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ORIGINAL PAPER

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ASSESSMENT OF ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF *CRAMBE* SPP. DURING VEGETATION

Olena Vergun[1], Dzhamal Rakhmetov[1], Oksana Shymanska[1], Oleksandr Bondarchuk[1], Eva Ivanišová[2], Miroslava Kačaniová[3], Olga Grygorieva[4]✉, Ján Brindza[5]

[1]Department of Cultural Flora, M. M. Gryshko National Botanical Garden of National Academy of Sciences of Ukraine
Timiryazevska 1, 01014 Kyiv, **Ukraine**
[2]Department of Technology and Quality of Plant Products, Slovak University of Agriculture in Nitra
Trieda Andreja Hlinku 2, 94676 Nitra, **Slovakia**
[3]Department of Fruit Growing, Viticulture and Enology, Slovak University of Agriculture in Nitra
Trieda Andreja Hlinku 2, 94676 Nitra, **Slovakia**
[4]Department of Fruit Plant Acclimatization, M. M. Gryshko National Botanical Garden of National Academy of Sciences of Ukraine
Timiryazevska 1, 01014 Kyiv, **Ukraine**
[5]Institute of Biological Conservation and Biosafety, Slovak University of Agriculture in Nitra
Trieda Andreja Hlinku 2, 94676 Nitra, **Slovakia**

ABSTRACT

Background. The search for new plant raw material as a potential source of antioxidants is still ongoing. This study aimed to evaluate the antioxidant and antimicrobial capacity of the plant raw material of *Crambe* spp. during vegetation.

Materials and methods. The free radical scavenging activity and molybdenum reducing power of the extracts were used to determine antioxidant activity. The quantification of polyphenol compounds was conducted with Folin-Ciocalteu reagent. Flavonoids and phenolic acids were also determined. The disc diffusion method was used to determine antimicrobial activity.

Results. It was determined that the free radical scavenging activity, assessed using the DPPH-method, was 4.38–8.20 mg TE/g DW, the molybdenum reducing power of the extracts was 40.07–129.12 mg TE/g DW, total polyphenol content was 20.24–70.88 mg GAE/g DW, total flavonoid content was 5.73–29.92 mg QE/g DW, and phenolic acid content was 3.00–10.63 mg CAE/g DW. Antimicrobial activity depended on the stage of growth and the part of the plant used.

Conclusion. *Crambe* spp. possess the antioxidant and antimicrobial potential to mean that they could be used in pharmaceutical studies and the food industry.

Keywords: *Crambe* spp., polyphenols, antioxidant capacity, antimicrobial activity

INTRODUCTION

Brassicaceae Burnett is a large family with economically important plants such as *Brassica* L. (Cömert Önder et al., 2020; Francisco et al., 2016), *Camelina* Crantz (Zubr, 2010), *Crambe* L. (Lovatto et al., 2017; Wang et al., 2000) etc. with a rich source of health-improving phytochemicals (Avato and Argentieri, 2015). Members of the Brassicaceae exhibit various biological functions including antimicrobial, antioxidant, antiviral, and anticancer, etc. (Shankar et al., 2019).

✉olgrygorieva@gmail.com, https://orcid.org/0000-0003-1161-0018

The genus *Crambe* L. consists of about 40 species in the world (Francisco-Ortega et al., 2002). Plants from this genus are mostly represented by perennial and economically important crops. Among others, the most common species is *Crambe hispanica* subsp. *abyssinica* (Hochst. ex R. E. Fr.) Prina wide is used as an oil plant, the seeds of which contain approximately 40% oil (Silva et al., 2019). In addition, the seed oil of this plant contains erucic acid, which has the potential for use in industry (Li et al., 2012). Bassegio et al. (2016) reviewed the potential application of *C. abissinica* mainly associated with the manufacture of biodiesel. *Crambe* meal is an important raw material that can contain up to 50% crude protein and has a digestibility level similar to soybean meal (Samarappuli et al., 2020). Some of the species from this genus are promising medicinal plants (Kalista, 2017). *C. maritima* (sea kale) and *C. hispanica* subsp. *abyssinica* are potential salt-tolerant crops (Francois and Kleiman, 1990; de Vos et al., 2010).

A study of the fatty acid composition showed that α-linolenic acid in the leaves and oleic acid in the seeds of samples of *C. tataria* Sebeók were the dominant fatty acids (Pushkarova et al., 2016). Previous studies of the makeup of *Crambe* spp. nutrients determined that 9.76–22.54% was dry matter, 6.54–33.18% was the total content of sugars, 139.85–987.02 mg/100 g was ascorbic acid, 0.38–2.41 mg/100 g was ß-carotene, 1.28–6.47% was tannins, 3.12–6.28% was titratable acidity, etc. during vegetation (Vergun et al., 2019b). Among the photosynthetic pigments, the highest content of carotenoids was found in the leaves of *C. koktebelica* during fruiting (Vergun et al., 2019a).

Study of the biological activities of *Crambe* spp. showed that selected species have been investigated. Extracts of *C. cordifolia* plants possessed antioxidant (Bukhari et al., 2013), antimicrobial, and hemolytic activities (Rashid et al., 2018). The study of antioxidant activity *in vivo* and *in vitro* samples of *C. tataria* was higher than the ascorbic acid solution in the same experiment (Pushkarova et al., 2016). Leaf extracts of *C. cordifolia* and *C. koktebelica* contained chlorogenic, ferulic, caffeic, p-coumaric, syringic, sinapic, cinnamic, hydroxyphenyl acetic, and quinic acids, as well as neohesperidin, and rutin, etc. (Marchyshyn et al., 2020).

Among the wild and cultured food plants of Brassicaceae, which are used as spices and analysis of which might be interesting, is *Armoracia rusticana* (P. Gaertn., B. Mey. & Scherb.). This is a well-known species with a rich content of biologically active compounds (Agneta et al., 2013; Tomsone et al., 2020). It is a well-known plant with biological activities such as antioxidant (Calabrone et al., 2015; Ivanišová et al., 2020), and antimicrobial ones (Park et al., 2013) that is also widely used as a food plant with a pungent smell, intense lachrymatory odor and bitter taste (Rivelli et al., 2017).

This study aimed to evaluate the antioxidant and antimicrobial capacity of the plant raw material of *Crambe* spp. in the M. M. Gryshko National Botanical Garden of the NAS of Ukraine as a potential source of antioxidants and antimicrobial agents.

MATERIALS AND METHODS

Plant materials

In this study we investigated the above-ground part of plants of the *Crambe* L. species from the experimental collection of M. M. Gryshko National Botanical Garden of the NAS of Ukraine: *C. cordifolia* Steven (1), *C. hispanica* subsp. *abyssinica* (Hochst. ex R. E. Fr.) Prina (2), *C. koktebelica* (Junge) N. Busch (3), *C. maritima* L. (4), *C. steveniana* Rupr. (5) at the stages of budding, flowering, and fruiting during 2018–2019 (Fig. 1). In this study, the above-ground parts of *Armoracia rusticana* (P. Gaertn., B. Mey. & Scherb.) were used for antioxidant activity determination and both above-ground and below-ground parts to compare antimicrobial activity. For antimicrobial activity, plant raw material was used at the start of vegetation (phase of the leaf rosette) and the flowering stage.

Chemicals

All chemicals were analytical grade and were purchased from Reachem (Slovakia) and Sigma Aldrich (USA).

Sample preparation

0.2 g of dried plant raw material was extracted with 20 ml of 80% ethanol for 2 hours. After centrifugation at 4000 g (Rotofix 32 A, Hettich, Germany) for 10 min, the supernatant was used for the next measurements: antioxidant activity, polyphenols, and flavonoids.

