Effect of mangrove plant extract on growth of four fungal pathogens

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ABSTRACT

In vitro assessment of the antifungal activities was evaluated in the present study for both aqueous and ethanol extracts prepared from leaves of *Avicennia Marina* and *Rhizophora Mucronata*. Leaves of mangrove species were collected from Qeshm and Syrik of south-east coast of Iran, mangrove forest respectively. Antimicrobial tests were carried out through disk diffusion method. Minimum fungicidal concentration was determined according to Agar Dilution method. Results showed that the ethanol extracts of both species had antifungal activities on P. pupurogenome, P. chrysogenum, P. notatum, A. niger, A. alternata and Penicillium italicum. However, none of the water extracts showed antimicrobial activity on the studied fungi. Among all pathogens tested, A. flavus was the most resistant fungi. Different concentrations of extracts from Avicennia Marina and Rhizophora Mucronata exhibited different degree of growth inhibition against tested fungal strains. This study indicates the capability of mangrove species for the utilization as source of natural antifungal.

Kewords: Mangrove; Natural Antifungal; Postharvest; Pathogen

INTRODUCTION

Fungal diseases are among the main problems against the crop production all around the world which mainly result in enormous economic loss. For many years, chemical fungicides have been used intensively against postharvest disease of fruits. Nevertheless, it also affects resistance in pathogens, in addition to contamination of the environment [1, 2]. Moreover, it may cause problems in human health through affecting the fruits by toxic residues [3]; this makes it quite necessary to search for natural pesticides. Recently, essential oils and plant extracts have been studied for their antifungal and antibacterial [4], nematicidal, [5] insecticidal [6] and antioxidant [7] activities. Researches from across the world have reported antimicrobial activities of different medicinal plants. It has been stated that the higher plants have shown to be a potential source for the new antimicrobial agents [8]. Scientific research throughout the world has found evidence to support the fact that mangrove foliar extracts have great potential against microbial pathogens. Mangrove plants are found in the interface of land and sea [9]; they possess antibacterial, antifungal and antiviral [10], anticancer [11] and antitumor [12] qualities. Recently, it has been powerfully suggested that mangroves would be considered as a valuable source for chemical components with possible medicinal values [13] despite the fact that the chemical components of most mangrove spices still have not been studied widely.

Rhizophora mucronata (family of Rhizophoraceae), usually identified as Asiatic mangrove and generally distributed along the coastal tropical and subtropical area, has been stated to have several medicinal properties [14]. This mangrove species belongs to Rhizophoraceae family and is widely observed on the south-east coast of India. For the past few decades, marine pharmacology researchers have

been focused on developing novel antimicrobial and anti-oxidant metabolites from marine halophytes and medicinal plants. *Avicennia Marina* is commonly identified as gray mangrove tree classified in the plant family *Avicenniaceae*, and is commonly used for ulcers [15]. There are two basic reasons that explain the pharmacological study of chemical constituents of mangrove plants. First, mangroves are among the easiest tropical forest types to generate. Mangroves can grow under stressful conditions such as high and low salinity of water, heavy metal pollution, and in abundance of living microorganisms and insects. According to the previuos research, mangrove plants were used as medicinal plants for the purpose of controling pathogen (second) [16]. However, only limited studies have been carried out to identify metabolites responsible for antimicrobial properties of these plants. Therefore our current study aimed at evaluating the antifungal properties of ethanol and water extracts of leaves of *Rhizophora Mucronata* and *Avicennia Marina* mangrove plant species against five postharvest pathogenic fungal. Our data can be useful for biological control of this pathogen during storage of fruits.

MATERIALS AND METHODS

Plant Collection Material and Preparation of the extracts

The plant collection was carried out within native stands. Leaves of *Avicennia Marina* and *Rhizophora mucronata* mangrove species were collected from Qeshm (*26°50'N and 56°0'E*) and Syrik, from south-east coast of Iran, mangrove forest respectively. The amount of 25 gram residue of *A. Marina* and *R. Mucronata* mangrove leaves was added to 125 ml ethanol 96% or distilled water. The ethanol and aqueous extracts mixture was well-maintained at 25 ˚C of laboratory temperature for 48 hours and was mixed every few hours with a glass rod. The collecting supernatant was centrifuged by 9000 rpm for 5 min. The supernatant was removed and reached to the original volume with ethanol or distilled water; then the samples were packed in dark containers and stored at 4 ˚C after being filtered by 0.45 µ Whitman filter paper [17].

