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Antimicrobial activity of *Amaranthus* spp. extracts against some mycotoxigenic fungi

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Abstract

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Plants, their parts and products have been used to treat diseases and pathogens. The aim of the present study was to test different extracts from three species of genus *Amaranthus* L. – *A. deflexus* L., *A. retroflexus* L. and *A. hybridus* L. for antifungal activities. The plant extracts (methanol and ethanol) from ground and underground plant parts were tested for antimicrobial activity by agar well diffusion method. Five fungal strains (*Penicillium verrucosum* var. *verrucosum* NBIMCC 2003 NRRL F-143, *Penicillium expansum, Fusarium graminearum* NBIMCC 2294 IMI 155426, *Aspergillus ochraceus* NBIMCC 2002 IM-BAS, *Aspergillus niger*) were used. Antimicrobial activity was evaluated by measuring zones of inhibition of microbial growth surrounding plant extracts in the wells. The most effective extracts, which showed activity against all tested strains of microorganisms, were *A.deflexus* and *A.hybridus* ethanol flower extract, *A.retroflexus* ethanol root extract and *A. retroflexus* methanol leaves and stem extract.

Keywords: antifungal; *Amaranthus deflexus*; *A.retroflexus; A.hybridus;* extracts

Introduction

The plants have an important role in our life and are one of the most productive ecosystems in the world. They provide a wide range of benefits to the population of the earth – nutritional, healing and purifying. People have used plants in the past for their curative and antimicrobial properties (Jani et al., 2012). Antibiotics, as microbial and plant products, are increasingly applied today to treat diverse infections. Antibiotics are harmful when utilized frequently and the patients even become resistant to them (Sevindik et al., 2018). The healthy intestinal micro flora is very important to the immune system and the usage of antibiotics damage and disrupts the work of beneficial microorganisms in the intestine. Various bacterial pathogens are treated with antibiotics and this lead to stress of the organism and environmental pollution (Ahmed, 2016). A good alternative to this are the plants and their extracts. Since ancient times, plants, their parts and products have been used to treat both human and animal diseases (Dadasoglu et al., 2016; Yilar et al., 2018). In connection with the intensive development of different animal production, there is an increasing need for more medicines to maintain animal health (Jani et al., 2012). A very important aspect of plant extracts usage is their antifungal activity during storage of different type of food and animal feeds. The fungal attacks during storage lead to large losses and decreased food and feed quality (Kaynar, 2017). The usage of fungicides can reduce the fungi growth in feed, but the pesticides have side effects which are dangerous to the health and life of the animals (Conte et al., 2018). Some pathogenic

microorganisms, such as *Salmonella* spp., *Escherichia coli, Fusarium* spp., *Aspergillus* spp. and *Rhizopus* spp., are cause for infection or food spoilage and this is very serious problems in the food industry (Kil et al., 2009). The herbal extracts have great potential as a source of natural compounds with antioxidant, antibacterial and antifungal properties and in recent years the interest for their usage is increasing (Hussain et al., 2008; Naili et al., 2010). Species from genus *Amaranthus* contain compounds with great nutritional value such as amino acids lysine, arginine, histidine, cystine, phenylalanine, leucine, isoleucine, valine, threonine, methionine, tyrosine etc. (Reyad-ul-Ferdous et al., 2015). Some authors reported a certain antimicrobial activity of the *Amaranthus* species (Maiyo et al, 2010; Tharun et al, 2012; Jin et al, 2013; Zhanh et al, 2013;). In view of this the aim of the present study was to test different extracts from three species of genus *Amaranthus* L. – *A. deflexus* L., *A.retroflexus* L. and *A.hybridus* L. for antifungal activities.

Material and Methods

Plant material

Materials of this three *Amaranthus* sp. were collected from: *A. deflexus* – from Stara Zagora town (42°25.145″N, 025°37.220″E) Thracian Plane, *A. retroflexus* – from Shipka town (42°42.832″N, 025°19.844″E) Tundzha Hilly Plain, *A.hybridus* – from the village Zvanichevo – Stara Zagora region (42°11.380″ N,024°15.000″, E) Thracian Plane. The plant species were determined through Flora of the Republic of Bulgaria (Jordanov et al., 1966). The harvested plants were herbarized and separated into roots, leaves, stems and flowers.

