African Journal of Biotechnology Vol. 11(90), pp. 15690-15694, 8 November, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB12.083
ISSN 1684–5315 ©2012 Academic Journals

Full Length Research Paper

Antibacterial activity of *Mentha piperita* against selected food spoiling bacteria

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Accepted 26 June, 2012

Leaf and stem extracts of *Mentha piperita* plant were tested against four bacterial isolates namely: *Bacillus* sp. (S1), *Bacillus* sp. (S2), *Bacillus halmapalus* and *Streptomyces sodiphilus*, isolated from commercial *Spirulina* products. The extraction was carried out with solvents such as petroleum ether, acetone, methanol and ethanol. Antibacterial assay revealed that *B. halmapalus* was highly sensitive to both leaf and stem extracts. *Bacillus* sp. (S2) and *S. sodiphilus* was moderately sensitive to all extracts.

Key words: Antibacterial activity, *Mentha piperita*.

INTRODUCTION

In general, plants are considered as important resources in providing novel antimicrobial compounds of commercial value. However, studies related to the isolation of bioactive potentials from plant resources against food borne pathogens are limited**.** Since ancient times, plants have been model source of medicines as they are a reservoir of chemical agents with therapeutic properties. The general population is increasingly using herbal medicines as dietary supplements to relieve and treat many different human disorders (Cutler, 1995). Herbs and spices are important part of the human diet. They have been used for thousands of years to enhance the flavour, colour and aroma of food (DeSouza et al., 2005). In addition to boosting flavor, herbs and spices are also known for their preservative and medicinal value, which forms one of the oldest sciences. Yet it is only in recent years that modern science has started paying attention to the properties of spices (Draughon, 2004). The search for natural antimicrobials to use in foods is encouraged by the high prevalence of food-borne diseases and the current popular preference of consuming only natural foods (Rasooli, 2007).

Furthermore, the resistance of microorganisms to common and novel antibiotics is on the rise (Schuenzel and Harrison, 2002). Some plant products have been historically used as natural antimicrobials to extend the shelf life of foods and as therapeutics used in folk medicine to treat diseases caused by pathogens (Adiguzel et al. 2009). Currently, plant products are considered to be important alternative sources of new antimicrobial drugs against antibiotic-resistant microorganisms (Sittiwet and Puangpronpitag, 2009) and as preservatives of food. According to this trend, the use of natural compounds derived from plants for the prevention of pathogenic and spoilage microorganisms in foods have been extensively reported (Rasooli, 2007). This communication reports the antibacterial activity of *Mentha piperita* extracts on spoilage causing bacteria isolated from *Spirulina* products.

MATERIALS AND METHODS

Source and description

The plant *Mentha piperita* (Family: Lamiaceae) was selected for this study. It is a perennial aromatic herb of wet places. The stems are quadrangular, usually erect or ascending from rhizomes and leave

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are simple, opposite, petiolate, petiole up to 2 cm long, leafblade 3-6 x 1.5-3.5 cm, ovate-elliptic, serrate, glabrous or slightly pubescent, especially along veins. The flowers are on terminal spike, with calyx 3-4 mm long, tubular, ribbed; calyx teeth subulate and corolla lilac in color. Samples of *M. piperita* were collected from farms in Wadi Hanifa, Riyadh, Kingdom of Saudi Arabia. Voucher specimens (No: KSUBM-MP25) deposited at Herbarium unit, Department of Botany and Microbiology, King Saud University.

Extraction

Leaf and stem extracts of *M. piperita* were prepared by cold percolation method. The plant materials were dried under shade for 10 days and ground into fine powder using electric blender. 20 g of dried powder was soaked in 150 ml of solvent and kept for extraction in mechanical shaker for 24 and 72 h. The solvents used for extraction were petroleum ether, acetone, methanol and alcohol. The plant extracts were filtered through Whatman No. 1 filter paper into pill vials. The filtrates were dried until a constant dry weight of each extract was obtained. The residues were stored at 4°C for further use. Discs were prepared at 25, 50, 75 and 100 µg concentration for further use.

Preparation of inoculum

Four bacterial strains isolated from commercially available *Spirulina* powder were used as test organisms. They were *Bacillus* sp. (S1), *Bacillus* sp. (S2). *Bacillus halmapalus* (*B. halmapalus*) and *Streptomyces sodiphilus* (*S. sodiphilus*). A loopful of stock culture was transferred to sterile nutrient broth and incubated at 24 h. The cultures were used for antibacterial assay.

