ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. XXIV, N 3, 22 SECTIO DDD 2011
Department of Biochemistry and Food Chemistry, University of Life Sciences in Lublin, Poland

URSZULA ZŁOTEK*, URSZULA SZYMANOWSKA,
MONIKA KARAŚ

Changes in the antioxidant activity of polyphenols purple basil leaves (Ocimum basilicum L. cv. Dark Opal) induced by selected abiotic elicitors

Zmiany aktywności antyoksydacyjnej polifenoli liści bazylii purpurowej (*Ocimum basilicum* L. cv. Dark Opal) indukowane wybranymi elicytorami abiotycznymi

INTRODUCTION

Basil (*Ocimum basilicum* L.) is a popular culinary herb commonly used in many kinds of food preparations in Mediterranean diets. In food industry basil is used for aromatizing dishes and herbal liqueurs [3]. Basil has also shown antiallergic, anticancer, antimicrobial, antiseptic, antispasmodic, antifungal, antiviral, anti-inflammatory, analgesic, immuno-stimulating, sedative and antioxidant activities due to its polyphenols and aromatic compounds [18]. Phenolics in fruit, vegetables and herbs are the major bioactive compounds known for health benefits especially due to their antioxidant properties. The antioxidant activity of phenolic compounds is mainly caused by their redox properties, which permit them to act as reducing agents, hydrogen donors and singlet oxygen quenchers [6]. The main phenolics reported in basil are phenolic acids (e.g. rosmarinic, lithospermic, vanillic, p-coumaric, hydroxybenzoic, syringic, ferulic, protocatechuic, caffeic and gentisic) and flavonol-glycosides [9, 10]. Therefore basil extracts are also used in the cosmetic and pharmaceutical products. Purple basils are also a good source of anthocyanins which are a class of flavonoids. Apart from role of anthocyanins as pigments there are many functions performed by this compounds in plants (e.g. UV protection, defense against pathogens and pests, protecting DNA) [8]. Numerous scientific studies have shown that these active compounds act also as antioxidants.

Recently scientists have carried out extensive research into production of nutraceuticals using bioactive compounds from fruit and vegetables residues or from herbs.

Therefore, there has been growing interest in developing methods of increasing polyphenols concentration and essential oil content in basil to further enhance their overall nutritional and pharmaceutical value [18].

It has been reported that various biotic and abiotic elicitors such as chitosan, jasmonic acid, methyl jasmonate, significantly increased production of secondary metabolites such as phenolic acid or anthocyanins in plants [7,15].

In this study, the effect of abiotic elicitors (jasmonic acid, arachidonic acid and β-aminobutyric acid) on the content of phenolics and antioxidant properties of purple basil was investigated.

MATERIALS AND METHODS

Plant materials and growth conditions: The seeds of purple basil (*O. basilicum* L. cv. Dark Opal) were purchased from W. Legutko Breeding and Seed Company. Basil seeds were sown into sowing boxes filled with universal ground for sowing seeds. Seven-days-old seedlings were transplanted to 600ml pots containing universal garden ground (four plants per pot). Plants were grown in growth chamber (SANYO MLR-350H) at 25/18°C, photoperiod 16/8h day/night, with PPFD (photosynthetic photon flux density) at plants level of 500-700μmol $m^{-2}s^{-1}$ and a relative humidity of 75%. Twenty one-day-old plants were sprayed with a solution of 10^{-6}M jasmonic acid (JA) (Sigma), 10^{-6}M arachidonic acid (AA) and 10^{-2}M β-aminobutyric acid (BABA) (Sigma) prepared in deionized water respectively. The control plants (C) were sprayed with deionized water. Fifteen days after elicitation the herbs were collected and frozen.

Sample preparation: Frozen leaf tissue (2g) was ground in a mortal and pestle with 15ml of acidified methanol (0.1% HCl) and the phenolics were extracted for 1h at 4°C, then centrifuged at 10 000xg for 30min – this procedure was repeated three times and the supernatants were combined and added to 50ml – it was a crude extract of polyphenols. In crude extracts the content of total phenolic, total anthocyanins, flavonoids, phenolic acids and antioxidant activities were determined.

Determination of polyphenols:

Total phenolics content of basil leaf extracts was determined using Folin – Ciocalteu method [16] and expressed as gallic acid equivalent in mg/g fresh weight (mg/g fw).

