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ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF FRUIT EXTRACTS FROM DIFFERENT FRESH CHILI PEPPERS

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ABSTRACT

Background. The aim of the current study was to assess the antimicrobial and antioxidant potential of ethanol extracts obtained from the fruit of five species of fresh chili pepper, *Capsicum* (*C.*) *baccatum* L. (Aji Amarillo), *C. chinense* (Fidalgo Roxa), *C. annuum* (Cherry Chocolate), *C. pubescens* (Rocoto Orange) and *C. frutescens* (Peruvian Purple).

Materials and methods. To obtain the ethanol extracts, accelerated solvent extraction (ASE) was applied. DPPH assay was used to determine the antioxidant activity of the extract samples. The disc diffusion method was used to measure antimicrobial activity against nine investigated microorganism species.

Results. The tested extract samples exhibited DPPH radical scavenging activities ranging from 0.24 ±0.01 (Peruvian Purple) to 0.72 ±0.02 (Aji Amarillo) mg TEAC·g^{-1} dw. The differences between all the varieties were statistically significant ($P < 0.05$; except for the Cherry Chocolate and Rocoto Orange), and the potential of antioxidant capacity increased in the following manner: Peruvian Purple < Fidalgo Roxa < Rocoto Orange < Cherry Chocolate < Aji Amarillo. The results from the antimicrobial evaluation showed that the *Capsicum* extracts had no uniform inhibition activity against tested gram-negative, gram-positive bacteria, and yeast. Specifically, Aji Amarillo fruit extract revealed the strongest antimicrobial activity against *S. pneumoniae* (6.33 ±0.58 mm), followed by Cherry Chocolate against *S. pneumoniae* (5.33 ±0.58 mm), Rocoto Orange against *S. enterica* (5.27 ±0.58 mm), Fidalgo Roxa against *C. albicans* (4.67 ±0.58 mm), and Peruvian Purple against *S. pneumoniae* (4.57 ±0.58 mm).

Conclusion. Considering these results, *Capsicum* spp. can be used as a source of novel antioxidant and antimicrobial compounds.

Keywords: fresh chili, extraction, DPPH assay, antimicrobial testing

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INTRODUCTION

Chili peppers belong to the genus *Capsicum* (*C.*) within the Solanaceae family. The genus consists of more than 30 species, of which *C. annuum*, *C. baccatum*, *C. chinense*, *C. frutescens*, and *C. pubescens* represent the major domesticated and economically important ones (Jaiswal et al., 2021). From these, cultivars belonging to *C. annuum* are the most commercially cultivated, but *C. chinense* and *C. frutescens* cultivars are currently also grown on large scale areas (Peñuela et al., 2020).

In general, different varieties of *Capsicum* fruits are considered to be an important part of the human diet worldwide. They may be consumed fresh, dried, fermented, or cooked, and their sensory attributes including color, flavor and pungency are highly valued (Pino et al., 2007). Apart from their use as food, *Capsicum* fruit are also applied in traditional medicine, especially in the treatment of gastrointestinal disorders (Xiang et al., 2021), respiratory diseases (Dumitrache et al., 2021), chronic diseases (Shi et al., 2019), and depression (Xia et al., 2021). Moreover, chili extracts are also used as pesticides in horticulture, as biochemical pest repellents (Chinn et al., 2011), and as pigments in the cosmetics industry (Lee et al., 2016).

A wide range of bioactive compounds, in particular secondary metabolites, are found and isolated from these plants (Sricharoen et al., 2017). Among them, capsaicinoids (especially capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homodihydrocapsaicin and homocapsaicin) (Yap et al., 2021), which are responsible for pungency (Naves et al., 2019), are recognized as the main phytochemicals of *Capsicum* fruits. Other bioactive substances also present in high amounts in chili peppers include carotenoids (Morales-Soriano et al., 2019), flavonoids and vitamins (mainly vitamin C, whose content is up to six times higher than in oranges) (Bae et al., 2012). Thanks to their presence, chili peppers exhibit strong antioxidant properties leading to protection against harmful reactive oxygen species and free radicals (Bhattacharya et al., 2010). Moreover, as a source of natural antioxidants, they possess many other biological functions including analgesic, anti-inflammatory (Baenas et al., 2019) and anticancer properties (Cao et al., 2015). In addition, recent studies have shown that the genus *Capsicum* also comprises diverse antimicrobial and antifungal compounds (Tajkarimi et al., 2010). Due to the fact that consumers currently try to avoid chemical preservatives, chili peppers seem to be a good natural alternative to inactivate or inhibit the growth of spoilage and pathogenic microorganisms in food (Omolo et al., 2014).

