Research Regarding the Antimicrobial Activity of Plant Extracts

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Abstract

The investigations regarding the use of plant extracts for their antimicrobial activity is becoming more intense nowadays. The goal of this paper was to evaluate the antimicrobial activity of several plant extracts in order to provide a better understanding of the essential role of the use of plant natural compounds as a primary source of antimicrobial products. Hydroalcoholic extracts of five medicinal plants were tested for their antimicrobial activity against *Pythium sp.* The plants considered for study were marigold (*Tagetes patula*), horsetail (*Equisetum arvense*), elderberry (*Sambucus nigra*), burdock (*Arctium lappa*) and liquorice (*Glycyrrhiza glabra*). The plant materials used for extractions were obtained from healthy dried plants collected from natural populations on non-polluted lands. The *experiments* demonstrated that plants possess strong antimicrobial activity and that plant based products can be effective in controlling microbial growth inhibition. Plant extracts may potentially control the growth and development of pathogenic microorganisms thus representing an alternative in plant protection management and human and veterinary medicine also.

Keywords: *antimicrobial activity, pathogenic microorganisms, plant extracts*

INTRODUCTION

From ancient to modern history, various traditional plant based products played a major role in healthcare and phytopathology (Prasad and Aggarwal, 2011; Vasanthi *et al.*, 2012). Lately, researchers from various fields such as chemistry, microbiology, pharmacology, agriculture and horticulture are investigating natural sources to find phytochemicals that may be used in the development of new antimicrobial products (Evans and Cowan, 2006). Medicinal and aromatic plants are presently the most investigated vegetal sources for their potential antimicrobial activity

and its applicability. Moreover, it is considered that the plant kingdom offers many biochemicals that can be used as pesticides, which are more environmentally safe than the synthetic chemicals (Riaz *et al.*, 2010). Pesticides based on natural components used in plant disease management gained increased attention lately, in search for eco-friendly methods that can be used complementary, either for mass production or for the organic and biofarming niche production.

Natural products derived from plants are considered a solution to mitigate the environmental issues caused by the intensive use

of synthetic pesticides and the negative effects on the human health through the food chain (Rodino *et al.*, 2014). Consequently, the current research is targeted on developing alternative treatments to synthetic chemicals for plant disease control.

Pythium species can cause serious diseases such as damping off, seed rot, root rot and soft rot (Agrios, 2005; Homer *et al.*, 2012; Le *et al.*, 2014) in wheat (*Triticum aestivum*), maize (*Zea mays*), soybean (*Glycine max*), peppers (*Capsicum annuum*), beans (*Pisum sativum*), cucumber (*Cucumis sativus*), tomato (*Solanum lycopersicum*), and many other crops resulting in significant economic losses. Chemical treatments with pesticides together with integrated disease management methods used for control of *Pythium* diseases seem not to be completely effective, and therefore it is recognized as a major pathogen of various crops and the diseases it causes remains a persistent issue for the farmers to deal with (Al-Sheikh, 2010).

In the present study, hydroalcoholic extracts of five medicinal plants were tested for their antifungal activity against *Pythium sp.* The plants taken into study were marigold (*Tagetes patula*), horsetail (*Equisetum arvense*), elderberry (*Sambucus*

nigra), burdock (*Arctium lappa*) and liquorice (*Glycyrrhiza glabra*).

MATERIALS AND METHODS

Plant material. The medicinal plants used in this study were selected from the locally available spontaneous flora and used as presented in Table 1. The plant materials used for extractions were obtained from healthy dried plants collected from natural populations on non-polluted lands from Southern Romania. The plant parts were chosen according to the traditional medicinal use of the refereed plants.

Preparation of plant extracts. A quantity of 10 grams of dried and powdered plant material was added to 100 ml of 70% ethanol. The mixture was left in sealed glass recipients for 96 hours, at room temperature, in darkness, with occasionally stirring. The obtained extract was filtrated through filter paper (Whatman no.1) under vacuum. The extracts obtained were kept at 4°C in sealed recipients until further use.

Fungal culture and growth conditions. The fungal strain of *Pythium sp.* belongs to the collection of the Faculty of Biotechnologies from the University of Agronomic Sciences and

Tab. 1. Plant material used for study.