Total flavonoid content was measured by aluminium chloride colorimetric assay [1] and expressed in mg/g fresh weight (mg/g fw) as quercetin equivalent.

The amount of phenolic acids was determined using Arnov's reagent [17] and expressed in μg/g fresh weight (μg/g fw).

The total anthocyanins content in *Ocimum basilicum* was determined using the pH differential method [4]. Total anthocyanin was calculated in the sample as mg/g fresh weight (mg/g fw).

Antioxidant activity:

The DPPH (1, 1-diphenyl-2-picryl-hydrazyl) radical scavenging assay was done according to the method of Brand-Williams et al. [2].

The scavenging activity against ABTS (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) radical was determined by the decolourisation assay [14]. The antioxidant activity was expressed as μmol of Trolox (a analogue of vitamin E) per gram of fresh weight (fw) (TEAC, Trolox equivalent antioxidant capacity).

The chelation of ferrous ions Fe^{2+} by purple basil leaves extracts was estimated following the method of Guo et al. [5].

Statistical analysis: All experimental results were mean ± S.D. of three parallel measurements and data were evaluated using the Tuckey's test at a significance level of α=0.05.

RESULTS

The conducted studies aimed at determining the influence of arachidonic acid (AA), jasmonic acid (JA) and β-aminobutyric acid (BABA) on the content of polyphenolic compounds in purple basil leaves. The contents of total phenolic, anthocyanins, flavonoids and phenolic acids were significantly increased after the application of AA and JA. The most effective elicitor was jasmonic acid, which has caused the change of phenolic acids level of about 36%, whereas flavonoids level about 20%. Arachidonic acid also has led to increased synthesis of polyphenols, especially phenolic acids (about 26%). The content of examined compounds has not changed significantly after application of BABA in relation to control (Table 1).

Table 1. The influence of abiotic elicitors on contents of phenolic compounds. Mean ± standard deviation. Statistically significant differences (p<0,05) indicated various letters.

Content Elicitor	Total phenolics [mg/g fw]	Anthocyanins [mg/g fw]	Flavonoids [mg/g fw]	Phenolic acids [µg/g fw]
Control	5.14 ± 0.22 ^{b}	0.84 ± 0.022 ^{a}	0.64 ± 0.03 ^{a}	5.35 ± 0.22 ^{a}
Arachidonic acid	6.66 ± 0.2 ^{a}	0.95 ± 0.04 ^{bc}	0.72 ± 0.008 ^{b}	6.78 ± 0.65 ^{b}
Jasmonic acid	6.6 ± 0.06 ^{a}	0.98 ± 0.068 ^{c}	0.77 ± 0.01 ^{b}	7.3 ± 0 ^{b}
β-aminobutyric acid	5.9 ± 0.29 ^{c}	0.87 ± 0.03 ^{ab}	0.65 ± 0.027 ^{a}	5.35 ± 0.14 ^{a}

In our studies the antioxidant activity against ABTS as a source of free radicals was determined. As shown in Fig. 1 the antioxidant activity increased significantly after stimulation of all used elicitors. The highest ability to scavenging of free radicals showed an extract of AA-stimulated basil (57.86% higher than control).

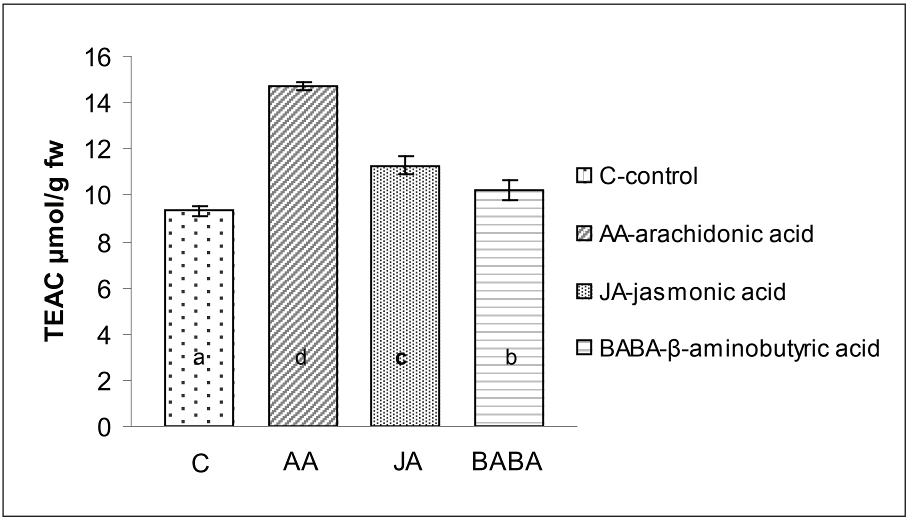

Fig. 1. Antioxidant activity (TEAC µmol/g fw) of purple basil extracts as determined by $ABTS^{\bullet+}$ assays. Results are means ±SD of three independent measurements. Different letters indicate significantly differences ($p<0.05$)