Extract preparation

The plant extracts were prepared by soaking of 5 g of dry, macerated plant material in

20 mL 100% ethanol for 24 h. Then the material was filtered through a 20 μm mesh before being placed in a rotary evaporator to reduce the volume to 5 mL. Each test compound was diluted by 1:10. The same procedure was used to prepare methanol plant extracts.

Tested microorganisms

Three of the fungi strains were provided by National Bank for Industrial Microorganisms and Cell Cultures in Bulgaria - *Penicillium verrucosum var. verrucosum* NBIMCC 2003 NRRL F-143, *Fusarium graminearum* NBIMCC 2294 IMI 155426 and *Aspergillus ochraceus* NBIMCC 2002 IM-BAS. *Penicillium expansum* and *Aspergillus niger* were isolated from plant diseases. The medium on which the five mould strains were inoculated was Potato-glucose agar (glucose 20.0 g, potatoes 200.0 g, yeast extract 2.0 g, agar 20.0 g, pH 5.6).

Antimicrobial activity

The methanol and ethanol plant extracts were screened for antifungal activity by agar well diffusion method (Perez et al., 1990). The 72 hours old fungal cultures were grown on Potato glucose agar. 20 mL of Potato glucose agar was poured in every *Petri* dish. After solidification, 0.1 mL inoculum of the fungal strains (1-2 x 10^4 CFU/mL) was introduced on the surface of the agar plate and the wells were made by using sterile cork borer of size 6.0 mm. 0.1 mL of methanol and ethanol plant extracts were introduced in the wells. An incubation period of 3-7 days at 23°C was maintained for observation of antifungal activity of plant extracts. Methanol and ethanol were used as negative solvent control.

The antimicrobial activity was evaluated by measuring the zones of inhibition of the microbial growth surrounding the plant extracts in the wells. The zones of inhibition were measured in millimeters. Antimicrobial activity was assumed in the presence of a growth inhibition zone ≥ 7 mm (Mohammadi-Sichani et al., 2012). The tests were performed in triplicate to determine the reproducibility of the results. The complete antimicrobial analysis was carried out under strict aseptic conditions.

Data analyses

Data analyses were conducted by using descriptive statistics, one-way Analysis of Variance ANOVA (MS Office, 2010).

Results and Discussion

The antibacterial activity of the three *Amaranthus* plant extracts is shown in Table 1. The results presented are with inhibition zones greater than the negative controls.

In this study *A. deflexus* ethanol flower extract is active against all tested fungi strains and the differences were statistically significant ($P < 0.05$). *A. deflexus* methanol leaf and stem extract have a high effect against *F. graminearum* (15.0±0.03) with statistically significant differences ($P < 0.05$).

A. retroflexus ethanol root, leaf and stem extracts are active against all tested fungi strains and the differences were statistically significant ($P < 0.05$). *A. retroflexus* ethanol flower extract also have a good inhibition affect against four tested mycotoxigenic strains, without *P. expansum.*

A. hybridus ethanol flower extract showed the highest activity against all tested fungi strains as the differences were

Table 1. Antifungal activity of *Amaranthus deflexus*, *Amaranthus retroflexus* and *Amaranthus hybridus* extracted with ethanol and methanol (mean ± SE).

