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In vitro antifungal activity of ethanol plant extracts against conidiospores of apple scab (Venturia inaequalis)

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Antifungal activity *in vitro* trials using ethanol extracts from leaves of *Yucca filamentosa*, stalks of *Saponaria officinalis*, roots of *Berberis vulgaris*, leaves of *Olea europaea*, roots of *Inula helenium* and flower twig of *Tamarix tetrandra*, prepared by maceration, were performed against *Venturia inaequalis* – a plant pathogen which causes apple scab disease. Results showed that some of the tested plant extracts had strong inhibition on germination of *Venturia inaequalis* conidiospores comparable to commercial synthetic pesticides.

Keywords: *Yucca filamentosa, Berberis vulgaris, Tamarix tetrandra, Saponaria officinalis, Oliva europea, Inula helenium, Venturia inaequalis*

INTRODUCTION

Since ancient times, plant extracts have been used as natural pesticides against agricultural pests including numerous plant pathogens. They are relatively cheap, easy to be prepared, in the most cases – non toxic or slightly toxic to the humans, non harmful for the environment and there is no resistance risk associated with their application in pest management (Pavela, 2016; Matthews, 2018).

In the present study, ethanol extracts of several plants were tested for antifungal activity against one of the most spread and harmful plant pathogen on apples – apple scab (Venturia inaeqialis): Yucca filamentosa, Saponaria officinalis, Berberis vulgaris, Oliva europea, Inula helenium (Gessler et al., 2006).

Yucca is a genus of perennial shrubs and trees in the family Asparagaceae, subfamily Agavoideae. About 40–50 of its species are notable for their rosettes of evergreen, tough, sword-shaped leaves and large terminal panicles of white or whitish flowers. They are native to the hot and dry (arid) parts of the Americas and the Caribbean. Yuccas are widely grown as ornamental plants in gardens. Many species also bear edible parts. Plant parts from Yucca are rich of saponines. Once the seeds have been removed, the fruits can be cooked and eaten. The large flower petals can also be eaten in salads. The leaves, stems and roots of this plant can be used to stun fish. In the traditional medicine, plant extracts were used to the joint pain, bleeding, urethral and prostate inflammations (Rau, 1945; Lim, 2014).

The antifungal activity of a crude steroidal glycoside extract from Yucca gloriosa flowers, named alexin, was investigated in vitro against a panel of human pathogenic fungi, yeasts as well as dermatophytes and filamentous species (Favel et al., 2005). The species also express antifungal effectiveness against strawberry soil-Borne Pathogens: Fusarium solani and Macrophomina phaseolina (Ruiz-Romero et al., 2018). The leaves of Yucca aloifolia extracted by using methanol showed an antimicrobial activity comparable with standard antibiotics towards a set of fungal and bacterial strains such as Aspergillus niger, A. flavus, Alternaria altrnata, Ganoderma lucidum, Pasturella multocida, Escherichia coli and Staphylococcus aureus (Zubair et al., 2013).

Treatment of seeds with an aqueous extract of yucca (Yucca schidigera), was evaluated for antifungal activity against seedborne pathogens as well as its effect on seed germination and seedling growth of sorghum (Sorghum bicolor). The antifungal effect of this plant was observed against Leptosphaeria sacchari when the extract was applied at 25 and 10% concentrations. At 10% concentration, Yucca schidigera significantly reduced not only the incidence of L. sacchari, but also that of Fusarium spp., Cochliobolus lunatus and Cladosporium spp. The effect of 10% Yucca schidigera on seedborne fungi was broader than the fungicide fludioxonil, particularly with regard to Fusarium. Furthermore, the number of normal, healthy-looking seedlings increased in a dose-responsive manner with Yucca treatment. Seedling vigour was also stimulated by Yucca schidigera (Wulff et al., 2012).

Saponin rich-extracts from Yucca schidigera have been tested against common phytopathogenic fungi (Pythium ultimum, Fusarium oxysporum, Alternaria solani, Colletotrichum coccodes and Verticillium dahliae). The inhibitory effect of these extracts was measured in vitro and the concentrations that will reduce the colony diameter of fungus to 50% of the control (DRC50 values) were determined (Chapagain et al., 2007). Another research studied the antifungal activity of Yucca gloriosa ethanol extract. The results showed that the antifungal effect of 60% ethanol extract on Botrytis cinerea (isolated from garlic bolt), Botrytis allii, Penicillium dititatum and Botrytis cinerea (isolated from grape) was significant, but there was no effect on Penicillium italicum (Qing-min et al., 2006). Antifungal activity of water, ethanol, lanolin and cocoa butter plant extracts derived from leaves of Yucca filifera were evaluated against Phytophthora cinnamomi and was established that they have strong effectiveness (Castillo-Reyes et al., 2015).