However, it is well-known that chemical composition and amounts of bioactive substances in *Capsicum* fruits are strongly affected by various factors such as variety (Vázquez-Espinosa et al., 2020), genetic factors (Tripodi et al., 2018), fruit ripening (Barbero et al., 2014) and maturity stages (Uarrota et al., 2021), environmental conditions (e.g., soil) (Montalvo et al., 2021), geographical locations (temperature, irrigations, altitudes, etc.) (Meckelmann et al., 2015), and processing methods (Topuz et al., 2011). Thus, our research aimed to investigate the antioxidant and antimicrobial activities of the five selected varieties of chili pepper growing in our experimental field in order to create a comprehensive view of such effects under precisely defined conditions. Keeping this in view, the study herein analyzed and compared the antioxidant activities and antimicrobial potentials of these different fruit extracts to choose the best one related to the activities investigated, which will be subsequently employed in further *in vitro* analyses on a cellular model system, as well as in the development of bakery products with an extended shelf-life. Such findings will be mainly useful in the alternative therapy of various human diseases and in the food industry.

MATERIALS AND METHODS

Plant material

Five different varieties of chili peppers: Aji Amarillo (*C. baccatum* L.), Fidalgo Roxa (*C. chinense*), Cherry Chocolate (*C. annuum*), Rocoto Orange (*C. pubescens*), and Peruvian Purple (*C. frutescens*) were used for the analyses. All samples were cultivated in the experimental plots of the Botanical Garden of the Slovak University of Agriculture in Nitra. The chili peppers were collected at full maturity and stored refrigerated (4°C) in resealable polyethylene bags until further analysis. Figure 1 shows the chili pepper varieties used in this study.

Fig. 1. Chili pepper varieties used for analyses: 1 – Aji Amarillo, 2 – Fidalgo Roxa, 3 – Cherry Chocolate, 4 – Rocoto Orange, 5 – Peruvian Purple

Chemicals

All chemicals were analytical grade and were purchased from Merck (Germany) and Sigma Aldrich (USA).

Sample extraction for further analysis

The extraction procedure was performed on a Dionex ASE 200 (Dionex Corp., Sunnyvale, CA) system. Fresh sliced peppers (1 g) were placed in between two layers of diatomaceous earth in a 22 mL Dionex (ASE 200) stainless-steel cell. The cells were equipped with a stainless-steel frit and a fiberglass filter (Dionex Corp.). The extraction cycle was automated. In the first step, the cell containing the sample was prefilled with the extraction solvent (80% ethanol), pressurised (1500 psi), and then heated for 5 min, followed by a static period of 5 min. In effect, the sample was extracted with 80% ethanol (60°C) during this 5 min period. Then, the cell was rinsed with fresh extraction solvent (60% of the extraction cell volume) and purged with a flow of nitrogen (150 psi for 90 s). Extracts (34 mL) were collected into 60 mL glass vials. These extracts were stored at 20°C in darkness until analysis. Then, the extract was filtered through 0.45 µm PTFE filters (Millipore, USA) before antioxidant activity and antimicrobial analyses.

Free radical scavenging activity

The free radical scavenging activity of the samples was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical according to the procedures described by Kačániová et al. (2020). The sample (0.4 mL) was reacted with 3.6 mL of DPPH solution (0.025 g DPPH in 100 mL ethanol). The absorbance of the samples was determined using a spectrophotometer Jenway 6405 UV/Vis (Keison, England) at 515 nm. The radical scavenging activity of the samples was expressed as Trolox equivalent antioxidant capacity (TEAC) in grams per kilogram of dry weight (dw). All analyses were performed in triplicates.