Antibacterial assay

The disc diffusion method (Bauer et al., 1966) was used to screen the antibacterial activity. 0.1% inoculum suspension was swabbed uniformly on Muller Hinton Agar (MHA) plates. The discs loaded with different concentrations were placed and incubated at 37°C for 24 h. After incubation, the zone of inhibition were measured with transparent ruler in millimeter. Standard methicillin discs were used as positive control.

RESULTS

The crude petroleum ether, acetone, methanol and ethanol extracts of *M. piperata* leaves and stem were tested for antibacterial activity. The antibacterial activity of the *M*. *piperita* was assessed through disc diffusion method and the zone of inhibition with different concentrations of extracts was tabulated (Tables 1 and 2). Acetone and ethanol extracts of leaf at 50, 75 and 100 µg showed inhibitory activity against *Bacillus* sp. (S1). The maximum inhibition was observed at 100 µg with acetone and ethanol extracts. Methanolic extracts did not show any inhibitory effect whereas petroleum ether extracts showed moderate inhibition zone at higher concentration. Extracts of stem did not show any effect on *Bacillus* sp. (S1) irrespective of the solvents used. All extracts of leaves shows activity against *Bacillus* sp. (S2) except 24 h extract of petroleum ether (Table 1). The maximum inhibitory effect (9 mm) was found with acetone extract at 72 h and minimum inhibition (6 mm) observed in petroleum ether. Acetone stem extracts did not show significant inhibitory effect on the bacteria. Methanol and ethanol extracts showed good and moderate inhibitory effects on bacteria respectively.

Both leaves and stem extracts showed inhibitory effect on *B. halmapalus.* Petroleum ether and ethanol extracts of leaves showed good inhibitory effect on *B. halmapalus* in 24 h. Acetone and methanolic extracts showed activity at higher concentrations (Table 1). The minimum inhibitory (5 mm) effect observed in 24 h extracts and the maximum effect showed in 72 h extracts of petroleum ether and ethanol extract at higher concentrations. All solvent extracts of stems showed good inhibitory effect on *B. halmapalus.* The minimum and maximum inhibitory effects were 5 and 12 mm respectively in stem extracts (Table 2).

Twenty four hours petroleum ether and acetone extracts showed minimum (7 mm and 6 mm) inhibition at higher concentration on *S. sodiphilus*. However, ethanolic and methanolic extracts inhibited the growth at all concentrations (Table 1) both in 24 and 72 h extracts. All extracts of stems showed inhibitory effect against *S. sodiphilus* in both 24 and 72 h extracts.

DISCUSSION

Different plants are rich in a wide variety of secondary metabolites such as tannins, terpenoides, alkaloids and flavonoides. Therefore various parts of the plants (flowers, buds, leaves, stem, skin and pulp) have been used for thousands of years to enhance the flavor and aroma of food. In addition, they have been found *in vitro* to have antimicrobial properties (Cowan, 1999). The results in this study revealed that the extracts with both polar and non polar solvents were active against tested bacteria that are common cause of food contaminations. In our report, *B. halmapalus* was highly sensitive than other bacterial isolates. *Bacillus* sp. (S2) and *S. sodiphilus* were moderately sensitive. Both leaf and stem extracts showed much inhibitory effect on the selected bacteria. This kind of significant activity might be due to active compounds such as menthol, menthone, menthyl acetate, menthofuran, and limnone (Fleming, 1998). These compounds have higher medicinal value especially in the treatment of dyspepsia, epigastric bloating, impaired digestion, eructions, and flatulence, tropically used to relieve nasal congestion in the common cold and itch relieving used as tropic protective agents (Alkofahi et al., 1990). The antibacterial activity was expressed at varying degrees relying on both strain and dosage. The various crude extracts of *M*. *piperita* showed significant activity against all the tested bacterial isolates. Similar to our result, the biological activity of *M*. *piperita* against some pathogenic bacteria were reported

Table 1. Antibacterial activity of leaf extracts of *M. piperita*.

