Asian Journal of Plant Sciences

ISSN 1682-3974

Asian Journal of Plant Sciences 11 (3): 160-162, 2012
ISSN 1682-3974 / DOI: 10.3923/ajps.2012.160.162

Antimycotic and Antifungal Activities of Amaranth and Buckwheat Extracts

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Abstract: Nowadays, there has been an increased interest in various natural plants as a source of biological active compounds including polyphenols. Important sources of these phytochemicals are less known pseudocereals such as amaranth and buckwheat. The objective of this study was determination of antimycotic and antifungal effects of mentioned pseudocereals. Antimicrobial screening against selected three yeast strains and four strains of fungi was carried out on DMSO buckwheat and amaranth extracts prepared by alkali hydrolyses. Results suggested variation in the antimicrobial properties of tested extracts. The studied extracts suppressed growth of *Aspergillus flavus, Fusarium culmorum* and *Alternaria alternata*. No inhibitory effect was observed against *Rhizopus oryzae* and tested yeast.

Key words: Pseudocereals, polyphenolics, flavonoids, antifungal effect, antimycotic properties

INTRODUCTION

In the last years, pseudocereals including amaranth and buckwheat have gained broad use not only in the common diet but also in diet of people with celiac disease or allergies to typical cereals (Pasko *et al*., 2009). They are eligible to grow in regions by low rainfall or drought, high temperature and low soil fertility (Ragaee *et al*., 2006). Their seeds have high nutritional and functional values. Pseudocereals contain essential amino acids, such as lysine, fats, dietary fibre, minerals and bioactive compounds with antioxidant potential (Jiang *et al*., 2007; Vogelmann *et al*., 2009; Pasko *et al*., 2009). Amaranth oil is also known with significant levels of squalene (De La Rosa *et al*., 2009). One of the compounds with antioxidant potential, occurring in pseudocereals, is polyphenolics. Buckwheat grains have been well known by significant content of rutin, quercetin, kaempferol-3-rutinoside and a trace quantity of a flavonol triglycoside (Van Hung and Morita, 2008). Polyphenolic compounds have been shown to exhibit many biological activities including antioxidative, antimicrobial, antimutagenic, anticancerogenic and cardioprotective effects (Sensoy *et al*., 2006; Podsedek, 2007; Naili *et al*., 2010).

Many pathogenic microorganisms, including *Salmonella* spp., *Escherichia coli*, *Fusarium* spp., *Aspergillus* spp. and *Rhizopus* spp., are considered as the causal agents of food borne diseases or food spoilage which are one of the most important problems in the food industry (Kil *et al*., 2009). In recent years, the interest about herbal extracts has still increase due to their potential as a source of natural ingredients with antioxidant, antibacterial or antifungal properties (Hussain *et al*., 2008; Naili *et al*., 2010).

Therefore, the aim of this study was to evaluate the antimycotic and antifungal activity of DMSO buckwheat and amaranth extracts.

MATERIALS AND METHODS

Microbial strains and standards: The yeast strains used in this study, *Candida albicans* CCM 8188, *Saccharomyces cerevisiae* CCM 8191and fungi *Aspergillus flavus* CCM 8363, *Fusarium culmorum* CCM F-163, *Alternaria alternate* CCM 8326 and *Rhizopus oryzae* CCM 8076 were obtained from the Collection of Microorganisms, Masaryk University, Brno (Czech Republic). *Candida maltose* YP1 was isolated from fruit yoghurt.

Plant materials: The amaranth seeds, variety PI 604671 and buckwheat seeds, variety Spaeinska 1, were acquired from the gene bank of the Research Institute of Plant Production, Piestany (Slovakia).

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Preparation of extracts: The tested extracts were prepared by method which served on isolation of mainly polyphenolic compounds (Krygier *et al*., 1982). Defatted flours (Kim *et al*., 2006) were hydrolysed with 2 M NaOH for 4 h at 50°C. Then, mixture was cooled, acidified with 6 M HCl to pH 2 and free polyphenolics were extracted with ethyl acetate at ratio 1:1 (v/v). Ethyl acetate extracts were evaporated to dryness in a rotary evaporator at temperature lower than 40°C and the residue was dissolved in DMSO for antioxidant and antimicrobial activity determination or 96% ethanol for phenolic and flavonoid content. Prepared pseudocereal extracts were kept at 5°C.

