as mg TE per g fresh weight sample.

Essential oil analysis. For the essential oil analysis the raw plant material was dried in a through-flow laboratory dryer set at 35°C. Dried flowers of *M. fistulosa* and *M. citriodora* (10 g, three replications) in a 1000 mL round-bottom flask along with 400 mL of

distilled water were separately hydrodistilled for 3 h using a Clevenger-type apparatus as recommended by European Pharmacopoeia [2010]. The obtained essential oils were dried over anhydrous sodium sulphate, filtered, weighed and stored at low temperature (4ºC) prior to GC-MS analysis.

The essential oil content was calculated based on dry weight of flowers and expressed as % (v/w) in Table 1.

Qualitative GC-MS analysis of the essential oils. Both *Monarda* essential oils were analyzed using an HP 6890 gas chromatograph with a HP-5MS (5% phenyl-methylpolysiloxane) capillary column (30 m × 0.25 mm, film thickness 0.25 µm; Agilent Technologies, Palo Alto, CA, USA) equipped with an HP 5973 mass selective detector. Helium (1 mL/min) was used as a carrier gas. Samples of 2 µL (30 mg of oil dissolved in 1.5 mL of dichloromethane) were injected in the split mode at a ratio of 5 : 1. The injector and detector temperatures were kept at 280°C. The ion source temperature was 230°C.

GC oven temperature was maintained at 40°C for 5 minutes initially, then increased to 60°C at a rate of 30°C/min, next to 230°C at a rate of 6°C/min (kept constant for 10 minutes), and then increased to a final temperature of 280°C at a rate of 30°C/min. The oven was kept at this temperature for 5 minutes. Mass spectra were taken at 70 eV. Mass range was from 40 to 550 m/z. Solvent delay time was 4 min. The total running time for a sample was about 51 minutes.

Relative percentage amounts of the essential oil constituents were calculated from the total peak area (TIC) by the computer.

Identification of components. The constituents of the essential oils were identified by comparison

Table 1. Content of some chemical compounds in the flowers of *Monarda fistulosa* L. and *M. citriodora* Cerv. ex Lag. ssp. *austromontana* 'Bees' Favourite' (means for 2015–2016)

Chemical compound	*Monarda fistulosa* L.	*Monarda citriodora* Cerv. ex Lag. ssp. *austromontana* 'Bees' Favourite'
Dry matter (%)	19.85^{b*} ±0.37	22.42^{a} ±0.52
Total ash (% FW)	1.64^{b} ±0.28	1.86^{a} ±0.16
Crude fibre (% FW)	1.82^{b} ±0.30	4.89^{a} ±0.32
Total sugars (% FW)	1.49^{a} ±0.38	1.30^{a} ±0.35
Reducing sugars (% FW)	1.46^{a} ±0.04	0.97^{b} ±0.03
Sucrose (% FW)	0.13^{b} ±0.07	0.31^{a} ±0.11
Titratable acidity (% citric acid FW)	0.342^{a} ±0.04	0.650^{a} ±0.16
Total sugars/titratable acidity ratio	4.05^{a} ±0.23	1.88^{b} ±0.08
Total carotenoids (µg g^{-1} FW)	128.14^{a} ±15.51	100.19^{a} ±8.08
Total chlorophyll (µg g^{-1} FW)	401.10^{a} ±23.01	253.34^{b} ±18.55
Chlorophyll a (µg g^{-1} FW)	271.74^{a} ±14.81	183.19^{b} ±15.64
Chlorophyll b (µg g^{-1} FW)	92.07^{a} ±12.69	48.23^{b} ±4.11
L-ascorbic acid (mg 100 g^{-1} FW)	48.99^{a} ±1.11	29.78^{b} ±0.68
Total flavonoids (% QE DW)	0.445^{b} ±0.01	0.795^{a} ±0.04
Total polyphenols (mg GAE g^{-1} FW)	7.64^{a} ±0.36	6.71^{b} ±0.26
Essential oil (% v/w)	3.25^{a} ±0.50	3.20^{a} ±0.66

* Data designated with same letter in line do not differ significantly at $P \leq 0.05$
± standard deviation
QE – quercetin equivalent
GAE – gallic acid eqivalent

of their mass spectra with those stored in NIST 2002 and Wiley NBS75K.L mass spectral libraries and confirmed by comparison of their calculated retention indices with data available online in the NIST Chemistry WebBook (http://webbook.nist.gov/chemistry/).

