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Antimicrobial activity of *Avicennia marina* extracts ethanol, methanol & glycerin against *Penicillium digitatum* (citrus green mold)

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ABSTRACT

Finding natural antimicrobial compound with minimum side effects on health the is important because of microorganisms are more antibiotics resistance. Avicenniaceae family is a member of true mangrove plants which has one genus, 11 species and several sub species. *Avicennia marina* (Forssk.) Vierh is the most current species among these plants in Iranian mangrove forest. In this study, mangrove leaves were dried in shadow and appropriate condition. After extraction with ethanol 96 degree, methanol 96 degree and 20% glycerin antimicrobial effect of extract were determined by "screening antimicrobial activity" and "disk agar diffusion test" in 20, 40, 60 and 80 Percent concentration of the extract against *Penicillium digitatum*. The results showed that mangrove leaf extract in screening antimicrobial activity method in 2000 µg/ml, were inhibited *Penicillium digitatum* of growth. In "disk agar diffusion test, mangrove extract, in 20, 40, 60 and 80 Percent concentration, the mentioned extract were shown inhibition effect on mold pathogen growth. Ethanol 96 degree extract was more effective than methanol 96 degree and 20% glycerin extract as antimicrobial against on *Penicillium digitatum* ($p<0.05$). Results showed extract of mangrove can be used as natural antimicrobial in food products.

1. Introduction

Penicillium digitatum is the most devastating pathogen of citrus fruit, being responsible for about 90% of production losses during post-harvest handling.In spite of the application of fungicides and the increased implementation of new biological control strategies green mold continues to exhibit high infection pressure on stored citrus commodities worldwide (Droby etal., 1998; Ghaouth etal., 2000). Avicennia marina is a mangrove tree species that is extraordinarily adaptable with a wide latitudinal range closely associated with its flexible growth pattern. It is common throughout the Indo-Pacific region within a latitudinal range of 30o N to 38o S (Duke, 1990). They have important functions, including water purification, coastal protection from wind and waves, and biodiversity enhancement. Mangrove habitat is an important component of the coastal ecosystem, an ecosystem characterized by high biological productivity. Recently, it has been strongly recommended that mangroves should be considered as a valuable source for chemical constituents with potential medicinal and agricultural values (Miles *et al*. 1998). Mangroves and mangal associates living in yet another different environment to that of marine and terrestrial plants, can produce metabolites, which may in turn, are unique to these plants and are of interest to the 'curious' chemist Although the chemical constituents of most mangrove plants still have not been studied extensively, investigations have led so far to the discovery of several novel compounds with prospective medicinal value for the discovery of new chemotherapeutic agents. In general, mangroves are trees and shrubs, which grow in saline coastal habitats in the tropics and subtropics. The mangrove dwellers get food and wide variety of traditional products and artifacts from mangroves. Extracts and chemicals from mangroves are used mainly in folkloric medicine *(e.g*. bush medicine), as insecticides and pesticides and these practices continue to this day (Bandaranayake, 2002). During mangrove restoration, mangrove seedlings often suffer from barnacles that attach themselves to the plants, seriously affecting photosynthesis and stem lenticels metabolism. The weight of barnacles may cause the seedlings to break off, and even die. Therefore, they can be serious fouling animals endangering mangrove ecological restoration engineering (He and Lai, 2001; Mo et al., 2003; Han et al., 2004; He and Mo, 1995; Li et al., 1998).Promising antibacterial activity of The effects of Mangrove extracts on some microorganisms including *Shigella sp.,Staphylococcus sp*., *Pseudomonas sp*(Ravikumar et al., 2010). has been reported in some studies in the area of pharmacology Also different type of solvents including Ethanol, Chloroform, Ethyl acetate have been used for extraction and antifungal activity of methanol extract of *Exoecaria agallocha* and *Bruguiera gymnorrhiza* trunks are some other examples of pharmaceutical potential of mangrove plants(Abeysinghe et al., 2006). Fungal diseases are one of the major problems facing the citrus production in all areas of the world and resulted in enormous economic losses. Jongsuvat (1981) found that the extracts of the plant were not toxic to experimental mice but displayed significant anti-leukemic activity. A novel alkaloid, acanthicifolin, has been isolated from the plant. The purpose of this study Antimicrobial effect of various concentrations of mangrove leaves on Penicillium digitatum is the most devastating pathogen of citrus fruit to increase the storage time of citrus fruit.

2. Materials and methods

2.1. Plant material

The leaves of Avicennia Marina were collected from the mangrove forests of Qeshm *Iran which extends from 26°50'N and 56°0'E. Branches and leaves of the plants chosen were cleaned with tap water, dried for 72h dried in shadow and appropriate condition, and then filtered using a 60-mesh sieve.*

2.2. Extract preparation

Ethanol & Methanol extracts of the samples were obtained by the following procedure. The extract was prepared by maceration 1 g sample was extracted with 50 mL ethanol 96˚or Methanol for 20 h. The mixture was filtered through Whatman No.1 and the filtrate was evaporated to dryness under vacuum at 40 °C. The dry extract

was weighed and the yield was calculated. 20% glycerin solution has been used as a solvent. 50 grams of fine powder of the leaves was added to 200 ml of prepared glycerin solvent and heated for 20 minutes. The extract was then filtered using paper filters and then centrifuged in 9000g for 15 minutes (Ahmad and beg., 2001).

2.3. Inucolum Preparation

Fungi penicillium digitatum was maintained on Sabouraud dextrose agar. Sterile distilled water containing 0.05% Tween 80 was added to the surface growth and spores and hypae were scraped off with a sterile wire loop. A spectrophotometer set at 530 nm used to adjust the sus-pension to 90% transmittance. This resulted in a con-centration of about 1×106 CFU/mL. (Collins etal., 1995).