A saponin fraction from the stems of Yucca schidigera (Mohave yucca) exhibited potent growth-inhibitory activities against certain food-deteriorating yeasts, film-forming yeasts, and dermatophytic yeasts and fungi (Miyakoshi et al., 2000). The effect of an extract of Yucca schidigera on the control and infection process of the apple scab pathogen, Venturia inaequalis, was examined and compared with the chemical resistance inducer, acibenzolar-S-methyl (ASM). In seedling assays, both materials significantly reduced apple scab symptoms and pathogen sporulation on leaves and both showed similar control efficacies as the reference treatment, sulphur. Whereas yucca extract and sulphur gave significant inhibition of conidial germination in vitro, ASM did not inhibit germination (Bengtsson et al., 2009). Four steroidal saponins were isolated from the leaves of Yucca. Their structures were established using one- and two-dimensional NMR spectroscopy and mass spectrometry. The isolated saponins were evaluated for their antitumor activity against HCT116, MCF7, HepG2, and A549 cell lines (Eskander et al., 2013).

Common barberry (Berberis vulgaris) is a shrub native to Central and Southern Europe, Northwest Africa and Western Asia. The berries of Berberis vulgaris are edible however plant is used mostly as decorative and for making hedges (Salehi et al.,2019). The problem is that Berberis vulgaris is a host for plant a pathogen of cereals such as Puccinia graminis f. sp. tritici. The plant parts of Berberis vulgaris are rich of biologically active substances which can express high antimicrobial efficacy and the plant is used in the traditional herbal medicine as remedy against dermal diseases, gastrointestinal inflammations and coughs (Bhardwaj and Kaushik 2012; Och and Nowak 2021). Plant extracts of Berberis vulgaris were can also express strong antimicrobial activity towards plant pathogens, dermatophytes and other fungal and bacterial pathogens.

The aqueous extracts from Berberis vulgaris (roots and bark) have strong effectiveness against Sclerotinia sclerotiorum (Pârvu and Pârvu 2011). The antifungal activity of Berberis vulgaris extracts was proved against Botrytis cinerea. It was found that B. vulgaris bark extract had significant antifungal activity against B. cinerea, and its effect was stronger than that of pure berberine. B. vulgaris bark extract might be recommended to be tested as a biocontrol agent against B. cinerea (Parvu et al., 2010).

In vitro antileishmanial activity of various extracts of Berberis vulgaris was evaluated thought its active component. Berberine was examined for antileishmanial activity against Leishmania tropica

and L. infantum species using in vitro experiments and expressed strong effectiveness (Mahmoudvand et al., 2014).

The effect of berberine sulphate salt on in vitro growth of Trichomonas vaginalis was compared to the efficacy of metronidazole as a reference drug. Results showed that berberine sulphate was comparable to metronidazole in regards to potency with the advantage of being more safe and possible replacement in metronidazole resistant cases (Soffar et al., 2001).

The methanolic macerated extract from Berberis vulgaris showed a strong antifungal activity on Candida albicans yeasts (Mezouar et al., 2014; Iauk et al., 2007).

70 % ethanol water solution extracts from stem and root bark of Berberis vulgaris were tested on Escherichia coli (Gram negative) and Staphylococcus aureus (Gram positive) bacteria. The extracts exhibited a stronger activity against S. aureus, which demonstrates that berberine extracts are useful in treatment of infections (Ungurean et al., 2018).

Tamarix tetrandra is a decorative plant native to south Eastern Europe, Turkey, Bulgaria and Crimea. Its plant parts contain biologically active substances that can express strong antimicrobial activity (Bahramsoltani et al., 2020). The extract from Tamarix dioica showed significant activity against A aspergiulus flavis and M. canis and moderate activity against Fusarium solani. The methanol extracts of the Tamarix gallica and Tamarix articulata could be a good source of antioxidants and antibacterials for food and pharmaceutical industries (Tabet and Boukhari, 2018). Flavonoid tamarixetin from Tamarix ramosissima exerted an anti-proliferative effect on human leukemia cells by blocking cell cycle, also hepatoprotective and anti-fibrotic activity (Bailon-Moscoso et al., 2017; Weiskirchen 2016).

In the present study, the antifungal effectiveness of ethanol extracts from roots of Berberis vulgaris and flower twigs of Tamarix tetrandra was conducted with mycelium of Monilia laxa and Phytophthora capsici in the in vitro trials.