	Popular name	Botanical name	Family	Part used	Uses in traditional medicine
1.	Marigold	<i>Tagetes patula</i>	Asteraceae	Flowers	gastric ulcers, conjunctivitis, bronchitis, fever, inflammation and wounds (Marottia <i>et al.</i> , 2011)
2.	Liquorice	<i>Glycyrrhiza glabra</i>	Fabaceae	Roots	eczema, peptic ulcers, upper respiratory infections (Fiore <i>et al.</i> , 2005)
3.	Burdock	<i>Arctium lappa</i>	Asteraceae	Leaves	skin infections, acne, boils, bites, eczema, herpes (Nejad <i>et al.</i> , 2012)
4.	Horsetail	<i>Equisetum arvense</i>	Equisetaceae	Whole Plant	disorders of the skin, kidneys and urinary tract (Brunet <i>et al.</i> , 2009)
5.	Elderberry	<i>Sambucus nigra</i>	Caprifoliaceae	Fruits	antitumor potential, antimicrobial, fungistatic, antioxidant activity (Barros <i>et al.</i> , 2011; Sidor and Gramza-Michalowska, 2014; Vlachoianis <i>et al.</i> , 2011)

Veterinary Medicine of Bucharest. The fungal culture was maintained on potato dextrose agar (PDA) at 20°C. The antimicrobial tests were carried out using mycelial plugs originating from an actively growing culture.

Evaluation of the antimicrobial activity.

The effect of the different concentrations of the selected plant extracts against the mycelial growth of *Pythium sp.* was tested by poisoned food technique. An appropriate quantity of each extract was incorporated in sterilized PDA medium to reach desired concentrations for each treatment. Three concentrations of ethanolic extracts were used in the assessment, respectively, 1%, 0.5% and 0.25%. Untreated PDA plates represented the positive control. The plates were left to solidify in the laminar flow hood and then mycelial discs of 6 mm diameter, taken from the margins of an actively growing culture of the phytopathogen were placed in the center of each Petri plates. Three replicates were used for each treatment. The Petri plates were incubated in dark at 25±2°C until the control colony reached the margins of the Petri plate. The measurement of the diameter of the mycelial growth was recorded on a daily basis, beginning with 24 hours after inoculation. The percentage of the growth inhibition of the mycelium (I) due to treatment with extracts was calculated using the following formula:

$$I (\%) = (1 - dt/dc) \cdot 100 (\%),$$

where, **dc** is the average fungal colony diameter measured in control plate, with no treatment, and **dt** is the average fungal colony diameter measured in treated plates.

Statistical analysis. The experimental data were subjected to one-way analysis of variance (ANOVA) using Microsoft Excel 2010.

RESULTS AND DISCUSSION

Pythium sp. *in vitro* growth proved to be sensitive to treatment with most of the plant extracts, demonstrated by the inhibition of the mycelial growth. The results revealed that the antimicrobial activity of the extracts was dose dependent, being negatively influenced by decreasing the concentration of the extracts in the growth media.

Regarding the growth inhibition dynamics of the variant using 1% extract concentration in the culturing medium it could be observed a delayed development of the *Pythium sp.* inoculum

compared to the not treated control. The mycelium started to develop on the control plates ten to twelve hours after inoculation and grew rapidly reaching the margins of the Petri plate in four days. On the treated plates, the growth was only observed after 24 hours since inoculation. The daily measurements of the radial growth of *Pythium sp.* colony at 1% concentration in the culture media are presented in Figure 1. At this concentration, the strongest extracts with more than 50% inhibition against *Pythium sp.* were *G. glabra* extract, with 72% growth inhibition, followed by *T. patula* 56%. The other three plant extracts showed an inhibition percentage below 50% as follows: *E. arvense* 46%, and *S. nigra* and respectively, *A. lappa* 2.4% (Fig. 2).

Going further to a 0.5% concentration of the plant extract, the inhibition of growth decreased, reaching 49% for *G. glabra* and 38% for *T. patula*. In this variant on the plates treated with extracts obtained from burdock, horsetail and elderberry the colonies had a slower growth than the control, but at the end of the 96 hours of incubation, they reached the margins of the plate. For a concentration of 0.25% the results are comparable. In the fourth day, the diameter of the colony treated with licorice being 48 cm corresponding to I=32%. For marigold, the mean diameter measured was 49 cm, I= 30%.

