

## Antimicrobial effect of mangrove extract on *Escherichia coli* and *Penicillium Digitatum*

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**Abstract.** Different levels of mangrove extract from *Avicenna Marina* have been used to consider its antimicrobial effect. A pathogen, gram negative bacteria *Escherichia coli* and a rotten fungi, *Penicillium digitatum* has been affected by mangrove extract using disc inhibitory zone. The length of inhibition zone was measured in millimeters. Results showed that mangrove extract has inhibitory effect on both tested fungi and pathogen but its effect on pathogen is stronger and more considerable.

**Keywords:** Mangrove plant, antimicrobial, food area

### 1. Introduction

Mangroves are usually found only in tropical climates, as they need consistently warm conditions for development and survival. They occur in approximately 112 countries and territories and are largely confined to the regions between 30° north and south of the equator.[1] Medicinal plants are known to produce certain bioactive molecules which react with other organisms in the environment; inhibiting bacterial or fungal growth. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world[2].

Mangrove plant extracts have been used for centuries as a popular method for treating several health disorders. Plant-derived substances have recently become of great interest owing to their versatile applications. Mangroves are biochemically unique, producing a wide array of novel natural products. Mangrove and mangrove associates contain biologically active antiviral, antibacterial and antifungal compounds[3]. The effects of Mangrove extracts on some microorganisms including *Shigella sp.*, *Staphylococcus sp.*, *Pseudomonas sp.* has been reported in some studies in the area of pharmacology[4, 5]. Also different type of solvents including Ethanol, Chloroform, Ethyl acetate have been used for extraction[4].

*Avicenna marina*, commonly known as gray mangrove, belongs to the family *Aviceniaceae*. It grows as a shrub or tree to a height of three to ten meters, or up to 14 meters in tropical regions, growing in the saline intertidal zones of sheltered coast lines. It has been reported to tolerate extreme weather conditions and high winds[6].

The increasing popularity of fresh products has been attributed to the associated nutritional and health benefits[7]. However, fruits and vegetables can be contaminated with pathogens at all stages from their growth to consumption due to several factors, such as soil, manure (human and other animal feces), water, insects, environment, post-harvest handling, washing, cutting and transportation. Although spoilage bacteria, yeasts and moulds usually dominate the micro-flora of raw food plants, pathogenic bacteria and viruses capable of causing human diseases are found in or on fruits and vegetables, such as leafy vegetables, cabbage, celery, cucumber, radish, tomato and bean sprouts [8, 9]. When fruits or vegetables are consumed raw, as is the case with salads, harmful viable micro-organisms may be present. The recent and increasing number of

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outbreaks of food-borne diseases associated with the consumption of fresh uncooked produce, like vegetables and fruits, has raised concern that these products may be an increasing source of food-borne infections[10]. Strains of *E. coli* have been shown to be the causative organism in many food-borne illnesses brought about by the consumption of contaminated raw vegetable salads available in markets and supermarkets[9]. The major postharvest problem of citrus is decay by fungi. Specifically, the diseases of oranges (*Citrus sinensis*) include green-rot by *Penicillium digitatum*, blue-rot by *Penicillium italicum* especially at refrigeration temperatures[11]

Little is known about the potential of mangroves grown in the Persian Gulf. Also there is no research using mangrove extract in the food area. The present research investigated the antimicrobial properties of mangrove plant as a new source of antimicrobial agents for the food industry as an inhibitor in fresh food packaging. So the main objectives of this study was to consider the possible antimicrobial activity of the glycerin extracts of mangrove plant *Avicenna Marina* against one pathogen(*E.coli*) and one fungal(*P. digitatum*) strain.

## **2. Materials and methods**

### **2.1. Plant material**

The leaves of *Avicennia Marina* were collected from the mangrove forests of Bandar Abbas- Iran which extends from 25- 27 ' N and 55-65' E. The leaves of the plant were shade dried for 15 days and then pulverized into fine powder using pestle and mortar.

### **2.2. Chemical composition**

The ash, moisture and fat content of the leaves were determined by AACC methods.

### **2.3. Extract preparation**

20% glycerin solution has been used as a solvent. 50 grams of fine powder of the leaves was added to 200ml 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. The concentration of the extract was 180g/l.

### **2.4. Test microorganisms**

One type of mould as a spoilage microorganism (*P. digitatum*) and one type of bacteria (*E. coli*) as a pathogen which are mainly important in the fruit and vegetables area have been selected.

*E. coli* has been isolated from poultry feces using a different dilution method. *P. digitatum* was isolated from rotten orange.

### **2.5. Determination of antimicrobial activity**

A method suggested by He and Zhou (2007) with some modification has been used [12]. Cotton discs were used and the diameter of the inhibitory zone was considered. The cotton discs (6mm) were wetted with the extracts at the levels of 180, 360, 540, 720, 1080, 1260 mg/ml for *P. Digitatum* and 18, 180, 540, 720, 1080 mg/ml for *E.coli*. Discs were left for some time to extract diffused on them. After 5 minutes, the disks were directly transferred to the surface of the pre cultured plates with sterile method. EMB plates for *E. coli* and YCG Agar plate for *P.digitatum* have been used. Finally incubation was done with the closed lid at 37°C for 24-48 hours for *E. coli* and 25°C for 48 hours for *P. digitatum* for all plates. The effects were compared with that of the standard culture loaded with sterile disc. Discs were observed for zone of inhibition by measuring diameter of inhibition zone (DIZ) using scale. For *E.coli* shiny green color was used as a sign for bacterial growth. All tests were performed in triplicate.

## **3. Result and discussion**

The moisture content and ash content of dried leaves was 1.24% and 50% respectively. The high ash content of the leaves was a very interesting result which shows the high amount of the minerals in mangrove leaves. The leaf extract has been tested for its antimicrobial effect against *E. coli* and *P. digitatum* .For *E.coli*, in the level of 18 mg/ml nearly 50 % of the growth was inhibited by the effect of mangrove extract. *E.coli* growth was successfully inhibited at the level of 720mg/ml and higher.

Table 1 shows the result of inhibition for *E. coli*. Microorganism activity and inhibition response was completely concentration dependent. However, some studies on natural antimicrobial agents reported that inhibition responses observed for of *E. coli* were concentration dependent up to a certain level and then get decreased at further higher concentration[2]. Abeysinghe et al used three different mangrove plant extracts. They showed that mangrove plant extracts have antimicrobial activity against pathogenic bacterial strains including antibiotic resistant strains. Antibacterial activity may be due to active components which are present in the plant extract[4].

Table 2 shows the result of inhibition zone for *P. Digitatum*. Results show that the extract has less effect on the fungi and complete inhibition happens at higher concentrations.

Table 1-Antimicrobial activity of Avicenna Marina on E. coli

Concentration(mg/ml)	Antibacterial activity(DIZ)mm
0	Whole plate
18	6.2
180	6.3
360	6.5
540	6.7
720	7
1080	7.6

Table 2-Antimicrobial activity of Avicenna Marina on P. Digitatum

Concentration(mg/ml)	Antibacterial activity(DIZ)mm
0	Whole plate
180	6*
360	6*
540	6.1
720	6.4
1080	7
1260	7.5

\*DIZ value is 6mm, that means there was no microbial growth

It seems that glycerol extract of mangrove plant has a better effect on *E.coli* growth rather than *P.digitatum*. Antibacterial activity may be due to active components which are present in plant extracts. This inhibitory effect can be noticed to use mangrove plant extracts as a new generation of antimicrobial agents in the food area.

#### 4. References

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