Olive leaves were also rich of biologically active substances with strong antifungal action. They are well known for many useful pharmacological effects. Olive leaves extracts have antimicrobial, anti-inflammatory, anti-oxidant anti-hypertensive, anti-hypercholestermic, anti-hyperglycemic, antithrombotic, diuretic and anti-tumor properties (Sabry, 2014; Erbay and Icier, 2010). The extracts using water, acetone, methanol and ethyl acetate were effective against many plant pathogens such as Alternaria alternata, Fusarium oxysporum, Rhizopus oligosporus and others (Korukluoglu et al., 2008). In Turkey, it was established that fresh olive leaf extracts prepared using various solvents (water, ethanol, acetone, ethyl acetate) can be effective against Saccharomyces cerevisiae, Schizosaccharomyces pombe, Saccharomyces uvarum, Candida oleophila, Metschnikowia fructicola and Kloeckera apiculata (Korukluoglu et al., 2006). Air-dried powdered olive leaves were defatted with hexane and the marc was then soaked in 80% methanol and successively extracted with CH2Cl2, EtOAc, and n-BuOH. Ethyl acetate fraction showed positive antibacterial activity and negative antifungal activity whereas water, 80% methanol, and butanol fractions have positive antifungal and negative antibacterial activity (Ghanem et al., 2019).

The hydroalcoholic extract of olive leaves were even more effective against herpes simplex virus type 1 (Motamedifar et al., 2007). Antimicrobial properties of olive leaf extract on some yeast were examined in the study. Fresh olive leaf extracts were prepared using various solvents (water, ethanol, acetone, ethyl acetate) in Soxhlet apparatus. Antimicrobial effects of these extracts were tested against Saccharomyces cerevisiae ATCC 9763, Schizosaccharomyces pombe, Saccharomyces uvarum, Candida oleophila, Metschnikowia fructicola and Kloeckera apiculata. All extracts showed various degrees of antifungal effects with 10-28 mu g/ml MIC, 20-48 mu g/ml MFC and 1.5-9.3 mm inhibitory zone values against yeasts utilised, except water (Karakaş, 2006).

In the fig (Ficus carica) and olive (Olea europaea) studies, leaves were extracted using water, hot

water and methanol. Antimicrobial activities of fig and olive leaf extracts were evaluated. Mixture of methanolic extracts of fig and olive leaves were the most effective extract and resulted in antimicrobial activities against some strains of food borne pathogenic bacteria and spoilage fungi isolated from kariesh cheese and yoghurt drink. Fig and olive leaf extracts which proved to be potentially effective can be used as natural preventive alternative to control food poisoning diseases and preserve food stuff avoiding health hazards in the applications of chemically antimicrobial agents (Yousef et al., 2019).

Saponaria officinalis is a common perennial plant from the family Caryophyllaceae, with native habitat range extending throughout Europe, Asia and to western Siberia. The plant parts contain saponin, in the roots at levels up to 20 percent when the plant is flowering. It has historically been used to clean delicate or unique textiles, especially woolen fabrics (Jia, 1998; Gonzalez and Sörensen, 2020; Chandra et al., 2021). Extracts from Saponaria officinalis can also show inhibitory effect against plant pathogenic fungi (Özbek et al., 2021). Saponaria officinalis hydroalcoholic extracts were examined for in vitro growth inhibition of an avian isolated fatal Escherichia coli. The study has revealed that saponin extract from S. officinalis had useful antibacterial effects (Nabinejad, 2013).

Inula helenium is a widespread plant species in the sunflower family Asteraceae. The plant is medicinal and is used in herbal medicine as an expectorant and for water retention (Bartram, 2013). Also, the roots contain sesquiterpene lactones with antiproliferative, antitumour and antioxidant action (Konishi et al., 2002; Li et al., 2012; Spiridon et al., 2013). In an in vitro study, dried roots of a Romanian indigenous population of Inula helenium showed antimicrobial activities. The powdered dried root of the plant was extracted in ethanol (using 30%, 50% and 70% v/v). The antimicrobial activity has been tested on five potential pathogenic bacteria species (Bacillus subtilis, Bacillus cereus, Enterococcus faecalis, Escherichia coli, Staphylococcus aureus) and four fungal species (Candida albicans, C. parapsilosis, C. lipolytica, Aspergillus niger), all of veterinary interest. The results indicated that ethanolic extracts (50% and 70%) from the roots of a Romanian cultivar of I. helenium showed significant antimicrobial activity against all tested microorganisms, except the pathogenic filamentous fungi A. niger. On the dermatophytic species (Candida sp.), the inhibitory effects of 50% and 70% extracts were very similar (Diguță et al., 2014). The antifungal activities of methanol extract and ethanol extract from Inula helenium were evaluated against Sphaerotheca fuliginea, Pseudoperonospora cubensis, Colletotrichum orbiculare, Botrytis cinerea and Fulvia fulva. The results indicated that extracts with different solvents exhibited different inhibitory activity against the five pathogens and the ethanol extract of I. helenium showed stronger antifungal activities than the methanol extract (Zhang, 2007).