From these results it can be concluded that the extract of marigold (*T. patula*) and licorice (*G. glabra*) possesses strong *in vitro* antifungal activity against *Pythium sp.* and can be subjected to further investigation regarding their chemical composition and their antimicrobial potential for *in vivo* efficacy study.

Occurrence of several active compounds, most of them terpenoids and flavonoids has been reported from these two plants. These compounds are known to exhibit antioxidant, antifungal, antibacterial and analgesic activities (Pérez Gutiérrez *et al.*, 2006; Barnabas *et al.* 1988). Many biological activities, such as anti-inflammatory, anti-viral, antitumour and hepatoprotective drug, antifungal, antioxidant, anti-atherogenic, anti-genotoxic have been reported for *G. glabra* extracts (Fatima *et al.*, 2009; Evans and Cowan, 2006; Visavadiya *et al.*, 2009; Siracusa *et al.*, 2011). These activities were reported due to two kinds of main constituents, the saponins and flavonoids present in *G. glabra* (Patil *et al.*, 2009).

Fig. 1. Diameter growth of *Pythium* sp. colony on PDA after treatment with plant extracts at 1% concentration.

Fig. 2. Percentage of the growth inhibition of the mycelium (I) due to treatment with extracts.

The phytochemical investigation of different parts of *T. patula* resulted in the isolation of the chemical constituents of several classes of secondary metabolites, such as flavonoids, benzofurans, carotenoids and thiophenes (Politi *et al.*, 2012; Marottia *et al.*, 2004). Other previous studies on the phytochemical composition of ethanolic floral extract of *T. patula* showed the presence of alkaloids, flavonoids, tannins and phenolics (Ramya *et al.*, 2012; Trombetta *et al.*, 2005). There are other previous reports on antimicrobial action of plants extracts against different *Pythium* species. Muthukumar *et al.* (2010) evaluated the fungitoxic effects of 66 medicinal plants against *Pythium aphanidermatum*, the causal agent of chili damping-off. According to their findings, Zimmu leaf extract (*Allium sativum* L. × *Allium cepa* L.) showed the highest inhibition of mycelial growth

of *P. aphanidermatum* (13.7 mm) (Muthukumar *et al.*, 2010). Ambikapathy *et al.* (2011) reported maximum antifungal activity (15 and 20 mm) of the n-butanol and methanol extract of *Lawsonia inermis*, followed by *Phyllanthus niruri* (15 and 20 mm), *Tephrosia purpurea* (10 and 15 mm) against *Pythium debaryanum*. The methanolic extract of *Mimosa pudica* (20 mm), *Vinca rosea* (10 mm) exhibited least activity against *P. debaryanum*. He also tested the antifungal effect of aqueous extract of all tested five plants but these ones showed no activity against *P. debaryanum* (Ambikapathy *et al.*, 2011).

Crude aqueous and methanolic extracts of 97 plant species belonging to 35 families collected from the west of Iran, were screened for antifungal activity against *Pythium aphanidermatum* by Bahraminejad (2012). Seventeen of 97 (17.5%) plant species showed inhibitory activity against the tested fungi. *Glycyrrhiza glabra*, *Xanthium strumarium* and *Portulaca oleraceae* were among the active plant species against *P. aphanidermatum*. The efficacy of the selected plant crude extracts against *P. aphanidermatum* was also evaluated in greenhouse condition and all tested plant extracts were reported to show superior effect in reducing the disease severity as compared to the control. Among the different treatments, seed treatments of *G. glabra* and *P. oleraceae* extracts reduced disease severity from 70% for infected control to 43% (Bahraminejad, 2012).

CONCLUSION

From the results, it can be concluded that the extract of flowers of marigold (*T. patula*) and roots of licorice (*G. glabra*) possesses strong *in vitro* antifungal activity against *Pythium* sp. and should be subjected to further investigation regarding their chemical composition, mechanism of action and their antimicrobial potential for *in vivo* efficacy study.

Plant extracts may potentially control the growth and development of pathogenic microorganisms thus representing an alternative in plant protection management and human and veterinary medicine also. These findings provide a basis for the use of naturally occurring compounds in the design of new solutions for pathogens control.

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