Assessment of antimicrobial activity with disc diffusion method against selected microorganism species

Three gram-negative bacteria (G^-): *Pseudomonas* (*P.*) *aeruginosa* CCM 1959, *Salmonella* (*S.*) *enterica* subsp. *enterica* CCM 3807 and *Yersinia* (*Y.*) *enterocolitica* CCM 5671, three gram-positive bacteria (G^+): *Enterococcus* (*E.*) *faecalis* CCM 4224, *Staphylococcus* (*S.*) *aureus* subsp. *aureus* CCM 4223 and *Streptococcus* (*S.*) *pneumoniae* CCM 4501, and three yeasts: *Candida* (*C.*) *albicans* CCM 8186, *C. krusei* CCM 8271 and *C. tropicalis* CCM 8223 were used to determine the antimicrobial activity of the samples. The microorganisms were obtained from the Czech Collection of Microorganisms (Brno, Czech Republic). Three antibiotics, Cefoxitin, Gentamicin and Fluconazole were used as controls for gram-negative, gram-positive bacteria and yeasts, respectively.

The bacteria and yeast cultures were incubated in Mueller Hinton broth (MHB, Oxoid, Basingstoke, UK) at 37°C, and in Sabouraud broth (Oxoid, Basingstoke, UK) at 25°C overnight, respectively, and subsequently they were seeded on their broths for 18 h to optimize their growth.

After that, bacterial and yeast suspensions were prepared with diluted distilled water and adjusted to a concentration of 0.5 McF. Bacterial and yeast suspensions were then streaked over the surface of Mueller Hinton agar and Sabouraud agar, respectively, using a sterile cotton swab to ensure uniform inoculation. Then, disks impregnated with 10 µL of selected chili fruit ethanol extracts were gently placed on the surface of the inoculated agar. Antibiotics were used as positive controls, and disks impregnated with distilled water served as negative controls. After 24 h incubation at 37°C (bacterial suspensions) and 25°C (yeast suspensions), the inhibition zone diameters were measured. The experiments were carried out in triplicates.

Statistical analysis

All obtained data was statistically evaluated using the GraphPad Prism 8.0.1 (GraphPad Software Incorporated, San Diego, California, USA). One-way analysis of variance (ANOVA) followed by the Tukey test were used for establishing statistical significance ($P<0.05$) in the investigated parameters between different *Capsicum* varieties.

RESULTS AND DISCUSSION

Antioxidant activity of chili extracts

Antioxidant activity is an important parameter in the assessment of the health function of foods. There are many methods used for its determination which differ in their reaction mechanisms, applications and complexity (Prior et al., 2005). The present study investigated the ability of five chili pepper extracts to sequester free radicals using DPPH assay. The method is widely used in studies of *Capsicum* spp. (Gomes et al., 2019). The values for antioxidant activity were found to range from 0.24 ±0.01 (Peruvian Purple) to 0.72 ±0.02 (Aji Amarillo) mg TEAC·g^{-1} dw (Table 1). In effect, the potential of the antioxidant capacity increased in the following manner: Peruvian Purple < Fidalgo Roxa < Rocoto Orange < Cherry Chocolate < Aji Amarillo. Furthermore, between individual varieties, significant differences ($P < 0.05$) in the investigated parameter were observed (except for between Rocoto Orange and Cherry Chocolate). In accordance with our study, differences between chili pepper varieties, as well as accessions were also noted by other authors (Gomes et al., 2019; Guil-Guerrero et al., 2006).

The antioxidant capacity of chili pepper extracts is directly correlated with their phytochemical and phytonutrient compositions, which can be attributed to their hydrogen-donating ability (Shimada et al., 1992). In general, the variations in the DPPH radical inhibition of our fruit extracts may result from the differences in their potency, diversity and complexity of contained antioxidants (e.g., phenolic compounds, carotenoids, ascorbic acid) or the concentrations of the reducing compounds. Moreover, antioxidant activity of chili extracts is also influenced by the degree of fruit maturity (Fredes et al., 2012). Since there is enormous divergence in geographic and agroecological conditions, it is very difficult to compare our results with the previous findings.

Antimicrobial activity of chili extracts

Generally, microorganisms are responsible for food spoilage, as well as several different foodborne illnesses. To inhibit their growth, various natural alternatives

Table 1. Antioxidant activity of different varieties of chili pepper

Analysis	Varieties				
	Aji Amarillo	Fidalgo Roxa	Cherry Chocolate	Rocoto Orange	Peruvian Purple
Antioxidant activity, mg TEAC·g^{-1} dw	0.72 ±0.02^{a}	0.35 ±0.03^{b}	0.57 ±0.05^{c}	0.56 ±0.04^{c}	0.24 ±0.01^{d}

Means ±standard deviation. Values followed by superscript within the same letters are significantly different ($P < 0.05$).