Fig. 1. Investigated plant species of *Crambe* L. genus at the flowering stage

Free radical scavenging assay (FRSA) by DPPH-method

The radical scavenging activity of the samples was measured using 2.2-diphenyl-1-picrylhydrazyl (DPPH) (Sánchéz-Moreno et al., 1998). The extracts (0.5 ml) were mixed with 3.6 ml of radical solution (0.025 g of DPPH in 100 ml ethanol). The absorbance of the sample extract was determined using a spectrophotometer Jenway (6405UV/Vis, England) at 515 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; 10–100 mg/l; $R^2 = 0.988$) was used as the standard and the results were expressed in mg/g Trolox equivalents.

Molybdenum reducing power of extracts (MRP)

The reducing power of the extracts was determined using the phosphomolybdenum method of Prieto et al. (1999) with slight modifications. The mixture of the sample (1 ml) of monopotassium phosphate (2.8 ml, 0.1 M), sulfuric acid (6 ml, 1 M), ammonium heptamolybdate (0.4 ml, 0.1 M) and distilled water (0.8 ml) was incubated at 90°C for 120 min, then rapidly cooled and detected by monitoring the absorbance at 700 nm using a spectrophotometer Jenway (6405UV/Vis, England). Trolox (10–1000 mg/l; $R^2 = 0.998$) was used as the standard and the results were expressed in mg/g Trolox equivalents.

Total polyphenol content (TPC)

The total polyphenol content of the extracts was measured using the method of Singleton and Rossi (1965) using a Folin-Ciocalteu reagent. 0.1 ml of each sample extract was mixed with 0.1 ml of the Folin-Chiocalteu reagent, 1 ml of 20% (w/v) sodium carbonate, and 8.8 ml of distilled water. After 30 min in darkness the absorbance at 700 nm was measured using a spectrophotometer Jenway (6405UV/Vis, England). Gallic acid (25–250 mg/l; $R^2 = 0.996$) was used as the standard and the results were expressed in mg/g gallic acid equivalents.

Total flavonoid content (TFC)

Determination of the total flavonoid content was conducted using the modified method of Shafii et al. (2017). 0.5 ml of the sample extract was mixed with 0.1 ml of 10% (w/v) ethanolic solution of aluminum chloride, 0.1 ml of 1 M sodium acetate, and 4.3 ml of distilled water. After 30 min in darkness the absorbance at 415 nm was measured using a spectrophotometer Jenway (6405UV/Vis, England). Quercetin (0.01–0.5 mg/l; $R^2 = 0.997$) was used as the standard and the results were expressed in mg/g quercetin equivalents.

Total phenolic acid content (TPAC)

Determination of the total phenolic acid content of the extracts was carried out using the method of Farmakopea Polska (1999). 0.5 ml of sample extract was mixed with 0.5 ml of 0.5 M hydrochloric acid, 0.5 ml Arnova reagent, 0.5 ml of 1 M sodium hydroxide (w/v), and 0.5 ml of distilled water. The absorbance at 490 nm was measured using a spectrophotometer Jenway (6405UV/Vis, England). Caffeic acid (1–200 mg/l, $R^2 = 0.999$) was used as a standard and the results were expressed in mg/g caffeic acid equivalents.

Antimicrobial activity

The ethanolic extracts of the investigated *Crambe* spp. were subjected to an evaporation procedure under reduced pressure at 40°C to remove the ethanol

(Stuart RE300DB rotary evaporator, Bibby scientific limited, UK, vacuum pump KNF N838.1.2KT.45.18, KNF, Germany). For the next procedure, the dried plant extracts were dissolved in dimethyl sulfoxide (DMSO) (Penta, Czech Republic). Nine strains of microorganisms (Czech Collection of microorganisms) were tested in this study, including three Gram-positive bacteria (*Bacillus cereus* CCM 869, *Clostridium perfingens* CCM 4435, *Staphylococcus aureus* subsp. *aureus* CCM 4223), three Gram-negative bacteria (*Haemophilus influenza* CCM 4456, *Klebsiella pneumoniae* subsp. *pneumoniae* CCM 4415, *Salmonella enterica* subsp. *enterica* CCM 7189), and three yeasts (*Candida albicans* CCM 8215, *C. glabrata* CCM 8270, *C. tropicalis* CCM 8264). The microorganism strains were grown in Mueller-Hinton agar (MHA, Biolife, Italy) in plates at a temperature of 37°C. The antimicrobial activity of each extract was determined using the disc diffusion method. 100 µl of microbial suspension was spread and grown in 1 ml of fresh media until they reached approximately 10^5 cells per ml. After this, 100 µl of microbial suspension was spread onto Mueller Hinton agar plates. The extracts were tested using 6 mm sterilized filter paper discs. The plates were incubated at 37°C for 24 hours. Blank discs were impregnated with Ampicillin (10 µg/disc, Oxoid, UK) for Gram-positive bacteria and yeasts, and Gentamicin (10 µg/disc, Oxoid, UK) for Gram-negative bacteria as a positive control. A 1% solution of DMSO (dimethyl sulfoxide) was used as a negative control. The diameter of the inhibition zone was measured in millimeters. Each antimicrobial assay was performed in triplicate.

Statistical analysis

The statistically treated data are given in tables as the arithmetical mean values and their standard errors. Data were submitted using ANOVA and the differences between means compared through the Tukey-Kramer test ($\alpha = 0.05$). Correlation analysis was performed using Pearson's criterion.

RESULTS AND DISCUSSION

Plant raw material, such as leaves, stems, roots, fruits, seeds, and peels, is a valuable source of antioxidants. Natural antioxidants are widely distributed in food and medicinal plants and exhibit sprawling biological effects such as anti-inflammatory, anti-aging, anti-atherosclerosis, and anticancer (Xu et al., 2017). The main group of natural antioxidants is phenolic compounds that are widely distributed in the plant world and involved in oxidative-reduction processes (Babenko et al., 2019). There are numerous methods to determine the antioxidant activity in plant extracts, among which the DPPH scavenging assay (Moharram and Youssef, 2014; Prior et al., 2005) and phosphomolybdenum methods (Alam et al., 2013; Gulcin, 2020) are widely used. Plants from Brassicaceae are characterized by a high antioxidant capacity when raw due to their content of vitamins, carotenoids, and phenolic compounds, etc. (Podsędek, 2007).

Free radical scavenging assay

Among other methods, the scavenging of the stable radical (DPPH) assay is based on the measurement of the scavenging ability of antioxidants against a radical solution that is also a simple and rapid test (Sánchéz-Moreno, 2002). The antioxidant activity of the investigated extracts, measured using the DPPH method, was from 4.38 (*C. koktebelica*, budding stage) to 8.20 (*C. koktebelica*, flowering stage) mg TE/g DW (Fig. 2). For the budding, flowering, and fruiting, we found the FRSA to be 4.38–8.19, 4.40–8.20, and 5.73–8.07 mg TE/g DW, respectively. In this experiment, the *A. rusticana* extracts showed FRSA from 6.56 to 9.11 mg TE/g DW. The highest value of its extracts was obtained at the budding stage among all investigated plants. At the flowering and fruiting stages, the best results were obtained for *C. koktebelica* and *C. hispanica* subsp. *abyssinica*, respectively.

Bukhari et al. (2013) determined the free radical scavenging activity of the methanolic extracts of *C. cordifolia* as 1106.65 µg/ml as compared to 21.69 µg/ml of ascorbic acid. Dubie et al. (2013) determined the antioxidant activity using DPPH assay in water and methanol extracts of *Brassica juncea* seed meal as 4.59 and 6.51 mg/g sinapic acid equivalent. Tomsone and Kruma (2017) detected the DPPH scavenging activity for *A. rusticana* leaf extracts during vegetation as 37.13–73.38 mM/100 g TE DW. The FRSA of other representatives of Brassicaceae, such as *Bunias orientalis,* at the stage of flowering was 8.94 mg TE/g DW in the ethanol extracts (Vergun et al., 2018). This parameter

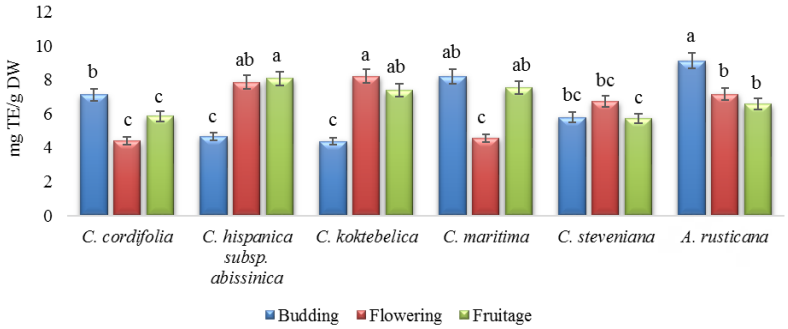

Fig. 2. Free radical scavenging activity using the DPPH-method of ethanol extracts of *Crambe* spp. during vegetation (different superscripts in each column indicate significant differences in the mean at $P < 0.05$): TE – Trolox equivalent

for *Brassica campestris* and *Sinapis alba* leaf extracts was 8.17 and 7.15 mg TE/g DW, respectively (Vergun et al., 2019c).