Antifungal activities

Plant extracts were purified through filtration, usinf 0.45 µg Millipore filters. Antifungal tests were then performed by the disk diffusion method using 100 µg of suspension containing 1.5×10^8 CFU/mL of fungi spread on Potato Dextrose Agar medium. Disks of 7 mm in diameter were impregnated with 20, 40, 60 and 80 mg/mL inoculated Agar. Negative controls were prepared with the same solvents employed to dissolve the mangrove extracts. The inoculated plates were incubated at 27 ˚C for 72 h. Antifungal activity was measured through determining the zone of inhibition against the test fungus in contrast to the negative control [18].

Determination of Minimum Fungicidal Concentration (MFC)

MFC was determined according to Agar Dilution method with slight modifications. Different concentrations of extracts (2-256 mg/ml) were incorporated in Sabouraud Dextrose (SD) broth in tubes. About 1 mL of spore suspension was added to them and incubated at room temperature for 3 days. The SD broth alone served as negative control and SD broth lacking incorporation of extract and 1 mL of spore suspension was used as positive control. The MFC was regarded as the lowest concentration of the extract which inhibited the growth of molds colony [19].

Statistical analysis

Data were generated for each assay in triplicate. A one-way ANOVA test was used. The computer program employed was SPSS for Windows version 10.0.

RESULT

Antifungal activity

Results of our study showed that ethanol extract of *Avicennia marina* reduced the growth of *Penicillium pupurogenome, Penicillium chrysogenum, Penicillium notatum, Penicillium niger, Penicillium alternata* and *Penicillium italicum.* However no inhibition of the growth of *Aspergillus flavus* was observed in ethanol extract of *A. marina.* Ethanol extracts of *Rhizophora mucronata* also showed the same effect on different fungi and this spices showed more

inhibition than Ethanol extracts of *Avicennia marina* against *Aspergillus flavus.* The rate of growth reduction of ethanol extract of two leaves was nearly the same. Results also showed that aqueous extracts had no antibacterial effect on *Aspergillus flavus, P. pupurogenome, P. chrysogenum, P. notatum, A. niger, A. alternata* and it was not able to prevent the growth of fungi on culture (Table 1).

Table 1. Antifungal activity of mangrove extracts against selected fungal strains

Extracts	Inhibition zone (mm)					
	Penicillium notatum	***Penicillium chrysogenum***	***Penicillium pupurogenome***	***Aspergillus alternata***	***Aspergillus niger***	***Aspergillus flavus***
Ethanol extract of *Avicennia marina*	12	12	13	14	13	0
Aqueous extract of *Avicennia marina*	0	0	0	0	0	0
Ethanol extract of *Rhizophora mucronata*	13	13	12	12	11	10
Aqueous extract of *Rhizophora mucronata*	0	0	0	0	0	0

No inhibition of the growth of different fungi was observed in water extracts of the two spices. Results showed that MFC of *Avicenna marina* ethanol extract for, *P. pupurogenome, P. chrysogenum, P. notatum* was 50 mg/mL. However MFC of this extract for *A. niger* and *A. alternata* was 75 and 25 mg/ml, respectively. Based on the results, MFC of ethanol extract of *Rhizophora mucronata* for *Aspergillus flavus* and *A. niger* was 100 mg/mL, while it was 50 mg/mL for *P. notatum and P. pupurogenome*. MFC of *Avicenna marina* ethanol extract for *P. chrysogenum* and *A. alternate* was 25 and 75 mg/mL, respectively (Table 2). In this study, the maximum size of inhibition zone for *Avicenna marina* ethanol extract in the disc method was 14 mm for *A. alternata* but had no effect on *Avicenna marina* aqueous extract (Table 2).