2.4. Screening for antibacterial activity

The method of Collins (1995) was used to test for antimicrobial activity of the plant extracts. 0.2g of the extract was reconstituted in 5ml sterile distilled water and vortexed for homogeneity. 1ml of the *Avicennia marina* extract was added to Petri dishes having sterile molten nutrient agar (Oxoid) to make a final concentration of 2000 µg/ml. The plates were prepared in duplicates and allowed to set at room temperature. A loopful each of the Standardized culture of test organisms was streaked on the solidified medium and incubated for 72h at 27C^{o}. Control plates comprising extract without inoculum and inoculum with extract were made in parallel(Collins *et al*., 1995).

2.5. Antimicrobial activity

The antimicrobial assay was performed by methods viz. agar disc diffusion method for solvent extract. The molten Mueller Hinton agar was inoculated with 100 µl of the inoculum (1 x 10^{8} cfu/ml) and poured into the Petri plate. For agar disc diffusion method, the disc (0.7 cm) was saturated with 100 µl of the test compound, allowed to dry and was introduced on the upper layer of the seeded agar plate. The plates were incubated72 h at 27C. Microbial growth was determined by measuring the diameter of zone of inhibition. For each fungi strain, controls were maintained where pure solvents were used instead of the extract. The result was obtained by measuring the zone diameter. The experiment was done three times and the mean values are presented (He and Zhou 2007).

3. Results and discussion

The results of antifungal effects mangrove leaf extract (ethanol, methanol & glycerin) in screening antimicrobial activity method were shown in Table 1& Figure 1.

The results showed that mangrove leaf extract in screening antimicrobial activity method in 2000 µg/ml, inhibit *Penicillium digitatum* growth. The results of the antifungal effect of mangrove leaf extract in agar diffusion method are shown in Table 2. The results show that mangrove leaf extract at all concentrations (20, 40, 60 and 80%) had inhibitory effect on *Penicillium digitatum.* Ethanol 96 degree extract was more effective than methanol 96 degree and 20% glycerin extract Against on *Penicillium digitatum,* Mangrove plant leaves caused by ethanol 96 degree extraction is more effective materials. This result, corresponds with findings of Mahasneh (2000) on the plant species Qatari, and is determined that the aqueous extract of this plant is antimicrobial activity; however butanol extract of it is capable to inhibition of *Pseudomonas aeruginosa* growth (Mahasneh 2000).

Table 1

Antimicrobial activity of *Avicenna marina* on *Penicillium digitatum* (Screening for antibacterial activity).

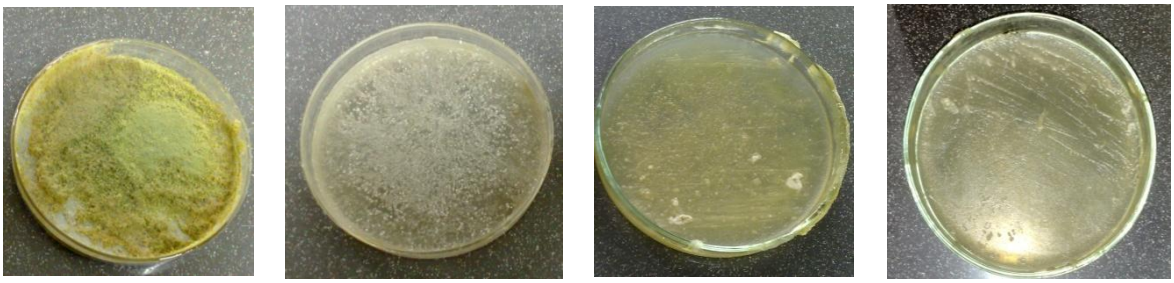
Concentration of 2000 µg/ml	**Methanol**	**Ethanol**	**Glycerin**
Penicillium digitatum	+	+	+

(+) no *Penicillium digitatum* growth on culture and antimicrobial activity of *Avicenna marina* extract

Antifungal metabolites Mangrove plant leaves include alkaloids, flavonoids and related compounds, modified fatty acids, oxygen heterocyclics, proanthocyanidins, quinones, stilbenes, terpenoids and triterpenoid saponins (Bandaranayake, 2002). The latex showed no activity against bacteria and yeast but some activity against fungi. The leaves were a rich source of a different class of terpenoids and along with stilbenes, inhibited histamine

release from rat mast cells and were active against *Bacillus* and *Staphylococcus* (Colmenares *et al*., 1998). Antibacterial activity of the leaves of Thespesia populnea is due to the known triterpene lupeol, and gossypol was the active ingredient in the flowers, which accounted for its antifertility activity (Goyal et al., 1998).

Xanthone is an active substance in these plants. These compounds have Toxicological characteristics, such as, anti-tumor, anti-inflammatory, anti-fungal. Results showed mangrove extract can be used as natural antimicrobial in food products.



A: Control | B: concentration of 2000 µg/ml Glycerin | C:concentration of 2000 µg/ml Methanol | D: concentration of 2000 µg/ml Ethanol

Fig. 1. Antimicrobial activity of *Avicenna marina* on *Penicillium digitatum* (Screening for antibacterial activity).

Table 2

Average diameter (mm) of microbial free zone area of by *Avicenna marina* on leaves extract, *Penicillium digitatum* (disk agar diffusion method)

Penicillium digitatum				
Concentration Ethanol(w /w)	20	40	60	80
	8.5	9.5	11.5	13
Penicillium digitatum				
Concentration Ethanol(w /w)	20	40	60	80
	8	8.5	10	11.5
Penicillium digitatum				
Concentration Ethanol(w /w)	20	40	60	80
	7.5	8	9	11

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