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Antioxidant and antifungal activities of two spices of mangrove plant extract

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ABSTRACT

Objective: To evaluate the antifungal and the radical scavenging capacity related to antioxidant potential of ethanol and water extracts of leaves of *Rhizophora mucronata* (*R. mucronata*) and *Avicennia marina* (*A. marina*) mangrove plant species against five postharvest pathogenic bacteria.

Methods: *In vitro* assessment of antioxidant and antifungal activities was evaluated in this present study for both aqueous and ethanol extracts prepared from leaves of *A. marina* and *R. mucronata*. The antioxidant activities of these mangroves were evaluated by using reducing power and 1,1-diphenyl-2-picrylhydrazyl assays with butylated hydroxytoluene and L-(+)-ascorbic acid as standards.

Results: The result showed that the antioxidant activities of all extracts increased with increasing concentration of extracts. However, the ethanol extracts of both species showed the highest antioxidant activities. Antimicrobial tests were then carried out by the disk diffusion method. The ethanol extracts of both species showed antifungal activities on *Penicillium purpurogenum*, *Penicillium chrysogenum*, *Penicillium notatum*, *Aspergillus niger*, *Alternaria alternata* and *Penicillium italicum*. However, none of the water extracts exhibited antifungal activity on the studied fungi. Among all the pathogens, tested *Aspergillus flavus* was the most resistant fungi. Different concentrations of extracts from *A. marina* and *R. mucronata* showed different amounts of control against tested fungal strains.

Conclusions: This study indicated that mangrove species has natural antioxidant and antifungal properties.

1. Introduction

Fungal diseases are one of the main problems opposite the crop production in all areas of the world and result in enormous economic losses. Four years ago, chemical fungicides have been applied for control of postharvest disease of fruit. However, it caused contamination of the environment and pathogen became more resistant to the main problems in human health caused by eating fruit with toxic residues, thus searching for natural pesticides is necessary[1]. Recently, essential oils and plant extracts have been studied for their antifungal, antibacterial and antioxidant

activities[2,3]. Antimicrobial activities of different medicinal plants from different parts of the world were reported by before research. It has been stated that the higher plants have compounds with antimicrobial agents[4,5]. Antioxidants act as a main defense in contrast to radical mediated toxicity by defending cells against the damage of free radicals. Some plant products like phenol and flavonoids can be act as biological effects, such as antioxidant and free radical scavenging[6,7]. Scientific research throughout the world has found evidence to support the fact that mangrove foliar extracts have great potential against microbial pathogens. Mangrove plants have found the interface between land and sea and have some biological activities like antibacterial and antifungal properties[7,8]. Recently, it has been powerfully suggested that mangroves would be considered as a valuable source for chemical components with possible medicinal values, although the chemical components of most mangrove spices still have not been studied widely[9].

Rhizophora mucronata (family of Rhizophoraceae) (*R. mucronata*)

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is usually identified as Asiatic mangrove generally distributed along the coastal tropical and subtropical area, which has been stated to have several medicinal properties[10]. *Avicennia marina* (Forssk.) (*A. marina*) is commonly identified as gray mangrove tree classified in the plant family Avicenniaceae and is commonly used for ulcers. *R. mucronata* is a mangrove species that belongs to Rhizophoraceae family and widely occurs on the southeast coast of India and Iran[11]. Mangroves can grow under stressful conditions such as high and low salinity of water, heavy metal pollution, abundant living microorganisms and insects. According to the research before, mangrove plants were used as medicinal plants for control of pathogen. However, only limited studies have been carried out to identify metabolites responsible for antimicrobial and antioxidant properties of these plants.

Our current study is aimed to evaluate the antifungal and the radical scavenging capacity related to antioxidant potential of ethanol and water extracts of leaves of *R. mucronata* and *A. marina* mangrove plant species against five postharvest pathogenic bacteria.

2. Materials and methods

2.1. Collection of plant materials and preparation of the extracts

The plant collection was carried out in native stands. Leaves of *A. marina* and *R. mucronata* mangrove species were collected from Geshm (26°50' N, 56°0' E) and Syrik of southeast coast of Iran, mangrove forest, respectively. The amount of 25 g residue of *A. marina* and *R. mucronata* mangrove leaves was added into 125 mL ethanol (96%) or distilled water. The ethanol and aqueous extracts mixture were well-maintained at 25 °C of laboratory temperature for 48 h and was moved every few hours with a glass rod. The collecting supernatant was centrifuged by 9000 r/min 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 filtered by 0.45 µm Whatman filter paper[12].