Plant extract	*A. ochraceus*	*A. niger*	*F. graminearum*	*P. verrucosum*	*P. expansum*
A.deflexus ethanol root	–	10.9±0.06*	–	8.9±0.08	–
A.deflexus ethanol leaf and stem	–	9.7±0.3*	–	9.9±0.3	10.0±1.0*
A.deflexus ethanol flower	12.0±2.3*	9.7±0.02*	10.0±1.0*	10.9±0.2*	10.2±0.3*
A.deflexus methanol root	–	12.9±0.3*	–	9.9±0.02*	–
A.deflexus methanol leaf and stem	12.3±0.2*	–	15.0±0.03*	–	10.3±0.2*
A.deflexus methanol flower	–	–	–	10.0±1.0*	10.0±1.0*
A. retroflexus ethanol root	9.7±0.3	14.2±0.1*	11.0±0.04*	12.8±2.2*	9.5±0.03
A. retroflexus ethanol leaf and stem	–	–	9.5±0.3	9.9±0.02*	–
A. retroflexus ethanol flower	9.3±0.06*	9.5±0.02*	10.2±0.2*	10.9±0.2*	–
A.retroflexus methanol root	–	–	–	–	–
A. retroflexus methanol leaf and stem	11.0±0.04*	11.3±0.2*	9.7±0.02*	9.2±0.06*	9.7±0.3*
A.retroflexus methanol flower	8.3±0.08	8.2±0.06	15.0±0.03*	–	–
A.hybridus ethanol root	–	–	–	12.5±0.2*	–
A.hybridus ethanol leaf and stem	10.0±1.0*	11.0±0.04*	10.0±1.0*	–	–
A.hybridus ethanol flower	12.2±2.0*	12.2±0.2*	12.0±2.0*	8.8±0.06*	10.0±1.0*
A.hybridus methanol root	–	–	–	–	–
A.hybridus methanol leaf and stem	–	12.0±2.3*	10.2±0.2*	–	–
A.hybridus methanol flower	–	–	–	10.9±0.06*	9.6±0.02
Ethanol	–	–	–	–	–
Methanol	–	–	–	–	–

– no activity, *Significantly different from the negative control values ($P < 0.05$).

statistically significant ($P < 0.05$). Extract from root of *A. hybridus* had not any effect against the tested fungi strains, except *A. hybridus* ethanol root extract which has an activity against *P. verrucosum* var. *verrucosum.*

Aqueous or alcoholic extracts from plants with proven antibacterial or antifungal function can be applied as antimicrobials against various pathogens and food spoilage microorganisms. Amaranth extract showed the strongest activity against *Aspergillus flavus* (Mosovska & Birosova, 2012). Therefore, it is important to investigate other species of genus *Amaranthus* because they have different antifungal activity. Dahiya et al. (2010) established that ethyl-acetate root extract of *A. hybridus* showed high antibacterial activity against *B. subtilis* and *S. aureus*. Fungi are spread all over the environment, and fungal infection has become more common (Dellavalle et al., 2011). Some fungi produce mycotoxins, which can accumulate in the food and feed. This leads to a health risk for human and animal consumers. *Fusarium graminearum* is a mycotoxigenic fungus that has a toxic effect on the reproductive system of animals (Milicevic et al., 2010). *Fusarium* species belong to widespread mycromycetes with a high potential of mycotoxins production. They are one of the important plant pathogens (Schollenberger et al., 2005). In this study flower ethanol and methanol leaf and stem extracts from three *Amaranthus* spp. had a large inhibition zone of 9.7 to 15 mm against *F.graminearum* and the differences were statistically significant ($P < 0.05$). *A. retroflexus* leaf extract has efficient antifungal activity against the plant fungus *F. oxysporum* (Bahrami-Teimoori et al., 2017). In this study *A. retroflexus* methanol flower extract was most effective against *F. graminearum* (15.0±0.03) ($P < 0.05$). Some species of *Aspergillus* and *Penicillium* generates ochratoxin A – a nephrotoxic and nephrocarcinogenic compound, which can be found in cereals (Zain, 2010). The protean extract of *Amaranthus* seeds showed high growth inhibition activity against *A. ochraceus* and *F. oxysporum* (Rivillas-Acevedo & Soriano-García, 2007). In our study ethanol extract of *Amaranthus deflexus*, *A.retroflexus* and *A.hybridus* were effective against *A. ochraceus* with 9.3-12.2 mm inhibition zone ($P < 0.05$). *A. deflexus* ethanol extract has low antifungal activity (below 40%) against *Aspergillus flavus green, Aspergillus nigar* and *Aspergillus fumigatus* (Sarwar et al., 2016). In this study *A. deflexus* root, flower, leaf and stem ethanol extract were effective against *Aspergillus niger* with 9.7-10.9 mm inhibition zone ($P < 0.05$).

Conclusion

The most effective extracts, which showed activity against all tested strains of microorganisms, were *A.deflexus*

and *A.hybridus* ethanol flower extract, *A.retroflexus* ethanol root extract and *A. retroflexus* methanol leaves and stem extract. Extracts from the three plants can be used as agents against food spoilage microorganisms when added to the food and feed. They could be used also for applications in the plant protection field.

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