Molybdenum reducing power of extracts

Determination of the antioxidant activity using the phosphomolybdenum method along with other assays allows the identification of the antioxidant capacity of plant raw material. The obtained results of antioxidant capacity can be expressed as α-tocopherol or Trolox equivalent (Gulcin, 2020). The MRP of the investigated extracts was from 40.07 (*C. maritima*, budding stage) to 129.13 (*C. steveniana*, fruitage) mg TE/g DW (Fig. 3). At the budding, flowering, and fruiting stages we determined 40.07–105.06, 62.01–111.19, and 64.34–129.13 mg TE/g, respectively. The *A. rusticana* extracts demonstrated an increase in MRP from the budding to the flowering stage and following a decrease in flowering to fruiting. A comparison of the obtained results of all the investigated extracts showed that at the budding stage similar values of MRP occurred for *A. rusticana* as well as for *C. hispanica* subsp. *abyssinica*. At the flowering stage, this parameter was the highest for *A. rusticana* extracts, and at fruiting, *C. steveniana* and *C. maritima* showed higher values than *A. rusticana*. The extracts of *C. koktebelica* and *C. cordifolia* demonstrated similar results.

Bukhari et al. (2013) determined antioxidant activity in *C. cordifolia* extract using the phosphomolybdenum

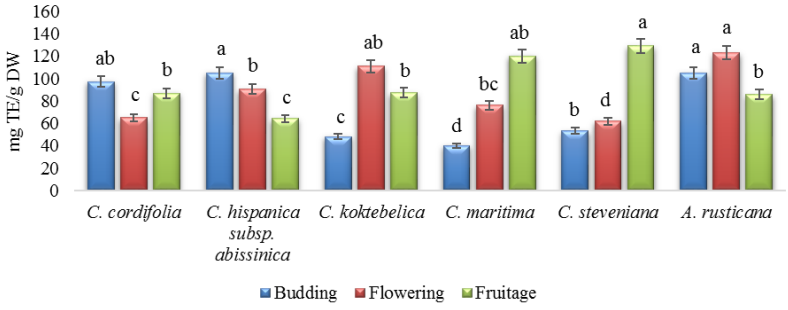

Fig. 3. Molybdenum reducing power of ethanol extracts using the phosphomolybdenum method of *Crambe* spp. during vegetation (different superscripts in each column indicate significant differences in the mean at $P < 0.05$): TE – Trolox equivalent

method as 205.73 µg/mg α-tocopherol equivalent. The reducing power of *A. rusticana* leaf extracts during vegetation was 2638–9573 mg/100 g of ascorbic acid equivalent DW (Tomsone and Kruma, 2017). The MRP of the ethanol extracts of *Bunias orientalis* showed the result of 184.59 mg TE/g DW (Vergun et al., 2018). In addition, ethanol leaf extracts of *Brassica campestris* and *Sinapis alba* showed an MRP of 91.94 and 148.43 mg TE/g DW, respectively (Vergun et al., 2019c).

Total polyphenol content

Polyphenol compounds are natural phytochemicals that form a major group of semi-water-soluble compounds which are generally found in nature as glycosides (Rasouli et al., 2017). Plants are a valuable source of polyphenols and crops from Brassicaceae are not an exception (Sikora et al., 2008).

The TPC of the investigated extracts of *Crambe* spp. during vegetation was from 20.24 (*C. koktebelica*, budding stage) to 70.88 (*C. cordifolia*, budding stage) mg GAE/g DW (Fig. 4). The TPC in the extracts of *A. rusticana* decreased during vegetation as it did in the extracts of *C. cordifolia* and *C. hispanica* subsp. *abyssinica* but values of this parameter were less. The TPC increased in plants of *C. koktebelica* and *C. maritima* during vegetation, where *C. maritima* accumulated these compounds 3 times more during fruiting than at the budding stage.

Rashid et al. (2018) investigated root extracts of *C. cordifolia* in different solvents and determined the TPC in methanol extracts to be 210.3 µg GAE/mg, chloroform extracts 122.5 µg GAE/mg, ethyl acetate extracts 35.1 µg GAE/g, and hexane extracts 10.7 µg GAE/mg DW. Due to less investigation of *Crambe* spp. polyphenol content and antioxidant activity, these were used to compare with other species from Brassicaceae in this study. Beevi et al. (2010) investigated different extracts of *Raphanus sativus* leaves and determined the TPC in water and methanol extracts to be 34.16 and 86.16 mg/g catechin equivalent, respectively. Deveci and Uzun (2011) identified TPC in the leaves of *Spinacia oleracea* L. at 119.88 mg GAE/100 g FW. Bukhari et al. (2013) found TPC in water extracts of *C. cordifolia* as 10.60 µg/g pyrocatechol equivalent. Dubie et al. (2013) determined the TPC in water and ethanol extracts of *Brassica juncea* seed meal to be 11.56 and 8.00 mg/g of sinapic acid equivalent. Goyeneche et al. (2015), in the leaf extracts of *R. sativus*, identified 695.07 mg GAE per 100 g DW of TPC. Leaf extracts of *A. rusticana* showed TPC of 1235–2705 mg GAE/100 g DW during vegetation (Tomsone and Kruma, 2017). For *Bunias orientalis* ethanol extracts, this parameter was 52.88 mg GAE/g DW, which was determined at the flowering stage (Comlekcioglu, 2019; Vergun et al., 2018). The TPC was determined for five species of *Isatis* as being between 8.90 and 19.16 mg GAE/g. In leaf extracts of *Brassica campestris* and *Sinapis alba* TPC was identified as 41.69 and 73.58 mg GAE/g DW, respectively (Vergun et al., 2019c).

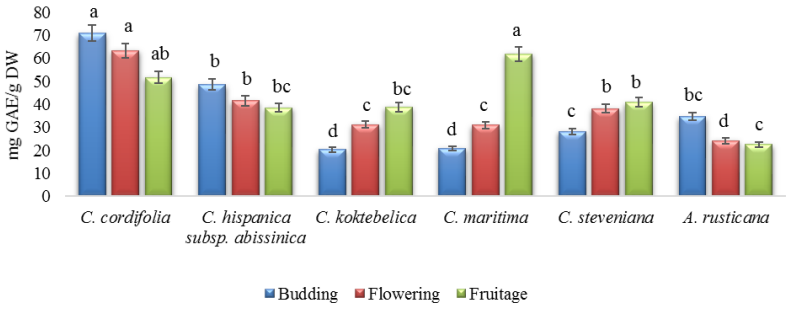

Fig. 4. The total content of polyphenol compounds of ethanol extracts of *Crambe* spp. during vegetation (different superscripts in each column indicate significant differences in the mean at $P < 0.05$): GAE – gallic acid equivalent