The retention indices (RI) were calculated for all volatile constituents using a homologous series of n-alkanes (C_7-C_{30}; Supelco, Bellefonte, PA, USA) under the same chromatographic conditions which were used for the analysis of essential oils.

Statistical analysis

The results of the study were subjected to an analysis of variance which was performed with AWAR software, made by the Department of Agrometeorology and Applied Informatics, Institute of Soil Science and Plant Cultivation in Puławy, Poland [Filipiak and Wilkos 1995]. The means were separated by the Tukey's test at p = 0.05

RESULTS AND DISCUSSION

The results of the study given in Table 1 and Figure 1 show that flowers of *M. fistulosa* and *M. citriodora* ssp. *austromontana* 'Bees' Favourite' are a good source of nutritional and biologically active compounds, especially of polyphenols. Until now, most of the results provided here have not been published by other authors.

M. citriodora flowers were characterised by a significantly higher content of dry matter (22.42%), total ash (8.30% DW) and crude fibre (21.81% DW) in comparison with *M. fistulosa* (respectively: 19.85%, 8.26 and 9.17% DW) (Tab. 1). Dry matter content of edible flowers varies from 6.9% (*Cucurbita pepo* flowers) to 28.5% (*Rosa micrantha* petals), total ash – from 2.6% (*Madhuca indica* flowers) to 15.9% DW (*Cucurbita pepo* flowers) and fiber – from 6.1% (*Allium schoenoprasum* flowers) to 55.4% DW (*Spilanthes oleracea* and *Tagetes erecta* flowers) [Fernandes et al. 2017].

Based on the study results we found that *M. citriodora* flowers also contained higher amounts of sucrose and total flavonoids than *M. fisulosa* flowers. The results obtained for the flavonoid content in *M. fistulosa* flowers are higher in comparison with *Calendula officinalis*, *Geranium macrorrhizum* and *Bougainvillea spectabilis* flowers [Petrova et al. 2016], while *M. citriodora* flowers contain more of the flavonoids