Antimicrobial susceptibility testing: The antimicrobial activity of DMSO pseudocereal extracts was determined by classical disk diffusion assay (Betina *et al*., 1987). Briefly, 20 μL extract in four tested concentration (6.25-50 mg mL^{-1}) was applied on filter discs placed malt agar which had previously been inoculated with the tested microorganisms. Disc with solvent was used as a negative control. The plates were incubated at 37°C for 24 h for yeast and at 25°C for 1 week for fungal strains. Antimicrobial activity of amaranth and buckwheat extracts was evaluated by measuring the diameter of the inhibition zones.

RESULTS

The antimicrobial effect of pseudocereal extracts was tested against three species of yeast and four species of fungi. Screening of the antimicrobial effects of buckwheat and amaranth extracts revealed different intense activity against tested microorganisms (Table 1).

Amaranth extract showed the strongest activity against *Aspergillus flavus*. The inhibition zones were noticed in all applied concentrations. Extract was also effective against *Fusarium culmorum* but inhibition of this fungal strain was not observed in the lowest used concentration. Buckwheat extract exhibited slight antifungal activity against mentioned two pathogens, the inhibition zones were reported in concentration from 12.5 mg mL^{-1}. Results of current study also indicated slight inhibitory effect against *Alternaria alternata* but the lowest concentrations (6.25 and 12.5 mg mL^{-1}) were not efficient to inhibit growth of this fungi. None of extracts showed antifungal activity against *Rhizopus oryzae*.

Table 1: Inhibition effect of amaranth and buckwheat extracts on fungi

		Inhibition zones (cm)			
Extract	Dose (mg mL^{-1})	*Rhizopus oryzae*	*Aspergillus flavus*	*Fusarium culmorum*	*Alternaria alternata*
Amaranth	0	NI	NI	NI	NI
	6.25	NI	0.8	NI	NI
	12.50	NI	1.0	0.9	NI
	25.00	NI	1.0	0.8	0.9
	50.00	NI	1.2	1.0	1.0
Buckwheat	0	NI	NI	NI	NI
	6.25	NI	NI	NI	NI
	12.50	NI	0.7	0.8	NI
	25.00	NI	1.0	0.9	1.0
	50.00	NI	1.0	1.1	1.0

NI: No inhibition

The antimycotic activity of pseudocereal extracts was tested on three yeast strains *Candida albicans*, *Candida maltosa* and *Saccharomyces cerevisiae*. Our results suggested that amaranth and buckwheat extracts did not inhibit growth of used yeast in tested concentrations.

DISCUSSION

In recent years, polyphenols gained much attention due to its biological properties (John *et al*., 2006). Our previous results (Mosovska *et al*., 2010) indicated that studied extracts are an important source of polyphenols including flavonoids. One of the potential biological effects of these compounds is their antimicrobial ability.

Aflatoxins are known to be potent hepatocarcinogens and receive attention as a potential hazard to human and animal health (Mostafa *et al*., 2011). *Fusarium* species belong to another mycromycetes with frequent occurrence and a high potential of mycotoxins production. They play an important role as plant pathogens (Schollenberger *et al*., 2005). In the present paper, amaranth and buckwheat extract displayed a potential antifungal activity against these fungi. Our results also suggested that higher tested extract concentrations inhibited strain of fungus *Alternaria alternata*. On the contrary, no inhibitory zones were detected in the presence of *Rhyzopus oryzae* and the tested yeasts. These variations in the antimicrobial activity of the analyzed extracts may be due to the differences in their active components content. Although, pseudocereal extracts have a high content of polyphenols, at this moment we cannot say which polyphenols are the effective compounds responsible for the mentioned properties. According to Kil *et al*. (2009) antimicrobial effect in plant extracts depends not only on the presence of polyphenolic compounds but also on the presence of other secondary metabolites. The other bioactive compounds, alone or in combination with polyphenols, might be responsible for the antimicrobial properties (Puupponen-Pimia *et al*., 2001).

In conclusion, present results indicated that the extracts from buckwheat and amaranth are a source of polyphenolic compounds including flavonoids with the antibacterial and antifungal activity, respectively. This study suggested that buckwheat and amaranth could be used as natural ingredients with their antimicrobial effects in food industry.

ACKNOWLEDGMENT

This study was supported by The Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic for the Structural Funds of EU, OP RandD of ERDF in the frame of the Project, „Evaluation of natural substances and their selection for prevention and treatment of lifestyle diseases“(ITMS 26240220040), VEGA 1/0094/10 and by the agency APVV in the frame of project Nr. 0310-06 and VMSP-II-0024-09. The authors thank to VURV Pieš_any for providing the amaranth and buckwheat seeds.

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