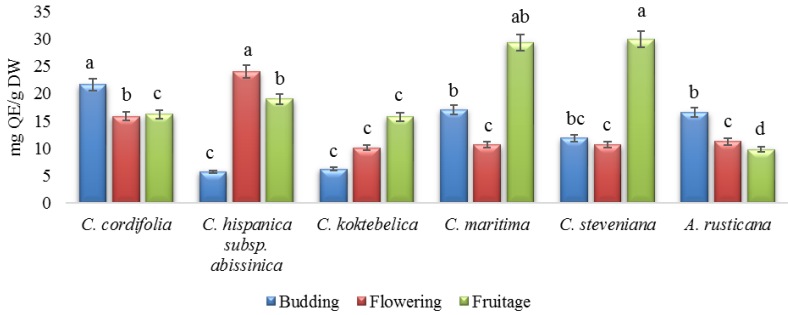

Fig. 5. The total flavonoid content of ethanol extracts of *Crambe* spp. during vegetation (different superscripts in each column indicate significant differences in the mean at $P < 0.05$): QE – quercetin equivalent

Total flavonoid content

Flavonoids are a large group of polyphenol compounds that are extensively distributed in plant raw material and are very effective antioxidants. They include flavones, flavanols, flavanones, and chalcones represented in the tissues of higher plants (Gulcin, 2020). In our research, the total content of flavonoids (TFC) was from 5.73 (*C. hispanica* subsp. *abissinica*, budding stage) to 29.92 (*C. steveniana*, fruitage) mg QE/g DW (Fig. 5). Accumulation of flavonoids during vegetation for all investigated species was uneven. Plants of species of *C. koktebelica*, *C. maritima*, and *C. steveniana* contained a maximal level of flavonoids at the fruiting stage before the death of generative shoots. The TFC in *A. rusticana* plants decreased from budding to fruiting. The maximal content of TFC at the budding stage was identified in extracts of *C. cordifolia* and *A. rusticana*, at the flowering stage for *C. hispanica* subsp. *abissinica*, and at the fruiting stage for *C. koktebelica*, *C. maritima*, and *C. steveniana*.

According to Aguinagalde and Gomez-Campo (1984), in the plant extracts of *Crambe* spp. kaempferol, quercetin, apigenin, luteolin, etc. were identified. The TFC of the ethanol extracts of *C. cordifolia* in the report of Bukhari et al. (2013) was 1.43 mg QE/mg.

Rashid et al. (2018) investigated root extracts of *C. cordifolia* in different solvents and determined the TFC in methanol extracts to be 75.5 µg QE/mg, hexane extracts 7.09 µg QE/mg, chloroform extracts 48.2 µg QE/mg, and ethyl acetate extracts 18.8 µg QE/mg of dry weight. In extracts of another species, *A. rusticana*, TFC was found from 2486 to 11 697 mg/100 g of catechin equivalent (Tomsone and Kruma, 2017). The comparison with other Brassicaceae species showed the presence of 1042.73 mg QE/100 g TFC in the leaf extracts of *Raphanus sativus* (Goyeneche et al., 2015). *Bunias orientalis* extracts demonstrated TFC at the stage of flowering of 39.91 mg QE/g DW (Vergun et al., 2018). Extracts of *Isatis* spp. contained flavonoids from 115.6 to 430.6 µg QE/g (Comlekcioglu, 2019). In the leaf extracts of *Brassica campestris* and *Sinapis alba* TFC of 28.37 and 62.91 mg QE/g, respectively, was found (Vergun et al., 2019c).

Total phenolic acid content

Phenolic acids are a class of polyphenol compounds that are widely distributed in plants and plant food. The content of these compounds in vegetables and fruits causes a potential protective role against damage by oxidative diseases (Gulcin, 2020). The TPAC of the investigated extracts of *Crambe* spp. during vegetation was from 3.00 (*C. hispanica* subsp. *abissinica*, budding stage) to 10.63 (*C. maritima*, fruiting) mg CAE/g DW (Fig. 6). The content of TPAC of *A. rusticana* extracts decreased from the budding stage to fruiting, as did TPC and TFC. An increase of TPAC from budding to fruiting was observed in extracts of *C. steveniana*. The peak of accumulation of TPAC was found at the budding stage for *C. cordifolia*, at the flowering stage for *C. hispanica* subsp. *abissinica* and *C. koktebelica*, and at the fruiting stage for *C. maritima* and *C. steveniana*.

As shown in other reports, the TPAC of *Bunias orientalis* extracts, for example, was 11.29 mg CAE/g

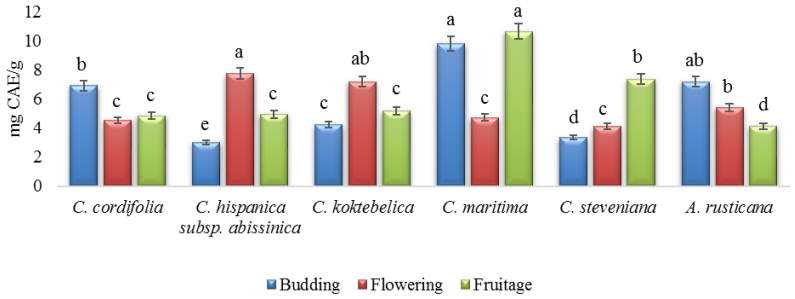

Fig. 6. The total content of phenolic acids of ethanol extracts of *Crambe* spp. during vegetation (different superscripts in each column indicate significant differences in the mean at $P < 0.05$): CAE – caffeic acid equivalent

DW at the stage of flowering (Vergun et al., 2018). For the leaf extracts of *Brassica campestris* and *Sinapis alba* the TPAC was 4.87 and 9.15 mg CAE/g DW, respectively (Vergun et al., 2019c).

Overall, the investigation of polyphenol compound accumulation in all *Crambe* species was uneven depending on the stage of growth, which could be connected with the biological peculiarities of these plants as perennial (besides *C. hispanica* subsp. *abissinica*) and their ability to create numerous shoots during the budding and flowering stages. Evidently, this ability can affect the distribution of biologically active compounds, however, it is necessary to investigate this more deeply.

In this study, the results of correlation analysis concerning polyphenols and antioxidant activity are presented. As the parameters of antioxidant activity in *Crambe* spp. extracts were affected by stages of growth and species, the antioxidant activity also changed. To determine the contribution of different polyphenol compounds to the molybdenum reducing power and free radical scavenging activity, and the relationship between antioxidants markers, correlation analyses were conducted independent of species but related to the phase of growth. The correlation between antioxidant activity and polyphenol compounds in the plant extracts depends on their phenolic composition and the different methods used based on different reaction mechanisms (Tusevski et al., 2014). Very strong correlation was found between TPAC and FRSA ($r = 0.907$) and between TFC and FRSA ($r = 0.885$) at the budding stage and between TPAC and TFC ($r = 0.851$) at the fruiting stage (Table 1). Strong correlation

Table 1. Pearson's correlation coefficients for investigated parameters of *Crambe* spp.

Parameter	TPC	TFC	TPAC	FRSA
Budding				
TFC	0.699**	1		
TPAC	0.423*	0.744**	1	
FRSA	0.536*	0.885**	0.907**	1
MRP	–0.169*	–0.432*	–0.775**	–0.473*
Flowering				
TFC	0.439*	1		
TPAC	–0.165*	0.489*	1	
FRSA	–0.231*	0.209*	0.777**	1
MRP	–0.515*	–0.685**	0.217*	0.557*
Fruitage				
TFC	0.383*	1		
TPAC	0.717**	0.851**	1	
FRSA	–0.053*	–0.174*	0.080*	1
MRP	0.791**	–0.249*	0.161*	0.020*

TPC – total polyphenol content, TFC – total flavonoids content, TPAC – total phenolic acid content, FRSA – free radical scavenging assay, MRP – molybdenum reducing power.
* Correlation is significant at the level of 0.05.
** Correlation is significant at the level of 0.01

between TPAC and FRSA (r = 0.777), TFC and TPAC (r = 0.744), TPAC and TPC (r = 0.717), and TPC and TFC (r = 0.699) was found at the budding stage.