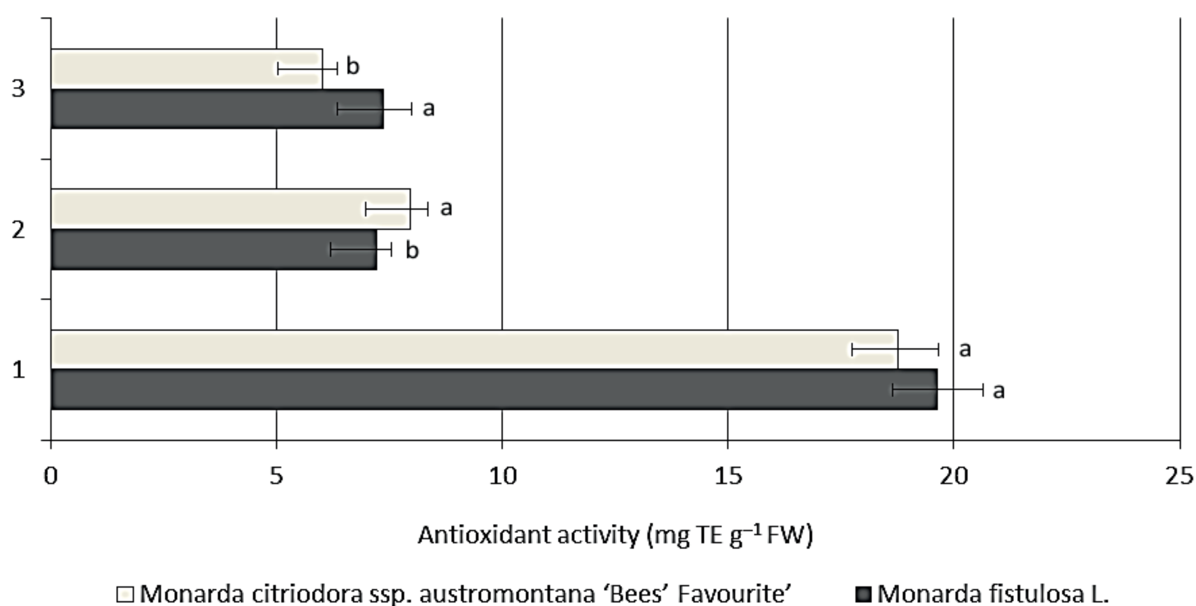

Fig. 1. Antioxidant activity of *Monarda fistulosa* and *Monarda citriodora* Cerv. ex Lag. ssp. *austromontana* 'Bees' Favourite' flowers assessed by using three different assays: 1 – ABTS, 2 – FRAP, 3 – DPPH (means for 2015–2016); Bars indicate standard deviation; TE – Trolox equivalent

than those mentioned above as well as *Tagetes erecta* flowers but less than *Helianthus tuberosus* flowers [Petrova et al. 2016].

In contrast, *M. fistulosa* flowers contained significantly higher amounts of reducing sugars, had a higher total sugar/titratable acidity ratio, higher content of total chlorophyll, chlorophyll a and b, and L-ascorbic acid. Only a few studies have detailed the reducing sugar content in edible flowers: 9.6% DW – *Rosa micrantha* [Guimarães et al. 2010], 10.6% DW – *Allium schoenoprasum* [Grzeszczuk et al. 2011], 53.8% DW – *Yucca filifera* [Sotelo et al. 2007], and 1.75% FW – *Monarda didyma* [Stefaniak and Grzeszczuk 2019]. L-ascorbic acid content in edible flowers varies from 16.3 to 241.2 mg 100 g^{-1} FW [Grzeszczuk et al. 2016, Fernandes et al. 2017]. In the presented study, the content of this compound was 48.99 mg 100 g^{-1} FW for *M. fistulosa* flowers and 29.78 mg 100 g^{-1} FW for *M. citriodora* flowers. In our previous study, for *M. didyma* flowers we recorded 33.38 mg of L-ascorbic acid per 100 g FW [Stefaniak and Grzeszczuk 2019].

The obtained results in the current investigation for chlorophyll and carotenoid content are higher than reported in the literature which is due to the green parts of the inflorescences have been taken into the analyses. Petrova et al. [2016] for the flowers of *Tagetes erecta*, *Calendula officinalis*, *Geranium macrorrhizum* and *Helianthus tuberous* recorded from 0.2 to 41.1 μg chlorophyll a g^{-1} FW, 0.3–7.6 μg chlorophyll b g^{-1} FW, 0.5–41.5 μg chlorophyll a+b g^{-1} FW and from 6.3 to 57.2 μg total carotenoids g^{-1} FW. There were no significant differences found in the content of total sugars, titratable acidity, total carotenoids and essential oil between the flowers of the two *Monarda* species and they were on average, respectively: 1.40% FW, 0.496% citric acid FW, 114.17 μg g^{-1} FW and 3.23%.

The content of total polyphenols was significantly higher for the flowers of *M. fistulosa* (7.64 mg GAE g^{-1} FW) in comparison with *M. citriodora* flowers (6.71 mg GAE g^{-1} FW). Its content in the plants depends on many factors e.g. plant origin, plant part, harvesting time, solvent and techniques chosen for extraction [Duletić-Laušević et al. 2016]. Petrova et al. [2016] assessed for 5 edible flower species from 0.35 (*Tagetes erecta* – water extract) to 19.79 mg GAE g^{-1} FW (*Geranium macrorrhizum* – ethanol extract) depending on the extraction method. On the other hand Navarro-González et al. [2015] in acidified ethanol extracts of *Tagetes erecta* determined 26.63 mg GAE g^{-1} FW, and for *Tropaeolum majus* – 12.95 mg GAE g^{-1} FW of total phenolic compounds. In our study for the plant material extraction we used methanol, the same as Rop et al. [2012] used in their experiment assessing total phenolic content in 12 edible flower species. Their results varied from 2.53 (*Chrysanthemum frutescens*) to 5.28 mg GAE g^{-1} FW (*Dianthus caryophyllus*). For *Tagetes patula* flowers they recorded 4.58 and for *Tropaeolum majus* – 3.31 mg GAE g^{-1} FW. Both of the *Monarda* species tested in our study were characterised by a higher content of total polyphenols in comparison with the study results of Rop et al. [2012].