Table 2. Minimum Fungicidal Concentration of *Avicenna marina* leaves ethanol extracts on fungal strains

Fungal strains	Minimum Fungicidal Concentration(mg/mL)	
	Ethanol extract of *Rhizophora mucronata*	**Ethanol extract of *Avicennia marina***
Penicillium. chrysogenum	25	50
Penicillium pupurogenome	50	50
Penicillium notatum	50	50
Aspergillus flavus	100	-
Aspergillus alternata	75	25
Aspergillus niger	100	75

DISCUSSION

Microorganisms, mainly fungi and bacteria, are the main pathogenic organisms having potential to reason fruit diseases. The limitations of existing antifungal drugs, increased occurrence of systemic fungal contaminations, and rapid development of drug resistance have highlighted the need for the finding of new antifungal agents, preferably with novel mechanism of action. Our results indicate that the ethanol extract of *Avicenna Marina* leaves generally had effect on *A. alternata* and had the least impact on *Aspergillus Flavus*. The results showed that MFC of ethanol extract of *Avicenna Marina* leaves for *A. alternata* was 25 mg/mL and for *A. Niger* was 75 mg/mL. However, MFC of ethanol extract of *Avicenna Marina* leaves for *P. Notatum and P. Pupurogenome* and *P. Chrysogenum* were the same (50 mg/mL). Effect of mangrove extract on microorganisms was reported in the previous references. Acetate extract of *Avecina Officianals* showed significant antibacterial activity with zone of inhibition of 13 mm against *Eseercia Coi* and 11 mm against *Staphylococcous Aeruos* [20]. Previous research revealed that chloroform and methanol extracts of *Avicennia Marina* can inhibit growth of *Erwinia Caratovara* and *Streptococcus Mutans* under in- vitro condition [21]. Literature [22] showed that among all extracts, *A. Marina*, *A. Officinalis* and *B. Sexangula* showed different grade of growth inhibition against tested strains of bacterial. Among all bacterial strains, Pseudomonas spp and E. coli showed considerable growth inhibition against almost all plant extracts. Through this study, the researchers [23] showed that the ethanol extract of *Avicennia marina* leaves inhibited the growth of *Aspergillus Flavus* and *Penicillium Italicum,* in "Disk Agar diffusion test" method. Theresults showed that MFC of ethanol extract of *Avicenna marina* for *Aspergillus Flavus* was 32 mg/mL, and for *Penicillium Italicum* was 16 mg/mL. Ethanol extract compared to the aqueous extract had a greater inhibition effect. Literature reported that mangrove leaf extract at all concentrations (20, 40, 60 and 80%) had inhibitory effect on *Penicillium digitatum.* Medium diameter (mm) of microbial free zone area of *Avicenna marina* was 13 in 80% concentration of leaf extract [23]. Antifungal activity may be because of active components which exist in plant extracts. The efficiency of the active composites causes the creation of growth inhibition zones that appear as clear areas near the disk. However, some plant extracts were not capable to exhibit antimicrobial activity against tested fungi strains. These fungi strains may have some kind of resistance mechanisms. e.g. target sites modification, enzymatic inactivation and decrease in intracellular drug accumulation [4]. previously performed studies in the literature showed that crude ethanol extract had better inhibition against fungi strains. It could be so because solubility of antimicrobial active complexes in polar solvent is better than in water [24]. Researchers have found [25] that the ethanol extract shown a higher anti-mutagenic activity than the water extract. They showed that *Avicennia Marina* leaf extract has inhibition effect on *Typhimurium* TA100 bacterial growth. Some researchers showed that ethanol fraction of *Aloe Vera* and *Coleus Aromaticus* exhibit more promising results in suppressing the fungal growth rather than aqueous extract [8].

CONCLUSION

The current research indicated the potential of *Avicennia Marina* and *Rhizophora Mucronata* for applications in biocontrol postharvest disease. Extra researches by different methodologies are essential to complete the research in the biological activities to identify the bioactive compounds of *Avicennia marina* and *Rhizophora mucronata.* To the best of our information, this it is the first scientific report that indicates the antifungal activity of the ethanol extracts of *Avicennia Marina* and *Rhizophora Mucronat* again *P. notatum and P. pupurogenome, A. Niger and P. Chrysogenum.*

"The authors declare no conflict of interest"

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