Correlation analysis was also performed for each species and showed differences in the accumulation of investigated compounds. So, in extracts of *C. cordifolia* a very strong correlation was found between TFC and TPAC (r = 0.997), FRSA and MRP (r = 0.987), TPAC and FRSA (r = 0.902), TFC and FRSA (r = 0.871), and TPAC and MRP (r = 0.823). A strong correlation was determined between TPC and TFC (r = 0.764), TPC and TPAC (r = 0.718).

A very strong correlation in the *C. hispanica* subsp. *abissinica* extracts was found between the following parameters: TFC and FRSA (r = 0.947), TFC and TPAC (r = 0.934), and TPC and MRP (r = 0.921). A strong correlation was determined between TPAC and FRSA (r = 0.772).

A very strong relation in the *C. koktebelica* extracts was determined between the following investigated parameters: FRSA and MRP (r = 0.984), TPC and TFC (r = 0.978), TPAC and MRP (r = 0.943), TPAC and FRSA (r = 0.870), TPC and FRSA (r = 0.811). A strong correlation was detected between TPC and MRP (r = 0.694), and TFC and FRSA (r = 0.673).

For *C. maritima* a strong correlation was found between TPC and MRP (r = 0.973), TPAC and FRSA (r = 0.954), TFC and TPAC (r = 0.841), and TPC and TFC (r = 0.834). A strong correlation was determined between TFC and MRP (r = 0.686), and TFC and FRSA (r = 0.641).

A very strong correlation was found between TPAC and MRP (r = 0.997), TFC and MRP (r = 0.987), and TFC and TPAC (r = 0.972) in extracts of *C. steveniana*. A strong relationship between the investigated parameters was found for TPC and TPAC (r = 0.786), TPC and MRP (r = 0.738), and TPC and TFC (r = 0.620). A significant correlation between the antioxidant activity and polyphenol compounds of *Crambe* spp. demonstrated that MRP and FRSA depended on the phenolic constituents. These results suggest that relationships between the investigated parameters depend on the species rather than on the stage of growth since in some cases, analyzing concrete species, the correlation between antioxidant activities and different groups of polyphenols was higher than in generalized analysis. In addition, the strong relationship between the investigated parameters depended on the antioxidant activity assay. It is interesting that the interrelationship between all groups of polyphenols and between all polyphenols and FRSA for *A. rusticana* was very strong (r = 0.954–0.999), while a moderate correlation was found between TPAC and MRP (r = 0.439). A weak or strong negative correlation between the investigated parameters may suggest that a strong correlation of FRSA and MRP in the *Crambe* spp. extracts was caused by other compounds rather than groups of polyphenols.

Tomsone and Kruma (2017) reported a strong positive correlation between TPC, TFC, DPPH scavenging activity, and the reducing power of *A. rusticana* extracts. We found a moderate correlation between these parameters at the flowering stage (r = 0.557). It should be noted that different methods of antioxidant activity determination can be correlated (Muzykiewicz et al., 2020).

Antimicrobial activity

The antimicrobial properties of ethanolic extracts of *Crambe* spp. were determined in above-ground parts and below-ground parts (Tables 2–4) of the plants. One of the most common methods to evaluate the antimicrobial potential of plant extracts is the disc diffusion method (Balouiri et al., 2016). Ethanol extracts of plants from Brassicaceae showed significant antimicrobial activity against selected pathogens (Prasad, 2014). For example, different extracts of *Brassica oleracea* var. *capitata rubra* showed inhibition against *Bacillus subtilis*, *Staphylococcus aureus*, etc. (Ayshwarya and Sudha Rameshwari, 2015).

At the start of vegetation, plant extracts of the above-ground parts of *C. cordifolia* and *C. koktebelica* weren't active against microbial strains. In this period, *C. hispanica* subsp. *abissinica* extracts showed an inhibition effect against *Staphylococcus aureus* subsp. *aureus*, *Bacillus cereus* (Table 2). *C. steveniana* extract inhibited *Haemophilus influenzae* and *Bacillus cereus*. In other cases, inhibition of microbial strains wasn't found. Extracts of *A. rusticana* also weren't effective against microbial strains at the start of vegetation, except *Salmonella enterica* subsp. *enterica*.

At the flowering stage, inhibition activity of the above-ground parts of the investigated plant extract was slightly higher. *C. cordifolia* and *C. maritima*

Table 2. Antibacterial activity of ethanol extracts of the above-ground parts of *Crambe* spp. by disc diffusion method, mm

Species	*Bacillus cereus*	*Clostridium perfingens*	*Staphylococcus aureus* subsp. *aureus*	*Haemophilus influenzae*	*Klebsiella pneumoniae* subsp. *pneumoniae*	*Salmonella enterica* subsp. *enterica*
Start of vegetation						
C. cordifolia	ND	ND	ND	ND	ND	ND
C. hispanica subsp. *abissinica*	2.60 ±0.70a	ND	1.60 ±0.70	ND	ND	ND
C. koktebelica	ND	ND	ND	ND	ND	ND
C. maritima	ND	ND	ND	ND	ND	ND
C. steveniana	1.40 ±0.70b	ND	ND	1.30 ±0.48	ND	ND
Armoracia rusticana	ND	ND	ND	ND	ND	1.20 ±0.42
AMP/GEN	21.17 ±1.17b	21.50 ±1.39b	22.12 ±1.18a	21.50 ±1.87b	20.67 ±1.03c	21.67 ±1.63b
DMSO	ND	ND	ND	ND	ND	ND
Flowering						
C. cordifolia	13.20 ±1.99a	2.20 ±1.03a	11.20 ±1.55a	1.10 ±0.32a	1.80 ±0.63b	4.10 ±1.20a
C. hispanica subsp. *abissinica*	2.10 ±0.99b	1.60 ±0.52	7.10 ±1.10b	ND	ND	4.70 ±0.95a
C. koktebelica	3.30 ±0.48b	2.90 ±0.88a	12.70 ±1.42a	ND	1.20 ±0.42	2.70 ±1.16b
C. maritima	1.08 ±1.03c	3.00 ±1.15a	5.10 ±0.99c	2.20 ±1.32a	3.80 ±0.79a	2.00 ±0.82c
C. steveniana	2.60 ±1.51b	ND	12.40 ±1.90a	ND	ND	1.40 ±0.52
Armoracia rusticana	1.20 ±0.42c	ND	ND	ND	ND	1.50 ±0.47c
AMP/GEN	20.50 ±1.05b	20.33 ±1.51b	22.47 ±1.73a	20.00 ±1.41b	21.33 ±1.37a	20.17 ±1.17b
DMSO	ND	ND	ND	ND	ND	ND

ND – not detected inhibition, AMP – Ampicillin, GEN – Gentamicin, DMSO – dimethyl sulfoxide.
Different letters in each column indicate significant differences in the mean at $P < 0.05$.

showed activity against all investigated microbial strains (1.10–13.20 and 1.08–5.10 mm, respectively). *C. hispanica* subsp. *abissinica* extracts showed inhibition effects (1.60–7.10 mm) against 7 strains, except *Haemophilus influenzae* and *Klebsiella pneumoniae* subsp. *pneumoniae*. *C. koktebelica* inhibited the growth of microbial strains on 1.20–12.70 mm, except *Haemophilus influenzae*. *C. steveniana* showed an antimicrobial effect from 1.40 to 12.40 mm, except on *Clostridium perfingens*, *Haemophilus influenzae*, and *Klebsiella pneumoniae* subsp. *pneumoniae*. Extract of *A. rusticana* at the flowering stage had low effectiveness against *Salmonella enterica* subsp. *enterica* and *Bacillus cereus*.