The antioxidant activity of the purple basil extracts was determined by measuring their ability to remove free DPPH radicals present in a methanol solution. The results are shown in Fig. 2. Basil plants pretreated with all used chemicals showed higher antioxidant properties in comparison to control, but in case of JA and BABA there were no statistically significant differences. The highest antioxidant activity was found in the extract of basil after arachidonic acid treatment (about 66% higher than control).

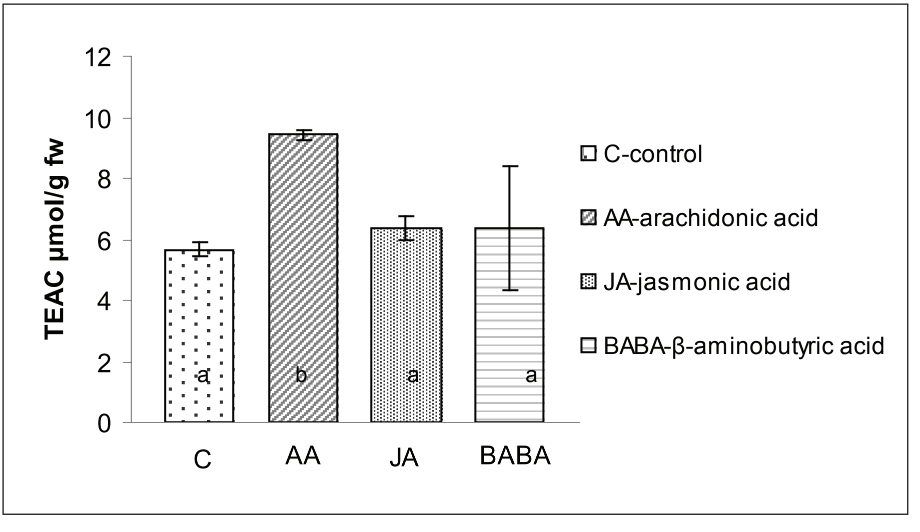

Fig. 2. Antioxidant activity (TEAC µmol/g fw) of purple basil extracts as determined by $DPPH^{\bullet}$ assays. Results are means ±SD of three independent measurements. Different letters indicate significantly differences ($p<0.05$)

The ferrous ions chelating ability of purple basil leaves extracts is shown in Fig. 3. The chelating activity increased only after treatment of BABA, but not statistically significantly. As a result of application AA and JA as elicitors chelating power decreased remarkably. Jasmonic acid led to reduction of Fe(II) chelating ability of almost 53%.

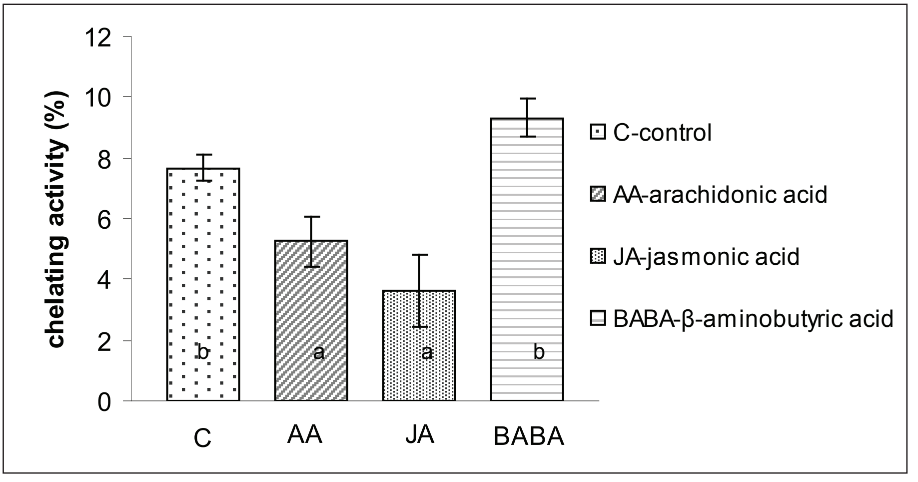

Fig. 3. Chelating activity on Fe^{2+} of purple basil extracts. Statistically significant differences ($p<0.05$) indicated various letters. Error bars represent standard deviations of three independent measurements