In this study, we evaluated in vitro ethanol extracts from leaves of Yucca filamentosa, stalks of Saponaria officinalis, roots of Berberis vulgaris, leaves of Oliva europea, roots of Inula helenium and flower twig of Tamarix tetrandra against Venturia inaequalis, a plant pathogen which causes apple scab disease.

MATERIALS AND METHODS

The plant extracts were prepared by method of maceration of the given dried and crushed plant parts with 96% (v/v) pure ethanol - 100 g of plant material in 1 liter ethanol in 5 liter dark-colored flask. The mixture stayed three days at room temperature at 20°C, after that shaken for 1 hour at 150 rpm. The extracts were then filtered using filter paper and ethanol was evaporated by Vacuum Rotational Evaporator (RVO 004 - INGOS Laboratory Instruments Ltd.) . Plant extracts prepared by this way were mixed with distilled water at the given concentrations for in vitro antifungal tests.

The germ tube inhibition tests were conducted in order to determine the ability of the salts to inhibit conidia germination of the plant pathogenic fungi. The microscopic slides “hanging drop” was sprayed with water solution of tested extracts at desired concentration. After drying of

solution, 20 µl conidial suspensions (3*104 spores/ml) was added. The slides were then incubated for 24-48 h in thermostat under 22-24°C. Observations with light microscope (10x) were conducted to determine germination of the spores (four observation on each slide). The percent of germination was calculated as follows: Percent germinated conidia=number of germinated spores*100 / (number of germinated spores + number of non-germinated spores). From the calculated percents of germination, effectiveness (inhibition) was calculated using with formula of Abbot (Abbot, 1925). Dose – Response Modeling was performed by R language of statistical computing (R Core Team, 2020), drc package (Ritz et al., 2015).

RESULTS

Figure 1 show the antifungal action of ethanol extracts from Yucca filamentosa leaves against conidiospores of Venturia inaequalis [NOAEL (LD 5) = 0.04 % (v/v); LOAEL (LD 25) = 0.057 % (v/v); LD 50 = 0.072 % (v/v); and LD 90 = 0.13 % (v/v)].

Above 0.3 % (v/v), the extract was able fully to block the germination of conidiospores.

The antifungal action of ethanol extracts from Berberis vulgaris root against conidiospores of Venturia inaequalis is shown in figure 2 [NOAEL (LD 5) = 0.04 % (v/v); LOAEL (LD 25) = 0.056 % (v/v); LD 50 = 0.076 % (v/v); and LD 90 = 0.17 % (v/v)]. Above 0.2 % (v/v) the extract was able to fully block the germination of conidiospores.

Figure 3 show the antifungal action of ethanol extracts from Inulla helenium root against conidiospores of Venturia inaequalis [NOAEL (LD 5) = 0.032 % (v/v); LOAEL (LD 25) = 0.076 % (v/v); LD 50 = 0.1 % (v/v); and LD 90 = 0.16 % (v/v)].

Above 0.2 % (v/v), the extract was able to fully block the germination of conidiospores.

Figure 4 show the antifungal action of ethanol extracts from Tamarix tetrandra flower twigs against conidiospores of Venturia inaequalis [NOAEL (LD 5) = 0.034 % (v/v); LOAEL (LD 25) = 0.053 % (v/v); LD 50 = 0.79 % (v/v); and LD 90 = 0.23 % (v/v)].

Above 0.25 % (v/v), the extract from Tamarix tetrandra flower twigs was fully able to block the germination of conidiospores.

In regards to trials using of Saponaria officinalis stalks, results showed that even with 10 % concentration, there is absolutely no effect against conidiospores of Venturia inaequalis. The ethanol extracts from olive leaves did not only express any antifungal activity but it even stimulated the germination of conidiospores.

Figure 5 shows generalized results from conducted in vitro trials with conidiospores of Venturia inaeqalis. It is obvious that the effectiveness of the different plant extracts is absolutely similar. The conducted ANOVA analysis showed that there was no significant differences ($p>0.05$).

CONCLUSION

The results of conducted trials showed the potential of ehanol plant extracts from leaves of Yucca filamentosa, roots of Berberis vulgaris, roots of Inulla helenium and flower twigs of Tamarix tetrandra to block the germination of conidiospores of Venturia inaequalis, a plant pathogen causing apple scab disease at concentrations completely comparable with commercial synthetic fungicides. Above 0.3 % (v/v) concentration, all of tested plant extracts (except those from stalks of Saponaria officinalis and olive leaves) completely inhibited the germination of conidiospores of apple scab.

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