In total, ethanol extracts of *Crambe* spp. underground parts were more effective against microbial strains than above-ground parts (Table 3). Extracts of *C. cordifolia* showed activity against microbial strains at the start of vegetation and the flowering stage of 2.40–4.00 mm and 4.40–16.10 mm, with *C. hispanica* subsp. *abissinica* at 1.70–3.10 mm and 4.70–11.10 mm, *C. koktebelica* at 1.70–7.50 mm and 4.60–13.10 mm,

Table 3. Antibacterial activity of ethanol extracts of the underground parts of *Crambe* spp. by disc diffusion method, mm

Species	*Bacillus cereus*	*Clostridium perfingens*	*Staphylococcus aureus* subsp. *aureus*	*Haemophilus influenzae*	*Klebsiella pneumoniae* subsp. *pneumoniae*	*Salmonella enterica* subsp. *enterica*
Start of vegetation						
C. cordifolia	2.40 ±0.52c	2.50 ±0.71a	4.00 ±0.82a	3.30 ±0.67a	3.90 ±0.99b	3.60 ±0.84b
C. hispanica subsp. *abissinica*	3.10 ±0.74b	2.70 ±0.67a	2.60 ±0.84c	1.70 ±0.67c	ND	1.70 ±0.67c
C. koktebelica	1.70 ±0.67	2.80 ±0.63a	4.70 ±0.95a	3.60 ±0.97a	3.90 ±0.99b	7.50 ±0.97a
C. maritima	4.40 ±1.26a	1.30 ±0.48b	4.30 ±0.82a	2.30 ±0.48b	3.30 ±0.82b	7.50 ±0.71a
C. steveniana	1.50 ±0.53c	2.50 ±0.71a	3.90 ±0.57b	2.40 ±0.52b	4.60 ±1.07a	6.80 ±0.79a
Armoracia rusticana	3.00 ±0.94b	2.10 ±0.74a	4.80 ±0.63a	2.30 ±0.67b	1.90 ±0.74c	2.00 ±0.82b
AMP/GEN	22.17 ±1.47a	21.67 ±1.03b	19.67 ±1.03c	20.00 ±0.89c	21.67 ±1.03b	23.33 ±1.21a
DMSO	ND	ND	ND	ND	ND	ND
Flowering						
C. cordifolia	4.50 ±1.08c	4.40 ±0.84d	16.10 ±0.74a	6.90 ±1.37b	4.60 ±2.22c	5.80 ±0.42c
C. hispanica subsp. *abissinica*	6.10 ±1.45b	10.70 ±0.82a	11.10 ±0.99b	4.70 ±0.67c	ND	8.70 ±0.67b
C. koktebelica	4.60 ±0.52c	7.50 ±1.84b	9.30 ±1.06c	8.30 ±0.67a	9.00 ±0.47a	13.10 ±1.45a
C. maritima	9.30 ±0.95a	5.90 ±0.57c	12.30 ±1.64b	5.80 ±1.14	7.40 ±1.51b	13.80 ±1.55a
C. steveniana	3.30 ±0.67c	5.60 ±0.97c	8.20 ±0.79c	7.50 ±0.97a	9.10 ±0.57a	12.60 ±1.71a
Armoracia rusticana	9.70 ±0.95a	10.10 ±1.52a	10.70 ±1.16b	7.80 ±1.40a	ND	13.70 ±2.10a
AMP/GEN	21.77 ±1.10b	21.33 ±0.98b	22.50 ±1.05a	21.92 ±0.86b	20.52 ±0.78c	22.15 ±1.23a
DMSO	ND	ND	ND	ND	ND	ND

ND – not detected inhibition, AMP – Ampicillin, GEN – Gentamicin, DMSO – dimethyl sulfoxide.
Different letters in each column indicate significant differences in the mean at $P < 0.05$.

C. maritima at 1.30–7.50 mm and 5.80–13.80 mm, *C. steveniana* at 1.50–6.80 mm and 3.30–12.60 mm, respectively, depending on microbial strains. Extracts of *C. hispanica* subsp. *abissinica* acted against *Klebsiella pneumoniae* subsp. *pneumoniae* in both periods and *A. rusticana* extracts were ineffective against this microbe at the flowering stage. In addition, the highest antimicrobial activity of *A. rusticana* extracts was found against *Bacillus cereus*.

The best anticandidal activity at the start of vegetation in the investigated extracts was observed in *C. maritima* (6 mm) in the underground parts against *Candida albicans* (Table 4). *A. rusticana* extract also showed the best inhibition (7.10 mm) in this period in the underground part. It should be noted that the above-ground part extracts of *C. cordifolia*, *C. koktebelica*, *C. steveniana*, and *A. rusticana* weren't effective against three *Candida* strains at the start of vegetation.

At the flowering stage, the highest anticandidal activity was identified for *C. hispanica* subsp. *abissinica* against *Candida albicans* (11.90 mm). *A. rusticana*

Table 4. Anticandidal activity of ethanol extracts of *Crambe* spp. by disc diffusion method, mm

Species	Above-ground part extracts			Underground part extracts		
	Candida albicans	*Candida glabrata*	*Candida tropicalis*	*Candida albicans*	*Candida glabrata*	*Candida tropicalis*
Start of vegetation						
C. cordifolia	ND	ND	ND	ND	1.30 ±0.48b	2.40 ±0.52a
C. hispanica subsp. *abissinica*	4.70 ±0.67a	6.60 ±0.70a	1.40 ±0.52	3.60 ±1.07c	1.80 ±0.79b	1.50 ±0.53b
C. koktebelica	ND	ND	ND	5.80 ±1.55b	2.50 ±0.71a	2.90 ±0.99a
C. maritima	3.50 ±0.97b	2.40 ±0.84b	ND	6.00 ±0.82a	3.30 ±0.48a	2.60 ±0.52a
C. steveniana	ND	ND	ND	5.20 ±0.79b	2.50 ±0.53a	1.90 ±0.88b
Armoracia rusticana	ND	ND	ND	7.10 ±1.29a	2.80 ±0.79a	1.90 ±1.10b
AMP	21.77 ±2.25b	21.50 ±1.05b	22.15 ±1.19a	21.50 ±1.05b	19.67 ±0.82c	21.00 ±1.41b
DMSO	ND	ND	ND	ND	ND	ND
Flowering						
C. cordifolia	8.30 ±0.67a	8.70 ±1.16a	4.60 ±1.17a	1.80 ±0.79c	2.90 ±0.57d	4.20 ±0.42c
C. hispanica subsp. *abissinica*	7.90 ±0.88a	3.70 ±0.82b	2.10 ±1.37b	11.90 ±1.60a	5.20 ±0.79c	8.30 ±0.82b
C. koktebelica	6.90 ±1.20b	2.10 ±0.57c	ND	8.50 ±0.53b	5.60 ±0.70c	7.30 ±1.06b
C. maritima	7.70 ±0.95a	7.40 ±0.97a	2.70 ±1.16b	10.50 ±2.68a	7.30 ±0.95b	5.00 ±0.67c
C. steveniana	8.30 ±0.67a	2.20 ±0.79c	ND	7.40 ±0.97b	6.30 ±0.95b	4.90 ±0.74c
Armoracia rusticana	ND	ND	ND	12.10 ±2.28a	9.10 ±0.99a	11.80 ±1.69a
AMP	21.83 ±1.60a	21.17 ±1.17a	20.17 ±0.75b	21.33 ±0.88a	20.05 ±0.81b	21.12 ±1.17a
DMSO	ND	ND	ND	ND	ND	ND

BC – *Bacillus cereus*, CA – *Candida albicans*, CG – *Candida glabrata*, CT – *Candida tropicalis*, CP – *Clostridium perfingens*, ND – not detected inhibition.
Different letters in each column indicate significant differences in the mean at $P < 0.05$.

extract showed the best result of inhibition among all the investigated extracts (12.10 mm).

CONCLUSIONS

This report demonstrated the potent antioxidant and antimicrobial capacity of ethanol extracts of five species of *Crambe* during vegetation. However, the accumulation of polyphenol compounds in the above-ground parts of the investigated plants was uneven and depended on the stage of growth and species. The highest level of TCP was found for *C. cordifolia* at the budding stage, TFC for *C. steveniana*, and TPAC for *C. maritima* during fruiting. The maximal values of the FRSA and MRP of extracts were found for *C. koktebelica* at the flowering stage and *C. steveniana* at the fruiting stage, respectively. Among the five plant species, *C. koktebelica*, *C. maritima*, and *C. steveniana* accumulated higher TFC during fruiting. The study of antimicrobial activity of the ethanol extracts in the underground parts showed minimal inhibition in extracts of *C. maritima* against *Clostridium perfingens* and

maximal inhibition against *Salmonella enterica* subsp. *enterica*. It can be concluded that the antimicrobial effectiveness of the investigated extracts appeared in the underground parts more than the above-ground ones. The obtained data on *Crambe* spp. is useful for food, pharmacological, and deep biochemical investigations.