Considering that edible flowers contain many different classes and types of antioxidants, the use of different methods of antioxidant activity determination was recommended [Priori et al. 2005, Loizzo et al. 2016]. For this reason in this study we applied different procedures: DPPH, FRAP and ABTS test. According to Chew et al. [2011], DPPH free radicals are more effectively scavenged by low molecular weight phenolic compounds than by high molecular weight polymers such as condensed tannins that were reported in Lamiaceae species [Carović-Stanko et al. 2016]. While FRAP assay allows for quantifying all antioxidants or reduced compounds in an extract sample [Dragland et al. 2003]. Moreover, despite high accuracy and common use of DPPH, its considerable limitation is the fact that it may be dissolved only in organic solvents and does not allow for determination of hydrophilic antioxidants. In contrast, the use of ABTS reagent enables the measurement of total antioxidant activity in both hydrophobic and hydrophilic samples [Wojdyło et al. 2007]. Antioxidant activity of *Monarda* flowers measured by three different methods were in the following order: ABTS assay > FRAP assay > DPPH assay. The results of antioxidant activity determined by FRAP and DPPH assay were similar to each other, and it is in agreement with the study results of Thaipong et al. [2006].

M. fistulosa flowers were characterized by a significantly higher antioxidant activity in DPPH test (7.35 mg TE g^{-1} FW) while using FRAP test higher results were noted for the *M. citriodora* flowers (7.96 mg TE g^{-1} FW). The ABTS test showed no signif-

icant differences between the two *Monarda* species and amounted on average to 19.20 mg TE g^{-1} FW. Similar antioxidant activity values were obtained in our previous study for *Monarda didyma* flowers [Stefaniak and Grzeszczuk 2019], and they were higher in comparison with the results obtained for *Mimulus* × *hybridus*, *Hemerocallis* × *hybrida* and *Antirrhinum majus*.

Many studies showed that the contents of flavonoids and phenolics are positively correlated to the antioxidant capacities in plant extracts [Luximon-Ramma et al. 2002, Zhou et al. 2011]. Similar results were also obtained in this research. Flowers of *M. fistulosa* containing higher amounts of total polyphenols had higher antioxidant activity in DPPH test. In contrast, *M. citriodora* flowers were characterized by a significantly higher flavonoid content and antioxidant activity values obtained in FRAP test. A positive correlation between FRAP values and the flavonoids content in 10 plant extracts was also found by Malinowska [2013].

The yields of essential oils of *Monarda fistulosa* and *Monarda citriodora* Cerv. ex Lag. ssp. *austromontana* 'Bees' Favourite' were 3.25 % (w/w) and 3.20% (w/w), respectively (Tab. 1). The results of GC-MS analysis of the essential oils obtained from the flowers of two *Monarda* species are presented in Tables 2 and 3. In the case of *M. fistulosa*, a total of 54 different compounds were identified representing 99.48% of the oil (Tab. 2). Carvacrol was the main constituent of flower oil (28.16 and 23.66% in 2015 and 2016, respectively), followed by p-cymene (20.10 and 17.94%) and thymoquinone (12.60 and 16.30%). γ-Terpinene (8.72 and 4.07%) and α-terpinene (5.03 and 2.94%) were identified in significant amounts.

In the oil from the flowers of *M. citriodora* 57 compounds were identified, representing 99.63% of the total oil composition (Tab. 3). This oil was rich in linalool (34.86 and 26.96%), thymol (17.83 and 37.34%), β-thujene (6.12 and 2.00%), camphene (5.80 and 5.64%), germacrene D (5.53 and 0.73%), α-pinene (3.48 and 1.11%) and 1,8-cineole (2.36 and 3.11%).