Table 2. Antimicrobial activity of fruit extracts of different chili varieties, inhibition zone in mm

Varieties	Gram-negative bacteria			Gram-positive bacteria			Yeasts		
	PA	SE	YE	EF	SA	SP	CA	CK	CT
	inhibition zone, mm								
Aji Amarillo	3.33 ±0.58^{a}	3.33 ±0.58^{a}	3.00 ±1.00^{ac}	3.67 ±0.58^{a}	4.67 ±0.58^{a}	6.33 ±0.58^{b}	3.33 ±0.58^{ab}	3.33 ±0.58^{ab}	5.33 ±0.58^{a}
Fidalgo Roxa	1.00 ±0.00^{bc}	2.33 ±0.58^{a}	2.33 ±0.58^{a}	1.33 ±0.58^{b}	2.67 ±0.58^{b}	4.33 ±0.58^{b}	4.67 ±0.58^{a}	4.33 ±0.58^{a}	1.67 ±0.58^{b}
Cherry Chocolate	2.67 ±0.58^{ac}	3.67 ±0.58^{a}	4.33 ±0.58^{c}	4.33 ±0.58^{a}	2.33 ±0.58^{b}	5.33 ±0.58^{b}	2.33 ±0.58^{b}	3.33 ±0.58^{ab}	4.67 ±0.58^{a}
Rocoto Orange	4.33 ±1.15^{a}	5.27 ±0.58^{b}	2.67 ±0.58^{ac}	4.00 ±1.00^{a}	2.67 ±0.58^{b}	0.00 ±0.00^{a}	4.33 ±0.58^{a}	2.67 ±0.58^{b}	4.00 ±1.00^{a}
Peruvian Purple	3.67 ±0.58^{a}	3.33 ±0.58^{a}	1.00 ±0.00^{b}	1.67 ±0.58^{b}	2.00 ±1.00^{b}	4.57 ±0.58^{b}	2.33 ±0.58^{b}	2.67 ±0.58^{b}	1.67 ±0.58^{bc}

Means ±standard deviation. Values followed by superscript within the same column are significantly different ($P < 0.05$). *Pseudomonas aeruginosa* – PA, *Salmonella enterica* – SE, *Yersinia enterocolitica* – YE, *Enterococcus faecalis* – EF, *Staphylococcus aureus* – SA, *Streptococcus pneumoniae* – SP, *Candida albicans* – CA, *Candida krusei* – CK, *Candida tropicalis* – CK.

are still being sought. *Capsicum* spp. was reported to possess such an ability (Das et al., 2018).

In our study, a disk diffusion method was used to assess the antimicrobial activity of selected chili extracts against *S. enterica* subsp. *enterica*, *P. aeruginosa*, *Y. enterocolitica*, *S. aureus* subsp. *aureus*, *E. faecalis*, *S. pneumoniae*, *C. albicans*, *C. krusei*, and *C. tropicalis*. Our results (Table 2) showed that fruit extracts had some inhibitory effects against the microorganisms tested; however, the antimicrobial activities were not highly effective. In Peruvian Purple and Aji Amarillo, the weakest antimicrobial activities were found against *Y. enterocolitica* (the zone of inhibition: 1.00 mm and 3.00 mm, respectively), whilst the greatest ones were reported against *S. pneumoniae* (4.57 mm and 6.33 mm, respectively). The inhibition zone of Fidalgo Roxa ranged from 1.00 mm (*P. aeruginosa*) to 4.33 mm (*S. pneumoniae*, *C. krusei*). Against *S. pneumoniae*, the strongest inhibitory action was also observed in Cherry Chocolate (5.33 mm). This fruit extract exhibited the weakest antimicrobial effect against *S. aureus* subsp. *aureus* and *C. albicans* (2.33 mm in both species). By contrast, Rocoto Orange was not effective against *S. pneumoniae*; however, it displayed the maximum zone inhibition (5.27 mm) against *S. enterica* subsp. *enterica*.