2.2. Antifungal activities

Plant extracts were pure by filtration through 0.45 µm Millipore filters. Antifungal tests were then done 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 with determining the zone of inhibition against the tested fungus in contrast to the negative control[13].

2.3. 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 that inhibited the growth of molds colony[14].

2.4. Reducing power

The reducing power of the extracts was determined by the method of Ye *et al.*[15]. Briefly, extracts (50–500 µg) in 1 mL of solvents were mixed with 2.5 mL of phosphate buffer (0.2 mol/L, pH 6.6) and 2.5 mL of potassium ferricyanide (1%) and then the mixture was incubated at 50 °C for 30 min. Then, 2.5 mL of trichloroacetic acid (10%) was added to the mixture, later centrifuged at 3000 r/min for 10 min. Finally, 2.5 mL of the upper layer solution was mixed with 2.5 mL of distilled water and 0.5 mL of FeCl₃ (0.1%) and the absorbance was read at 700 nm.

2.5. 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity

Free radical scavenging activity of crude extracts was tested by DPPH using the method of Milella *et al.*[16]. About 1 mL of DPPH (0.1 mmol/L) solution in methanol was added to 3 mL of crude extracts (7.5 µg/mL–25 mg/mL) in appropriate solvents and was shaken vigorously and allowed to stand at room temperature for 30 min. The absorbance was read at 517 nm in a UV-visible spectrophotometer (Model Cecil3200 England UV). A low absorbance of the reaction mixture indicated a high free radical scavenging activity. Ascorbic acid (AA) and butylated hydroxyanisole (BHA) were used as positive control and phosphate buffer was used as a blank. The percentage of DPPH scavenging effect was calculated with the following equation:

$$\text{DPPH scavenging effect (\%)} = \frac{A_c - A_t}{A_c} \times 100$$

where A_c was the absorbance of the control reaction and A_t was the absorbance in presence of the sample of the extracts.

2.6. 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.

3. Result

3.1. Reducing power

The reducing power of ethanol extract of leave of *A. marina* was the highest among all the extracts and it increased as the amount of extract increased (Figure 1). The water extraction of *R. mucronata* also showed the high reducing power in the highest extraction (700 and 900 µg/mL). Ethanol extract of *A. marina* showed significantly higher reducing power rather than water extract. According to Figure 1, reducing power of all extract was increased by increasing concentration. The reducing power of all of these extracts was markedly lower than those of ascorbic acid in lower concentration (Figure 1).