REFERENCES

Agneta, R., Möllers, Ch., Rivelli, A. R. (2013). Horseradish (*Armoracia rusticana*), a neglected medical and condiment species with a relevant glucosinolate profile: a review. Genet. Res. Crop. Evol., 60, 1923–1943. https://doi.org/10.1007/s10722-013-0010-4

Aguinagalde, I., Gomez-Campo, C. (1984). The phylogenetic significance of flavonoids in *Crambe* L. (Cruciferae). J. Linn. Soc., Bot., 89, 277–288.

Alam, M. N., Bristi, N. J., Rafiquzzaman, M. (2013). Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. Saudi Pharm. J., 21, 143–152. http://dx.doi.org/10.1016/j.jsps.2012.05.002

Avato, P., Argentieri, M. P. (2015). Brassicaceae: a rich source of health improving phytochemicals. Phytochem. Rev., 14, 1019–33. https://doi.org/10.1007/s11101-015-9414-4

Ayshwarya, M., Sudha Ramashwari, K. (2015). Antimicrobial activity of the plant extracts of *Brassica oleracea* var. *capitata rubra*. JIARM, 3(10), 149–156.

Babenko, L. M., Smirnov, O. E., Romanenko, K. O., Trunova, O. K., Kosakivska, I. V. (2019). Phenolic compounds in plants: biogenesis and functions. Ukr. Biochem. J., 91(3), 5–18. https://doi.org/10.15407/ubj91.03.005

Balouiri, M., Sadiki, M., Ibnsouda, S. K. (2016). Methods for *in vitro* evaluating antimicrobial activity: A review. J. Pharm. Anal., 6, 71–79. https://dx.doi.org/10.1016/j.jpha.2015.11.005

Bassegio, D., Zanotto, M. D., Ferreira Santos, R., Werncke, I., Pereira Dias, P., Olivo, M. (2016). Oilseed crop crambe as a source of renewable energy in Brazil. Renew. Sust. Energ. Rev., 66, 311–321. https://dx.doi.org/10.1016/j.rser.2016.08.010

Beevi, S. S., Narasu, M. L., Gowda, B. B. (2010). Polyphenolics profile, antioxidant and radical scavenging activity of leaves and stem of *Raphanus sativus* L. Plant Foods Hum. Nutr., 65, 8–17. https://doi.org/10.1007/s11130-009-0148-6

Bukhari, S. M., Simic, N., Siddiqui, H. L., Ahmad, V. U. (2013). Determination of antioxidant activity of *Crambe cordifolia*. World Appl. Sci. J., 22, 11, 1561–1565.

Calabrone, L., Larocca, M., Marzocco, S., Martelli, G., Rossano, R. (2015). Total phenols and and flavonoids content, antioxidant capacity and lipase inhibition of root and leaf horseradish (*Armoracia rusticana*) extracts. Food Nutr. Sci., 6, 64–74. https://dx.doi.org/10.4236/fns.2015.61008

Cömert Önder, F., Doğrular, N., Gunduzalp, E., Barlak, S., Ay, M. (2020). A comparative study of three Brassicaceae vegetables grown in Canakkale: determination of total phenolic content and antioxidant activity of pulp and juice samples of radish (*Raphanus sativus* L.), cabbage (*Brassica oleracea* L. var *capitata* L.) and cauliflower (*Brassica oleracea* L.). J. Grad. School Nat. Appl. Sci., 6(1), 30–38. https://doi.org/10.28979/comufbed.633456

Comlekcioglu, N. (2019). Bioactive compounds and antioxidant activity in leaves of endemic and native *Isatis* spp. in Turkey. Braz. Arch. Biol. Technol., 62, e19180330. http://dx.doi.org/10.1590/1678-4324-2019180330

Deveci, M., Uzun, E. (2011). Determination of phenolic compounds and chlorophyll content of spinach (*Spinacia oleracea* L.) at different growth stages. Asian J. Chem., 23(8), 3739–3743.

Dubie, J., Stancik, A., Morra, M., Nindo, C. (2013). Antioxidant extraction from mustard (*Brassica juncea*) seed meal using high-intensity ultrasound. J. Food Sci., 78(4), 542–548. https://doi.org/10.1111/1750-3841.12085

Farmakopea Polska (1999). The Polish Pharmaceutical Society. Retrieved January 1, 2017, from: http://www.ptfarm.pl/?pid=1&language=en

Francisco, M., Tortosa, M., Martínez-Ballesta, C. M., Velasco, P., García-Viguera, C., Moreno, D. A. (2016). Nutritional and phytochemical value of *Brassica* crops from the agri-food perspective. Ann. Appl. Biol., 170, 273–285. https://doi.org/10.1111/aab.12318

Francisco-Ortega, J., Fuertes-Aguilar, J., Kim, S.-Ch., Santos-Guerra, A., Crawford, D. J., Jansen, R. K. (2002). Phylogeny of the Macaronesian endemic *Crambe* section *Dendrocrambe* (Brassicaceae) based on internal transcribed spacer sequences of nuclear ribosomal RNA. Am. J. Bot., 89(12), 1984–1990. https://doi.org/10.3732/ajb.89.12.1984

Francois, L. E., Kleiman, R. (1990). Salinity effect on vegetative growth, seed yield, and fatty acid composition of *Crambe*. Agron. J., 82(6), 1110–1114. https://doi.org/10.2134/agronj1990.00021962008200060017x

Goyeneche, R., Roura, S., Ponce, A., Vega-Galvez, A., Quispe-Fuentes, I., Uribe, E., Di Scala, K. (2015). Chemical characterization and antioxidant capacity of red radish (*Raphanus sativus* L.) leaves and roots. J.

Funct. Foods, 16, 256–264. http://dx.doi.org/10.1016/j.jff.2015.04.049

Gulcin, I. (2020). Antioxidants and antioxidant methods: an updated overview. Arch. Toxicol., 94, 651–715. https://doi.org/10.1007/s00204-020-02689-3

Ivanišová, E., Vasková, D., Zagula, G., Grynshpan, D., Savitskaya, T. A., Kačaniová, M. (2020). Phytochemical profile and biological activity of selected kind of medicinal herbs. Potr. Slov. J. Food Sci., 14, 573–579. https://doi.org/10.5219/1370

Kalista, M. (2017). Underutilized medicinal species of *Crambe* L. from the flora of Ukraine. Agrobiodiv. Impr. Nut. Health Life Qual., 1, 216–220. http://dx.doi.org/10.15414/agrobiodiversity.2017.2585-8246.216-220

Li, X., van Loo, E. N., Gruber, J., Fan, J., Guan, R., Frenzen, M., Stymne, S., Zhu, L.-H. (2012). Development of ultra-high erucic acid oil in the industrial oil crop *Crambe abissinica*. Plant Biotechnol. J., 862–870. https://doi.org/10.1111/j.1467-7652.2012.00709.x

Lovatto, N. M., Goulart, F. R., Loureiro, B. B., Speroni, C. S., Bender, A. B. B., Giacomini, S. J., ..., Silva, L. P. (2017). Crambe (*Crambe abissinica*) and sunflower (*Helianthus annuus*) protein concentrates: production methods and nutritional properties for use in fish feed. An. Acad. Bras. Ciênc., 89, 2495–2504. https://dx.doi.org/10.1590/0001-3765201720140630

Marchyshyn, S., Skrynchuk, O., Buidniak, L., Mosula, L. (2020). Investigation of phenolic compounds of the leaves of *Crambe cordifolia* Steven and *Crambe koktebelica* (Junge) N. J. Pharm. Innov., 9(1), 14–17.