Test organism	Concentration (µg /ml)	Zone of inhibition including disc diameter (mm)								
		Solvent extract								Methicillin (1.25 µg)
		Petrol. ether		Acetone		Methanol		Ethanol		
		24*	72	24	72	24	72	24	72	
Bacillus sp. (S1)	25	-	-	-	7	-	-	-	7	35
	50	-	-	7	9	-	-	-	7	
	75	7	-	7	8	-	-	5	9	
	100	7	7	10	10	-	-	5	10	
	Solvent control	-	-	-	-	-	-	-	-	
Bacillus sp. (S2)	25	-	6	-	8	5	7	-	7	35
	50	-	6	-	10	5	8	5	7	
	75	-	7	5	10	7	8	5	8	
	100	-	7	5	12	6	10	5	8	
	Solvent control	-	-	-	-	-	-	-	-	
B. halmapalus	25	10	10	-	8	-	9	5	10	18
	50	10	11	6	8	-	8	5	10	
	75	10	10	8	10	8	10	5	10	
	100	11	12	8	10	8	10	5	12	
	Solvent control	-	-	-	-	-	-	-	-	
S. sodiphilus	25	-	-	-	7	5	6	-	-	18
	50	-	8	-	8	5	7	5	10	
	75	-	11	-	10	6	10	6	10	
	100	7	12	6	10	6	10	6	11	
	Solvent control	-	-	-	-	-	-	-	-	

*Duration of extracts**; (-)** No zone of inhibition.

Table 2. Antibacterial activity of stem extracts of *M. piperita.*

Test organism	Concentration (µg /ml)	Zone of inhibition including disc diameter (mm)								
		Solvent extract								Methicillin (1.25 µg)
		Petrol. ether		Acetone		Methanol		Ethanol		
		24*	72	24	72	24	72	24	72	
Bacillus sp. (S1)	25	-	-	-	-	-	-	-	-	35
	50	12	-	-	-	-	-	-	-	
	75	12	-	-	-	-	-	-	-	
	100	12	-	-	-	-	-	-	-	
	Solvent control	-	-	-	-	-	-	-	-	
Bacillus sp. (S2)	25	-	-	-	-	5	7	-	-	35
	50	-	-	-	-	5	8	5	6	
	75	-	-	5	-	7	9	5	7	
	100	-	7	5	7	6	9	5	7	
	Solvent control	-	-	-	-	-	-	-	-	18

Table 2. Continued.

	25	10	10	-	8	-	9	5	10	
	50	10	11	6	8	-	8	5	10	
B. halmapalus	75	10	10	8	10	8	10	5	10	
	100	12	11	8	10	8	10	5	12	
	Solvent control	-	-	-	-	-	-	-	-	
	25	-	-	-	7	4	6	-	-	
	50	-	8	-	8	4	7	5	10	
S. sodiphilus	75	-	11	-	10	5	10	6	10	18
	100	7	12	6	10	5	10	6	11	
	Solvent control	-	-	-	-	-	-	-	-	

*Duration of extracts**; (-)** No zone of inhibition.

previously (Deans and Baratta, 1998). In this investigation acetone, methanol and ethanolic extracts produced more effect on the tested bacteria than petroleum ether. This corresponds to the previous reports which stated that the ethanol extracts expressed higher activity against the microorganisms (Akroum et al., 2009). Another report revealed the highest antibacterial effect of the ethanolic extract of *M. longifolia* may be due to its high content on flavonoids. In fact these compounds are known for their strong antimicrobial activity (Martini et al., 2004).

Reportedly the lipophilic nature of ethanol extract was cyclic monoterpenes will preferentially part of an aqueous phase and has affinity into membrane structures. This resulted in membrane expansion, increased membrane fluidity and inhibition of a membrane-embedded enzyme (Sikkema et al., 1995). Thus Gram positive bacteria are considered more sensitive to essential oil than Gram negative bacteria (Lambert et al., 2001; Lis-Balchin, 2003) because of their less complex membrane structure. Moreover, there might be several reasons for the lower antibacterial activity shown by the plant extracts as suggested by Parekh and Chanda (2007) either the plant part used or the type of extraction might have resulted in the lower activity in this study, or the time of collection of herbal material and climate, which might, in turn, affect the amount of active constituents in the plant material.

The present study showed that the compounds from *M. piperita* possess potent antibacterial activity. They may contain effective active constituents responsible for eliminating the bacterial contaminants. The active chemical compounds present in *M. piperita* could find place in treatment of various bacterial infections. The results from the present study are encouraging and suggest more investigations of their potentials in the control of food contaminants.

ACKNOWLEDGEMENT

This work is supported by Addiriyah Chair for Environmental Studies, Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia.

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