Moreover, data from statistical analysis revealed that the strongest antibacterial effect of Rocoto Orange against G^- bacteria was even statistically significant ($P < 0.05$) as compared to Fidalgo Roxa (*P. aeruginosa*) and Aji Amarillo, Fidalgo Roxa, Cherry Chocolate and Peruvian Purple (*S. enterica*). In addition, *Y. enterocolitica* was the most sensitive against Cherry Chocolate, and the antimicrobial activity of the extract considerably ($P < 0.05$) differed from those of Fidalgo Roxa and Peruvian Purple. Regarding G^+ bacteria, the maximum inhibition zones of Aji Amarillo against *S. aureus* and *S. pneumoniae* were found to be markedly ($P < 0.05$) different from Fidalgo Roxa, Cherry Chocolate, Rocoto Orange and Peruvian Purple; and from Rocoto Orange, respectively. In *E. faecalis*, significant differences were observed between the strongest antibacterial activity identified in Cherry Chocolate and that of Fidalgo Roxa and Peruvian Purple. Interestingly, the fruit extract from Fidalgo Roxa was shown to be the most effective inhibitor against the growth of *C. albicans* and *C. krusei*, and its antifungal activity considerably differed from those of Cherry Chocolate and Peruvian Purple, and Rocoto Orange and Peruvian Purple, respectively. Finally, against *C. tropicalis*, the maximum antifungal activity of Aji Amarillo was reported which was significantly different in comparison

with Fidalgo Roxa and Peruvian purple. Based on the results, it can be seen that Rocoto Orange is the most effective mainly against growth of *P. aeruginosa* and *S. enterica*, Cherry Chocolate against *Y. enterocolitica* and *E. faecalis*, Aji Amarillo against *S. aureus*, *S. pneumoniae* and *C. tropicalis*, and Fidalgo Roxa against *C. albicans* and *C. krusei*.

Differences in the antimicrobial activities of the fruit extracts tested are in general attributed to their different chemical compositions responsible for such activities. It was found that the antimicrobial activity of *Capsicum* spp. is at least partially associated with their two pungent compounds – capsaicin and dihydrocapsaicin (Segura-Campos et al., 2016). The investigation of the antimicrobial mechanism of capsaicin revealed that the compound exerts its toxic effect on the growth of yeast cells via induced osmotic stress and damage in the membrane structure (Kurita et al., 2002). Moreover, a study by Omolo et al. (2018) implies that there might also be other *Capsicum* constituents or peptides with antimicrobial properties since the cultivar with very little capsaicin content also showed some antimicrobial effects, and vice versa, chili peppers with the highest capsaicin concentrations did not always correlate with the greatest antimicrobial activity.

Similar to our study, the antibacterial effects of extract from *Capsicum* spp. against *S. aureus* and/or *P. aeruginosa* have been investigated in research conducted by other authors (Das et al., 2018; Koffi-Nevry et al., 2012). However, due to dissimilarities in the varieties tested, the extraction methods used, and other experimental conditions, it is hard to compare the studies with our findings and to draw some hypotheses.

In summary, the antioxidant and antimicrobial activity of chili pepper varieties are influenced by the presence of individual biologically active substances, as was previously described (Hassimotto et al., 2005). Therefore, the assessment of the content of beneficial components in such studies should not be omitted and will be involved in the methodology of our future investigations on chili peppers.

CONCLUSION

In the current study, the diverse antioxidant capacity and antimicrobial activity of fruit extracts from five different varieties of *Capsicum* spp. growing in experimental conditions were demonstrated. The analysed samples did not display uniform DPPH radical scavenging activity. Indeed, its values ranged from 0.24 ±0.01 (Peruvian Purple) to 0.72 ±0.02 (Aji Amarillo) mg TEAC·g^{-1} dw. The *Capsicum* spp. fruit extracts also had some inhibitory effects against the microorganisms tested, which were, however, not highly effective. From them, Aji Amarillo fruit extract showed the highest antimicrobial activity against *S. pneumoniae* (6.33 ±0.58 mm). Regardless, our results allow for the conclusion that Aji Amarillo seems to be the most effective fruit extract in the context of antioxidant and antimicrobial properties of all those investigated. Thus, the extract will be further employed in our additional parallel experiments in which its antioxidant activity will be assessed using a cellular model system exposed to oxidative stress, and its antimicrobial potential will be used in the development of bakery products with prolonged storage terms. Such research can be valuable for alternative therapies of various diseases and the food industry.

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