Moharram, H. A., Youssef, M. M. (2014). Methods for determining the antioxidant activity: a review. AJFS, 11(1), 31–42. https://doi.org/10.12816/0025348

Muzykiewicz, A., Zielonka-Brzezicka, J., Siemak, J. (2020). Antioxidant activity and polyphenol content in extracts from various parts of fresh and frozen mangosteen. Acta Sci. Pol. Technol. Aliment., 19(3), 261–270. https://doi.org/10.17306/J.AFS.2020.0788

Park, H.-W., Choi, K.-D., Shin, I.-Sh. (2013). Antimicrobial activity of isothiocyanates (ITCs) extracted from horseradish (*Armoracia rusticana*) root against oral microorganisms. Biocontrol Sci., 18(3), 163–168. https://doi.org/10.4265/bio.18.163

Podsędek, A. (2007). Natural antioxidants and antioxidant capacity of *Brassica* vegetables: A review. LWT – Food Sci. Technol., 40(1), 1–11. https://doi.org/10.1016/j.lwt.2005.07.023

Prasad, M. P. (2014). Antimicrobial potential of *Brassicaceae* family against clinical isolates. Int. J. Pure Appl. Biosci., 2(2), 158–162.

Prieto, P., Pineda, M., Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal. Biochem., 269, 337–241. https://doi.org/10.1006/abio.1999.4019

Prior, R. L., Wu, X., Schaich, K. (2005). Standartized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. J. Agricult. Food Chem., 53, 4290–4302. https://doi.org/10.1021/jf0502698

Pushkarova, O., Kalista, M., Kharhota, M., Rakhmetov, D., Kuchuk, M. (2016). *Crambe tataria* Sebeók seeds and plants grown *in vitro* and *in vivo* fatty acid composition comparison. Potrav. Slov. J. Food Sci., 10(1), 494–498. https://doi.org/10.5219/646

Rashid, M. A., Akhtar, M. N., Ashraf, A., Nazir, S., Ijaz, A., Omar, N. A., ..., Tareen, R. B. (2018). Chemical composition and antioxidant, antimicrobial and haemolitic activities of *Crambe cordifolia* roots. Farmacia, 66(1), 165–171.

Rasouli, H., Farzaei, M. H., Khodarahmi, R. (2017). Polyphenols and their benefits: A review. Int. J. Food Prop., 20(2), 1700–1741. https://doi.org/10.1080/10942912.2017.1354017

Rivelli, A. R., Caruso, M. C., Maria, S., Galgano, F. (2017). Vitamin C content in leaves and roots of horseradish (*Armoracia rusticana*): seasonal variation in fresh tissues and retention as affected by storage conditions. Emir. J. Food Agric., 29(10), 799–806. https://doi.org/10.9755/ejfa.2017.v29.i10.1294

Samarappuli, D., Zanetti, F., Berzuini, S., Berti, M. T. (2020). Crambe (*Crambe abissinica* Hochst): a non-food oilseed crop with great potential: a review. Agronomy, 10, 1380. https://doi.org/10.3390/agronomy10091380

Sánchéz-Moreno, C., Larrauri, A., Saura-Calixto, F. (1998). A procedure to measure the antioxidant efficiency of polyphenols. J. Sci. Food Agric., 76(2), 270–276. https://doi.org/10.1002/(SICI)1097-0010(199802)76:2%3C270::AID-JSFA945%3E3.0.CO;2-9

Sánchéz-Moreno, C. (2002). Review: methods used to evaluate the free radical scavenging activity in foods and biological systems. Food Sci. Technol. Int., 8(3), 121–137.

Shafii, Z. A., Basri, M., Malek, E. A., Ismail, M. (2017). Phytochemical and antioxidant properties of *Manilkara zapota* (L.) P roen fruit extracts and its formulations for cosmeceutical application. Asian J. Plant Sci., 7(3), 29–41.

Shankar, S., Segaran, G., Sundar, R. D. V., Settu, S., Sathiavelu, M. (2019). Brassicaceae – a classical review on

its pharmacological activities. Int. J. Pharm. Sci. Rev. Res., 55(1), 107–113.

Sikora, E., Cieślik, E., Topolska, K. (2008). The sources of natural antioxidants. Acta Sci. Pol., Technol. Aliment., 7(1), 5–17.

Silva da, M. F., Araújo, E. F., Silva da, L. J., Amaro, H. T. R., Santos Dias dos, L. A., Santos Dias dos, D. C. F. (2019). Tolerance of crambe (*Crambe abyssinica* Hochst) to salinity and water stress during seed germination and initial seedling growth. Sci. Agrotec., 43, e025418. http://dx.doi.org/10.1590/1413-7054201943025418

Singleton, V. L., Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagent. Am. J. Enol. Vitic., 16(3), 144–158.

Tomsone, L., Kruma, Z. (2017). Influence of gravest time on the phenolic content of horseradish leaves. Foodbalt, 45–50. https://doi.org/10.22616/foodbalt.2017.019

Tomsone, L., Galoburda, R., Kruma, Z., Dirrieu, K., Cinkmanis, I. (2020). Microencapsulation of horseradish (*Armoracia rusticana* L.). Foods, 9, 1332. https://doi.org/10.3390/foods9091332

Tusevski, O., Kostovska, A., Iloska, A., Trajkovska, L., Gazdovska Simic, S. (2014). Phenolic production and antioxidant properties of some Macedonian medicinal plants. Cent. Eur. J. Biol., 9(9), 888–990. https://doi.org/10.2478/s11535-014-0322-1

Vergun, O., Kačaniová, M., Rakhmetov, D., Shymanska, O., Bondarchuk, O., Brindza, J., Ivanišová, E. (2018). Antioxidant and antimicrobial activity of *Bunias orientalis* L. and *Scorzonera hispanica* L. ethanol extracts. Agrobiodiv. Impr. Nut. Health Life Qual., 2, 29–38. https://doi.org/10.15414/agrobiodiversity.2018.2585-8246.029-038

Vergun, O., Rakhmetov, D., Shymanska, O., Fishchenko, V. (2019a). Estimation of photosynthetic pigments in the leaves of *Crambe* spp. during vegetation. Agrobiodiv. Impr. Nut. Health Life Qual., 3, 373–381. https://doi.org/10.15414/agrobiodiversity.2019.2585-8246.373-381

Vergun, O., Shymanska, O., Rakhmetov, D., Fishchenko, V., Bondarchuk, O., Rakhmetova, S. (2019b). Accumulation of nutrients in the raw of *Crambe* species. Agrobiodiv. Impr. Nut. Health Life Qual., 3, 323–332. https://doi.org/10.15414/agrobiodiversity.2019.2585-8246.323-332

Vergun, O. M., Rakhmetov, D. B., Shymanska, O. V., Fishchenko, V. V., Ivanišová, E., Brindza, J. (2019c). Leaves extracts of selected crops as potential source of antioxidants. Introdukt. Rosl., [Plant Introduction], 84(1), 82–88. https://doi.org/10.5281/zenodo.3566626

Vos, A. C. de, Broekman, R., Groot, M. P., Rozema, J. (2010). Ecophysiological response of *Crambe maritima* to airborne and soil-borne salinity. Ann. Bot., 105, 925–937. https://doi.org/10.1093/aob/mcq072

Wang, Y. P., Tang, J. S., Chu, C. Q., Tian, J. (2000). A preliminary study on the introduction and cultivation of *Crambe abyssinica* in China, an oil plant for industrial uses. Ind. Crop. Prod., 12, 47–52.

Xu, D.-P., Li, Y., Meng, X., Zhou, T., Zhou, Y., Zheng, J., ..., Li, H.-B. (2017). Natural antioxidants in food and medicinal plants: extraction, assessment, and resources. Int. J. Mol. Sci., 18, 96. https://doi.org/10.3390/ijms18010096

Zubr, J. (2010). Carbohydrates, vitamins and minerals of Camellina sativa seed. Nutr. Food Sci., 40(5), 523–531.