DISCUSSION

Polyphenols (flavonoids, phenolic acid) occurring in fruit, vegetables and herbs are protective against a variety of diseases, particularly cardiovascular disease and some types of cancer [8]. Recently, there is a high interest in increasing the amount of functional phytochemicals like phenolic compounds [15]. It is already known that some elicitors induce expression of genes encoding enzymes of secondary metabolites biosynthesis what can also have an indirect effect on phenolics accumulation [15]. In this experiment the amounts of all phenolic compounds (total phenolics, phenolic acids, flavonoids and anthocyanins) increased by the arachidonic acid and jasmonic acid treatment as compared to those in the control (Table 1**).** This result is similar with Kim et al. [12] study where at the second day after the treatment by 0.1 and 0.5 M methyl jasmonate total phenolic contents were 27 and 57%, respectively, higher than in the control [12]. However as our study indicates the β-aminobutyric acid is not effective elicitor in inducing phenolics in basil (Table 1).

Antioxidant activities of basil extracts are shown in Fig1, Fig.2 and Fig 3. Our results correspond well with study of Kim et al.[11]. In this research extracts of chitosan treated plants showed higher antioxidant activities against $DPPH^{\bullet}$ in comparison to the control extract [11].What is more, according to Kim et al. [13], methyl jasmonate (MeJa) treatment also resulted in the increased antioxidant capacities determined by $ABTS^{\bullet+}$ assay and $DPPH^{\bullet}$ assay in lettuce leaves. There is a positive correlation between phenols content and antioxidant properties in plants after MeJa stimulation [13].

These results emphasized the importance of abiotic elicitors for enhancement of the content and the antioxidant activity of phenolic compounds of basil plant extracts.

CONCLUSION

Our study demonstrates that elicitors such as arachidonic acid and jasmonic acid can effectively induce phytochemicals in herb plants, which might be alternative and effective means instead of genetic modification. Stimulating purple basil by abiotic elicitors causes an increase of phenolics content, including anthocyanins. Therefore this plant can be a good source for food and pharmaceutical industries. Because of the high relative antioxidant activity of stimulated basil, these plants could constitute new sources of antioxidant phenolics in the diet.

Acknowledgements. This work was partly supported by Polish Ministry of Science and Higher Education under the grant no. IP2010 042070.

REFERENCES

1. Bahorun T et al.: Total phenol, flavonoid, proanthocyanidin and vitamin C levels and antioxidant activities of Mauritian vegetables. J Sci Food Agric, 84**,** 1553, 2004
2. Brand-Williams W, Cuvelier E, Berset CM: Use of free radical method to evaluate antioxidant activity. LWT, 28, 25, 1995.
3. Dzida K: Biological value and essential oil content in sweet basil (*Ocimum basilicum* L.) depending on calcium fertilization and cultivar. Acta Sci. Pol., Hortorum Cultus, 9(4), 153, 2010.
4. Giusti MM, Worlstad RE (2001): Unit F1.2: Characterization and measurement of anthocyanins by UV-visible spectroscopy. In: Current protocols in food analytical chemistry. New York: John Willey and Sons.
5. Guo J-T et al.: Antioxidant properties of the extracts from different parts of broccoli in Taiwan. J Food Drug Anal, 9(2), 96, 2001.
6. Hakkim FL, Shankar CG, Girija S: Chemical composition and antioxidant property of Holy Basil (*Ocimum sanctum* L.) leaves, stems, and inflorescence and their in vitro callus cultures. J. Agric. Food Chem, 55, 9109, 2007.
7. Horbowicz M et al.: The effect of methyl jasmonate and phenolic acids on growth of seedlings the effect of methyl jasmonate and phenolic acids on Growth of seedlings and accumulation of anthocyanins in common buckwheat (*Fagopyrum esculentum* Moench). Acta Agrobotanica, 62 (1), 49, 2009.
8. Horbowicz M et al: Anthocyanins of fruits and vegetables – their occurrence, analysis and role in human nutrition. Veget. Crops Res. Bull, 68, 5, 2008.
9. Javanmardi J et al.: Chemical characterization of basil (*Ocimum basilicum* L.) found in local accessions and used in traditional medicines in Iran. J. Agric. Food Chem.**,** 50*,* 5878, 2002.
10. Jungmin L, Carolyn FS: Chicoric acid found in basil (*Ocimum basilicum* L.) leaves. Food Chem., 115, 650, 2009.
11. Kim H et al.: Effect of chitosan on the biological properties of sweet basil (*Ocimum basillicum* L.). J. Agric. Food Chem. 53, 3696, 2005.
12. Kim H et al.: Effect of methyl jasmonate on secondary metabolites of sweet basil (*Ocimum basilicum* L.). J. Agric. Food Chem., 54, 2327, 2006.