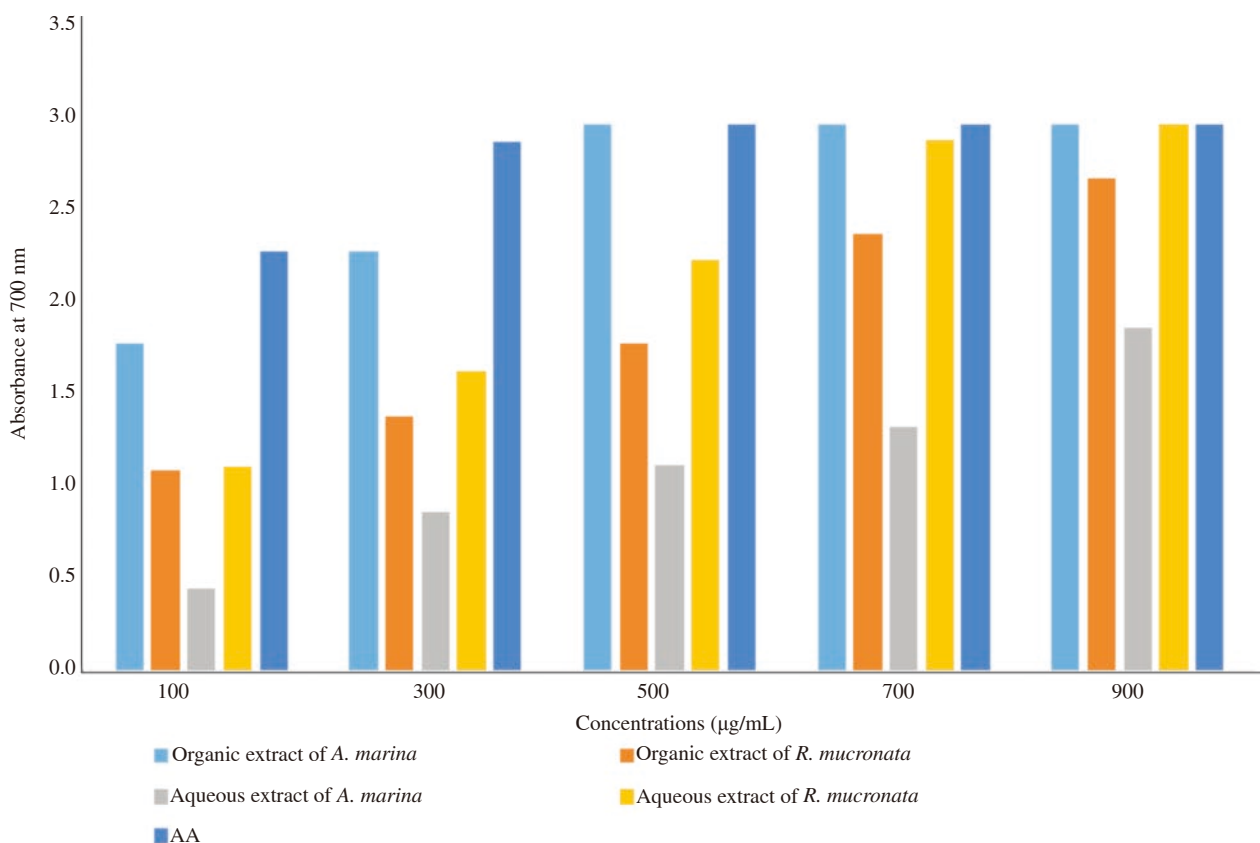


Figure 1. Total reducing power of mangrove extracts in comparison with standard AA.

3.2. DPPH radical scavenging activities

Percent DPPH scavenging activities of ethanol and water extracts of two species of mangrove were concentration-dependent. Like reducing power, the highest percent DPPH scavenging activity was detected in the ethanol extract of *A. marina* and the lowest activity was determined in the water extract of that. In the concentration of 0.015 mg/mL of water or ethanol extracts of mangroves, scavenging activities were between 5% and 25%, respectively, whereas in the concentration of 25 mg/mL of each mangrove extract, scavenging activities were about 64% and 83%, respectively (Figure 2). A radical scavenging method was chosen in this study, because radical scavenging was the main mechanism, by which antioxidants acted in foods. The DPPH assay had been widely used for screening antioxidants due to its simplicity and reproducibility.

3.3. Antifungal activities

Results of our study showed that ethanol extract of *A. marina* reduced the growth of *Penicillium purpurogenum* (*P. purpurogenum*), *Penicillium chrysogenum* (*P. chrysogenum*), *Penicillium notatum* (*P. notatum*), *Aspergillus niger* (*A. niger*),

Alternaria alternata (*A. alternata*) and *Penicillium italicum* (*P. italicum*). However, no inhibition of the growth of *Aspergillus flavus* (*A. flavus*) was observed in ethanol extract of *A. marina*. Ethanol extracts of *R. mucronata* also showed the same effect on different fungi and this species showed more inhibitions than ethanol extracts of *A. marina* against on *A. flavus*. The rate of reducing growth of ethanol extract of two leaves was nearly the same. Our result showed that aqueous extracts had no antibacterial effect on *A. flavus*, *P. purpurogenum*, *P. chrysogenum*, *P. notatum*, *A. niger* and *A. alternata* and it was not able to prevent the growth of fungi on culture (Table 1). No inhibition of the growth of different fungi was observed in water extracts of two species. However, the maximum size of inhibition zone (14 mm) was detected in ethanol extract of *A. marina*, against *A. alternata* (Table 1). Results showed that MFC of ethanol extract of *A. marina* for *P. purpurogenum*, *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. Results showed that MIC of ethanol extract of *R. mucronata* for *A. flavus* and *A. niger* was 100 mg/mL, while for *P. notatum* and *P. purpurogenum* was 50 mg/mL. MFC of ethanol extract of *A. marina* for *P. chrysogenum* and *A. alternata* was 25 and 75 mg/mL, respectively (Table 2).

Table 1

Antifungal activity of mangrove extracts against selected fungal strains.