Linalool, one of the main constituents of *M. citriodora* ssp. *austromontana* oil was not detected in the oil of *M. fistulosa*. Moreover, thymoquinone (TQ), one of the main constituents of *M. fistulosa* oil was not present in the oil of *M. citriodora.* TQ shows anti-inflammatory, antimicrobial, antioxidant, hematoprotective, and immunomodulatory effects [Randhawa and Alghamdi 2011]. Taborsky et al. [2012] detected TQ in *M. didyma*, *M. media* and *M. menthifolia*. In case of *Monarda didyma* the highest content of TQ was noted for inflorescences in comparison with the leaves and stems.

The content of carvacrol (0.56% in 2016) in *M. citriodora* oil was significantly lower as compared with *M. fistulosa* oil (28.16 and 23.66%). However, the higher content of thymol (17.83 and 37.34%) was found in the oil of *M. citriodora* ssp. *austromontana.*

Generally, oxygenated monoterpenes (43.94–45.16% for *M. fistulosa* and 60.54–79.85% for *M. citriodora*) and monoterpene hydrocarbons (30.78–41.24% for *M. fistulosa* and 13.40–26.97% for *M. citriodora*) dominated in both the oils (Tabs. 2, 3). Oxygenated sesquiterpenes were present in amounts lesser than 3% (Tabs. 2, 3).

According to Mazza and Marshall [1992], the chemical composition of *Monarda fistulosa* essential oil depends on genotype and geographical origin. In the leaf oil of *M. fistulosa* var. *menthaefolia* from California, carvacrol (70.24%) was the main component, whereas oil from leaves of plants of the same species from Manitoba contained mainly geraniol (85%). However, Lawrence [1985] found high percentages of thymol (60.8%) and carvacrol (54.5%) in the *M. fistulosa* spp. *fistulosa* oil and high thymol content (81.6%) in the oil of *M. fistulosa* spp. *menthaefolia.* Zamureenko et al. [1989] reported that p-cymene (32.52%), carvacrol (23.90%) and thymol (12.61%) were major constituents of the essential oil of *M. fistulosa* from Krasnodarsk Krai (Siberia). Similarly, carvacrol (39.10%) and p-cymene (35.40%) rich *M. fistulosa* was collected from the South of Mississippi [Tabanca et al. 2013]. Thymol (28.38–33.42%), β-phellandrene (16.87–18.02%), α-phellandrene (13.70–14.04%), p-cymene (13.18–13.27%) and myrcene (8.64–8.70%) dominated in the essential oil of *M. fistulosa* from Italy [Mattarelli et al. 2017], while the dominant components of the *M. fistulosa* oil from Canada [Adebayo et al. 2013] were geraniol (61.83%), geranyl formate (16.61%) and geranial (10.58%).

Essential oils obtained from *M. fistulosa* in this study were also rich in carvacrol (23.66–28.16%) and p-cymene (17.94–20.10%), however the content of