13. Kim H et al.: Effect of methyl jasmonate on phenolic compounds and carotenoids of romaine lettuce (*Lactuca sativa* L.). J. Agric. Food Chem., 55, 10366, 2007.
14. Re R et al.: Antioxidant activity applying an improved ABTS radical cation decolorisation assay. Free Rad Biol Med, **26**, 1231, 1999.
15. Saniewski M, Horbowicz M, Puchalski J: Induction of anthocyanins accumulation by methyl jasmonate in shoots of *Crassula multicava* Lam. Acta Agrobotanica, 59(2), 43, 2006.
16. Singleton VL, Rossi JA: Colorimetry of total phenolics with phosphomolybdic-phosphotungstics acid reagents. American J Enol Vitic, 16, 144, 1965.
17. Szaufer-Hajdrych M: Phenolic acids in leaves of species of the *Aquilegia* genus. Herba Pol, 50, 10, 2004.
18. Taie HAA, Salama ZAR, Radwan S: Potential activity of basil plants as a source of antioxidants and anticancer agents as affected by organic and bio-organic fertilization. Not. Bot. Hort. Agrobot. Cluj, 38 (1), 119, 2010.

SUMMARY

Purple basil (*Ocimum basilicum* L. cv. Dark Opal), cultivated in grown chamber, pretreated with abiotic elicitors: arachidonic acid (AA), jasmonic acid (JA) and β-aminobutyric acid (BABA). The content of: total phenolics, flavonoids, phenolic acids and anthocyanins in methanolic extracts of basil leaves were determined. Antioxidant activity of obtained extracts against $DPPH^{\bullet}$ and $ABTS^{\bullet +}$ and Fe(II) chelating ability also were investigated. In extracts of basil pretreated with AA and JA increase of the content of all analyzed polyphenols was observed. The most effective inductor was jasmonic acid, which caused increase of phenolic acids content by 36%, flavonoids by 20% in comparison to control. The increase of antioxidant activity of analyzed extracts was detected – the best antiradical effects were obtained after arachidonic acid treatment. The β-aminobutyric acid was not effective elicitor to inducing polyhenols in basil.

Keywords: purple basil, elicitors, phenolics, antioxidant activity

STRESZCZENIE

Bazylię purpurową (*Ocimum basilicum* L. cv. Dark Opal) uprawianą w fitotronie potraktowano elicytorami abiotycznymi: kwasem arachidonowym (AA), kwasem jasmonowym (JA) i kwasem β-aminomasłowym (BABA). W metanolowych ekstraktach z liści bazylii oznaczono zawartość: związków fenolowych ogółem, flawonoidów, kwasów fenolowych oraz antocyjanów. Zbadano również aktywność przeciwutleniającą uzyskanych ekstraktów wobec $DPPH^{\bullet}$ i $ABTS^{\bullet +}$ oraz zdolność do chelatowania jonów Fe(II). W ekstraktach z bazylii poddanej stymulacji AA i JA odnotowano wzrost zawartości wszystkich analizowanych frakcji polifenolowych. Najbardziej efektywnym induktorem okazał się kwas jasmonowy, który spowodował wzrost zawartości kwasów fenolowych o 36%, flawonoidów o 20% w stosunku do kontroli. Odnotowano też wzrost aktywności przeciwutleniającej badanych ekstraktów – najlepsze efekty przeciwrodnikowe uzyskano po zastosowaniu kwasu arachidonowego. Natomiast kwas β-aminomasłowy okazał się mało efektywnym induktorem polifenoli w bazylii.

Słowa kluczowe: bazylia purpurowa, elicitory, związki fenolowe, aktywność przeciwutleniająca