Extracts	Inhibition zone (mm)					
	<i>P. purpurogenum</i>	<i>P. chrysogenum</i>	<i>P. notatum</i>	<i>A. flavus</i>	<i>A. niger</i>	<i>A. alternata</i>
Ethanol extract of <i>A. marina</i>	13	12	12	0	13	14
Aqueous extract of <i>A. marina</i>	0	0	0	0	0	0
Ethanol extract of <i>R. mucronata</i>	12	13	13	10	11	12
Aqueous extract of <i>R. mucronata</i>	0	0	0	0	0	0

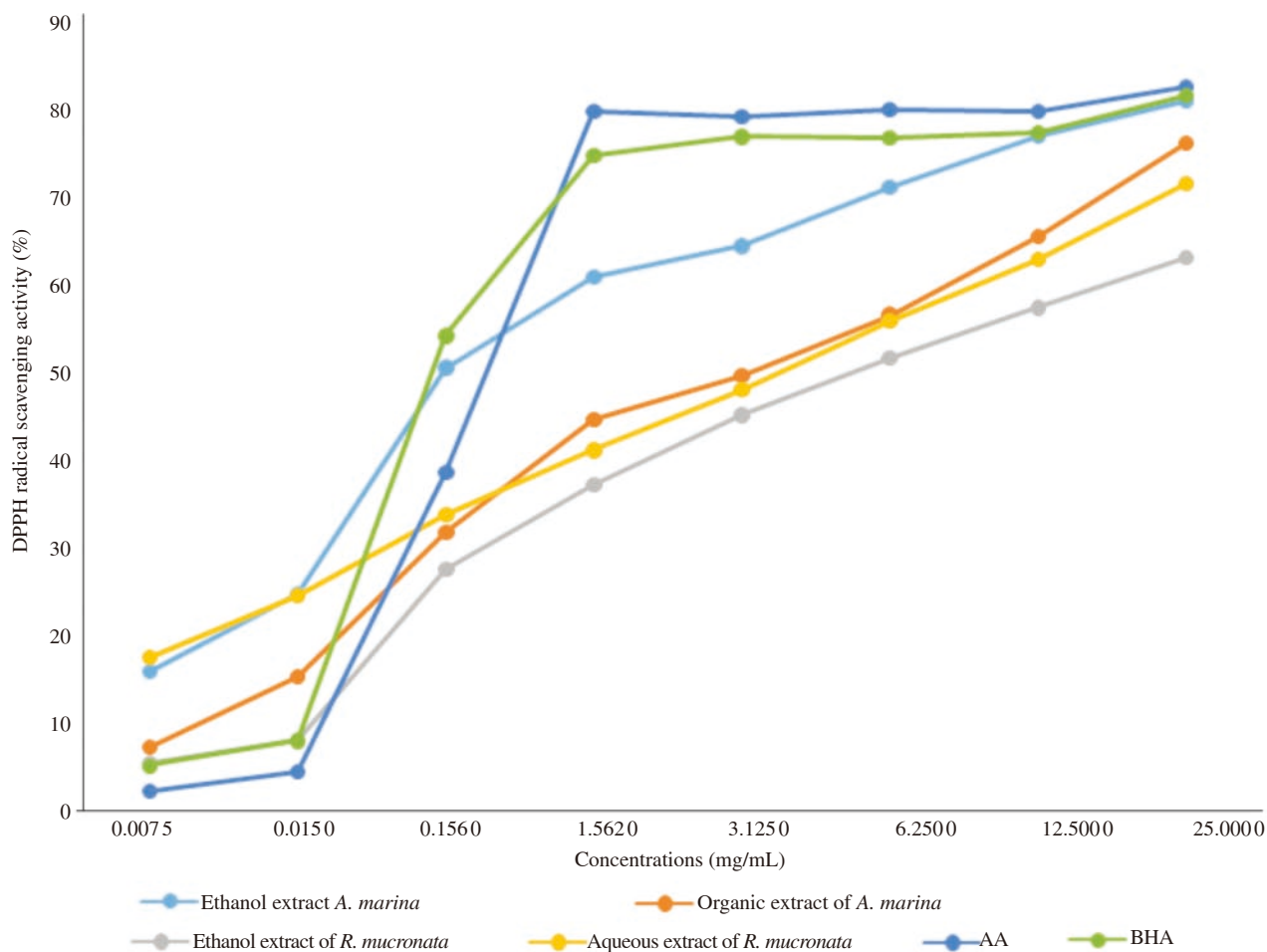


Figure 2. DPPH scavenging activity of mangrove extracts in comparison with standard AA and BHA.

Table 2

MFC of ethanol extracts of *A. marina* leaves on fungal strains.