Table 2. Essential oil composition of *Monarda fistulosa* L. flowers

No.	Compounds	RI	2015	2016
1.	α-Thujene	928	2.15 ±0.32	1.39 ±0.28
2.	α-Pinene	934	0.66 ±0.11	0.49 ±0.09
3.	Camphene	949	0.10 ±0.01	–*
4.	β-Thujene	974	0.21 ±0.03	0.11 ±0.02
5.	β-Pinene	976	0.19 ±0.02	0.15 ±0.01
6.	1-Octen-3-ol	982	2.30 ±0.09	1.38 ±0.25
7.	3-Octanone	989	0.45 ±0.02	0.28 ±0.06
8.	β-Myrcene	993	1.15 ±0.07	0.44 ±0.10
9.	3-Octanol	996	1.06 ±0.04	0.72 ±0.16
10.	α-Phellandrene	1004	0.45 ±0.04	0.21 ±0.04
11.	3-Carene	1010	0.25 ±0.03	0.17 ±0.03
12.	α-Terpinene	1017	5.03 ±0.48	2.94 ±0.33
13.	p-Cymene	1027	20.10 ±1.22	17.94 ±1.60
14.	Limonene	1029	2.08 ±0.18	2.87 ±0.48
15.	γ-Terpinene	1060	8.72 ±0.36	4.07 ±1.04
16.	*cis*-Sabinene hydrate	1068	1.01 ±0.09	0.69 ±0.14
17.	α-Terpinolene	1089	0.15 ±0.04	–
18.	*cis*-β-Terpineol	1099	0.28 ±0.06	0.20 ±0.02
19.	Camphor	1148	–	0.16 ±0.01
20.	Borneol	1169	0.13 ±0.01	0.14 ±0.03
21.	Terpinen-4-ol	1180	0.87 ±0.05	0.88 ±0.10
22.	α-Terpineol	1195	0.22 ±0.01	0.21 ±0.01
23.	Thymoquinone	1257	12.60 ±0.30	16.30 ±0.79
24.	Bornyl acetate	1288	0.07 ±0.02	0.05 ±0.01
25.	Thymol	1294	1.82 ±0.78	1.65 ±1.06
26.	Carvacrol	1308	28.16 ±1.69	23.66 ±2.60
27.	α-Cubebene	1353	0.13 ±0.06	0.07 ±0.02
28.	α-Copaene	1381	0.53 ±0.46	0.21 ±0.04
29.	β-Bourbonene	1391	0.10 ±0.03	0.09 ±0.01
30.	β-Elemene	1396	0.15 ±0.03	0.15 ±0.02
31.	β-Caryophyllene	1425	0.86 ±0.02	1.12 ±0.17
32.	β-Cubebene	1435	0.17 ±0.03	0.24 ±0.04
33.	Aromadendrene	1450	0.05 ±0.01	–

Table 2 cont.

No.	Compounds	RI	2015	2016
34.	α-Caryophyllene	1460	0.11 ±0.01	0.12 ±0.04
35.	Alloaromadendrene	1470	0.03 ±0.00	–
36.	γ-Muurolene	1482	0.11 ±0.01	0.30 ±0.07
36.	γ-Muurolene	1482	0.11 ±0.01	0.30 ±0.07
37.	Germacrene D	1487	2.25 ±0.13	1.47 ±0.16
38.	Bicyclogermacrene	1501	0.11 ±0.01	0.18 ±0.07
39.	α-Muurolene	1505	0.10 ±0.01	0.17 ±0.02
40.	γ-Cadinene	1520	0.06 ±0.00	0.20 ±0.04
41.	δ-Cadinene	1529	0.14 ±0.06	0.45 ±0.09
42.	Caryophyllene oxide	1592	0.04 ±0.03	0.13 ±0.04
43.	β-Eudesmol	1650	–	0.20 ±0.07
44.	τ-Cadinol	1654	0.18 ±0.01	–
45.	α-Cadinol	1665	0.08 ±0.01	–
46.	1-Heptadecene	1696	–	0.11 ±0.02
47.	Heptadecane	1702	0.12 ±0.01	0.23 ±0.06
48.	3-Methyleicosane	2068	–	0.16 ±0.06
49.	(E)-Phytol	2119	–	0.16 ±0.05
50.	Linoleic acid	2139	0.46 ±0.02	3.63 ±1.40
51.	7-Methylheneicosane	2145	1.70 ±0.03	5.95 ±1.90
52.	Ethyl linoleate	2162	0.95 ±0.05	2.95 ±1.10
53.	Docosane	2201	0.15 ±0.00	0.84 ±0.29
54.	1-Octadecanol acetate	2206	0.69 ±0.11	1.44 ±0.36
	Total identified		99.48	97.67
	Monoterpene hydrocarbons		41.24	30.78
	Oxygenated monoterpenes		45.16	43.94
	Sesquiterpene hydrocarbons		4.90	4.77
	Oxygenated sesquiterpenes		0.30	0.33
	Others		7.88	17.85

± standard deviation
* not detected

Table 3. Essential oil composition of *Monarda citriodora* Cerv. ex Lag. ssp. austromontana 'Bees' Favourite' flowers

No.	Compounds	RI	2015	2016
1.	α-Tricyclene	922	0.31 ±0.02	0.23 ±0.05
2.	α-Thujene	928	0.28 ±0.00	0.16 ±0.02
3.	α-Pinene	934	3.48 ±0.13	1.11 ±0.18
4.	Camphene	949	5.80 ±0.07	5.64 ±0.82
5.	β-Thujene	974	6.12 ±0.09	2.00 ±0.25
6.	β-Pinene	976	1.32 ±0.04	–*
7.	1-Octen-3-ol	982	1.17 ±0.04	0.36 ±0.16
8.	3-Octanone	989	0.14 ±0.01	–
9.	β-Myrcene	993	1.10 ±0.03	0.32 ±0.04
10.	α-Phellandrene	1004	0.06 ±0.01	–
11.	α-Terpinene	1017	0.30 ±0.03	0.44 ±0.07
12.	p-Cymene	1027	0.46 ±0.16	0.43 ±0.09
13.	Limonene	1029	1.55 ±0.08	–
14.	1,8-Cineole	1031	2.36 ±0.02	3.11 ±0.43
15.	(Z)-β-Ocimene	1038	2.81 ±0.23	–
16.	(E)-β-Ocimene	1048	1.60 ±0.07	–
17.	γ-Terpinene	1060	0.53 ±0.04	0.59 ±0.10
18.	*cis*-Sabinene hydrate	1068	0.49 ±0.01	0.42 ±0.04
19.	*cis*-Linalool oxide	1071	0.21 ±0.02	2.42 ±0.09
20.	Artemisia alcohol	1082	–	0.11 ±0.02
21.	α-Terpinolene	1089	0.57 ±0.02	1.99 ±0.11
22.	Linalool	1107	34.86 ±1.65	26.96 ±0.43
23.	β-Thujone	1113	0.86 ±0.13	0.94 ±0.11
24.	*cis*-p-Ment-2-en-1-ol	1126	0.18 ±0.02	–
25.	(Z)-Alloocimene	1129	0.68 ±0.19	0.49 ±0.06
26.	Isopinocarveol	1142	0.17 ±0.02	0.62 ±0.08
27.	Camphor	1148	0.10 ±0.01	0.81 ±0.08
28.	Pinocarvone	1161	–	0.34 ±0.05
29.	Menthofuran	1166	–	0.21 ±0.03
30.	Borneol	1169	1.03 ±0.04	1.90 ±0.17
31.	Terpinen-4-ol	1180	1.32 ±0.06	2.66 ±0.17
32.	α-Terpineol	1195	0.92 ±0.07	0.65 ±0.12
33.	p-Menth-1-en-8-ol	1200	0.21 ±0.01	0.53 ±0.05

Table 3 cont.

No.	Compounds	RI	2015	2016
34.	*cis*-Verbenone	1214	–	0.27 ±0.02
35.	Thymol	1294	17.83 ±0.17	37.34 ±2.54
36.	Carvacrol	1308	–	0.56 ±0.27
37.	α-Copaene	1381	0.14 ±0.03	0.10 ±0.01
38.	β-Bourbonene	1391	0.17 ±0.03	0.51 ±0.05
39.	β-Elemene	1396	0.24 ±0.01	0.18 ±0.01
40.	β-Caryophyllene	1425	0.56 ±0.04	0.21 ±0.02
41.	β-Cubebene	1435	0.21 ±0.04	0.12 ±0.02
42.	Aromadendrene	1450	0.07 ±0.01	–
43.	α-Caryophyllene	1460	1.47 ±0.62	0.30 ±0.14
44.	Alloaromadendrene	1470	0.32 ±0.11	–
45.	γ-Muurolene	1482	0.13 ±0.01	–
46.	Germacrene D	1487	5.53 ±0.77	0.73 ±0.20
47.	α-Muurolene	1505	0.20 ±0.02	–
48.	β-Bisabolene	1512	0.15 ±0.09	–
49.	γ-Cadinene	1520	0.10 ±0.03	–
50.	δ-Cadinene	1529	0.41 ±0.09	–
51.	2-Methylpentadecane	1562	–	0.34 ±0.02
52.	Spathulenol	1583	0.40 ±0.07	–
53.	Caryophyllene oxide	1592	–	0.57 ±0.10
54.	Humulene epoxide II	1620	–	0.41 ±0.05
55.	τ-Cadinol	1654	0.17 ±0.02	0.34 ±0.17
56.	α-Cadinol	1665	0.54 ±0.03	0.68 ±0.22
57.	1-Heptadecene	1696	–	0.16 ±0.09
	Total identified		99.63	98.26
	Monoterpene hydrocarbons		26.97	13.40
	Oxygenated monoterpenes		60.54	79.85
	Sesquiterpene hydrocarbons		9.70	2.15
	Oxygenated sesquiterpenes		1.11	2.00
	Others		1.31	0.86