Fungal strains	MFC (mg/mL)	
	Ethanol extract of <i>A. marina</i>	Ethanol extract of <i>R. mucronata</i>
<i>P. purpurogenum</i>	50	50
<i>P. chrysogenum</i>	50	25
<i>P. notatum</i>	50	50
<i>A. flavus</i>	–	100
<i>A. niger</i>	75	100
<i>A. alternata</i>	25	75

4. Discussion

In recent years, the investigation on antioxidants from medicinal plants is a fast increasing field of research and several antioxidants have been studied such as flavonoids and other phenolic compounds[17]. Our result showed that ethanol extracts of both species have high antioxidant activities. These results approve with other results on antioxidant ability of plant. Researcher showed that methanol extracts of some mangroves exhibited high antiradical activity contrary to DPPH. Antioxidant activity of mangrove plants was correlated with total phenolic and flavonoid contents. Prior reports also showed that mangroves are rich in polyphenols and tannins[18].

The results of Wei *et al.* showed that all the extract mangrove plants like *Kandelia candel* have strong antioxidant activity[19]. There was a significant correlation between the total phenol concentration and the

reducing power of the plant. Cruz *et al.* studied biological evaluation of leaves, roots and barks of *Rhizophora mangle*[2]. They showed that ethanol extracts of roots and leaves had the highest antioxidant activity.

Microorganisms mainly fungi and bacteria are the main pathogenic organisms having potential to reason fruit diseases. Our results indicated that the ethanol extract of *A. marina* leaves generally had effect on *A. alternata* and had the least impact on *A. flavus*. The results showed that MFC of ethanol extract of *A. marina* leaves for *A. alternata* was 25 mg/mL and for *A. niger* was 75 mg/mL. However, MFC of ethanol extract of *A. marina* leaves for *P. notatum*, *P. purpurogenum* and *P. chrysogenum* was the same (50 mg/mL). Effect of mangrove extract on microorganisms was reported in before references.

Bhimba *et al.* reported that acetate extract of *Avicennia officinalis* showed significant antibacterial activity with zone of inhibition of 13 mm against *Escherichia coli* and 11 mm against *Staphylococcus aureus*[20]. Gurudeeban *et al.* showed that alkaloid-rich extract of some mangrove spices had antibacterial and antioxidant chemicals[8]. Abeyasinghe *et al.* studied 12 different plant extracts[21]. They found that the extracts of *A. marina*, *Avicennia officinalis* and *Bruguiera sexangula* showed different grade of growth inhibition against tested bacteria. Alizadeh-Behbahani *et al.* showed that the ethanol extract of *A. marina* leaves was inhibited the growth of *A. flavus* and *P. italicum* by disk agar diffusion test[22]. The results showed that MFC of ethanol extract of *A. marina* for *A. flavus* was 32 mg/mL and for

P. italicum was 16 mg/mL. Ethanol extract compared to the water extract was more effective against pathogens. Alizadeh-Behbahani *et al.* also presented that mangrove leaves extract at all concentrations (20%, 40%, 60% and 80%) had inhibitory effect on *Penicillium digitatum*[23]. In their study, medium diameter (mm) of microbial free zone area of *A. marina* was 13 in 80 % concentration of leaf extract.

Antifungal activity may exist in plant extracts due to active components. However, some plant extracts were not capable to exhibit antimicrobial activity against tested fungi strains. John *et al.* reported that most of the antimicrobial active complexes dissolve in polar solvent better than in water[24]. Karami *et al.* reported that the ethanol extract revealed a higher antimutagenic activity than the water extract[25]. They showed that *A. marina* leaf extract has inhibition effect on typhimurium Tel 100 bacterial growth. Malini *et al.* showed that ethanol fraction of *Aloe vera* and *Coleus aromaticus* exhibited more effect on suppressing the fungal growth rather than water extract[4]. Mouafi *et al.* studied four extracts from *Rhizophora stylosa* (*R. stylosa*) and *A. marina* leaves[9]. They showed that ethyl acetate had best results as inhibition zones. In their experiments, leaf extracts of *R. stylosa* showed inhibition zones ranged from 11 to 19 mm by leaf extracts of *R. stylosa*, while *A. marina* showed 11–20 mm against tested pathogens.

The current search indicated the potential of some mangrove to find new active compounds with future applications in postharvest disease control. Extra researches are essential to complete the research in the biological activities and identify the bioactive components of *A. marina* and *R. mucronata*. To the best of our information, this is the first scientific report which showed the antifungal activity of ethanol extracts of *A. marina*, *R. mucronata*, *P. notatum*, *P. purpurogenum*, *A. niger* and *P. chrysogenum*.

Conflict of interest statement

We declare that we have no conflict of interest.

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