± standard deviation
* not detected

those valuable compounds was lower when compared with the results obtained by Tabanca et al. [2013]. The content of thymol found in our oils did not exceed 2.00%. Interestingly, thymoquinone (12.60–16.30%), which was found to be a major compounds in our oils, has not been reported as the main constituent in previously studied *M. fistulosa* plants. Although, Rohlfsen [2016] patented a method of cultivation of *M. fistulosa* rich in thymoquinone and thymohydroquinone.

The chemical composition of essential oils isolated from the flowers and the aerial parts of *Monarda citriodora* have been previously studied by several authors.

The major constituents identified in the essential oil of *M. citriodora* var. *citriodora* flowers growing in the United Kingdom [Collins et al. 1994] were thymol (61.77%), γ-terpinene (13.30%) and p-cymene (4.19%). The volatile oil from flowers of *M. citriodora* cultivated in China [Lu et al. 2011] contained mainly thymol (44.59%), 1,8-cineole (23.61%), α-phellandrene (4.81%), m-cymene (4.01%) and carvacrol (3.21%). Pathania et al. [2013] reported thymol (82%), carvacrol (4.82%), β-myrcene (3.45%), terpinene-4-ol (2.78%) and p-cymene (1.53%) as the major constituents of flower oil of *M. citriodora* Cerv. ex Lag. from India. In the essential oil obtained from the aerial parts of *M. citriodora* cultivated in Egypt [Salama et al. 2016], the main components identified were thymol (32.73–63.88%), carvacrol (6.54–29.56%), p-cymene (1.24–17.72%) and γ-terpinene (0.37–19.6%). In another study, Dorman and Deans [2004] found a high level of thymol (70.60%), p-cymene (10.60%) and carvacrol (6.10%) in *M. citriodora* var. *citriodora* Cerv. ex Lag. from the commercial supplies in the United Kingdom.

Based on these results, it can be concluded that the essential oil isolated from different varieties of *M. citriodora* has different chemical composition but thymol is always one of its major components.

The content of thymol (17.83–37.34%), 1,8-cineole (2.36–3.11%), γ-terpinene (0.53–0.59%), carvacrol (0–0.56%) and p-cymene (0.43–0.46%) found in *M. citriodora* Cerv. ex Lag. ssp. *austromontana* 'Bees' Favourite' flower oil were lower when compared to the cited literature. Generally, linalool (26.96–34.86%) was its major constituent. The high content of linalool (59%) was found by Lawrence [1980] in the volatile oil of *M. didyma*. Similarly, linalool rich essential oils (45.74–66.99%) were obtained from hybrids of *M. fistulosa* var. *menthaefolia* and *M. didyma* [Mazza and Marshall 1992].

CONCLUSIONS

The chemical analysis results showed that the flowers of *Monarda fistulosa* and *Monarda citriodora* Cerv. ex Lag. ssp. *austromontana* 'Bees' Favourite' are a good source of nutritional and bioactive compounds. The highest contents of reducing sugars chlorophylls, L-ascorbic acid, total polyphenols and total sugars/titratable acidity ratio were found in flowers of *M. fistulosa*. On the other hand flowers of *M. citriodora* demonstrated higher content of dry matter, total ash, crude fibre, sucrose and total flavonoids. Flowers of both species were characterized by a high antioxidant activity assessed in DPPH, FRAP and ABTS tests.

The results of this study have also demonstrated that the essential oil isolated from the flowers of *M. fistulosa* is rich in carvacrol, p-cymene and thymoquinone, whereas *M. citriodora* Cerv. ex Lag. ssp. *austromontana* 'Bees' Favourite' flower oil is dominated by linalool and thymol.

We think that the flowers of both *Monarda* species may be useful as natural antioxidant and antimicrobial agents in food industry or may be applied for medicinal purposes.

SOURCE OF RESEARCH FOUNDING

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