

## The Antibacterial Activity of Selected Edible Plant Extracts against *Bacillus Cereus*

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### ABSTRACT

Six presumptive *Bacillus cereus* isolates were isolated from 49 samples of food, soil, manure and eggshells in Amman, Jordan. One isolate from eggshells was identified, characterized and confirmed to be *B. cereus* and designated as *B. cereus* (J<sub>u</sub>). Among the 11 water and ethanol plant extracts, which demonstrated the highest antibacterial action against *B. cereus* (J<sub>u</sub>), were sumac fruits (*Rhus coriara*. L) and rossle flowers (*Hipiscus sabdariffa*. L). It was found that the inhibition of the growth of bacteria was proportional to the increase of extract concentrations. Using 1, 2 and 4 mg/ml of each of water and ethanol extracts of rossle and sumac resulted in an inhibition activity (mm diameters) of the growth of *B. cereus* of 2, 6 and 16 and 4, 9 and 12 for rossle and 3, 7 and 15 and 3, 8 and 14 mm, respectively. A complete inhibition of the growth (100%) was demonstrated at the highest concentrations used (1.5mg/ml nutrient agar for sumac and 3.45-4.12 mg/ml media for Rossle). These levels are also found to be as the MIC for the two plant extracts. Heat treatment at 70°C for 3min did not affect the anti *B. cereus* activities of the two extracts. These two edible plants may have the potential to be used as food preservatives after further investigation on their safety and effectiveness against wider spectra of spoilage microorganisms in food.

**KEYWORDS:** Antibacterial, anti *B. cereus*, plant extracts, sumac, rossle.

### 1. INTRODUCTION

Shinagawa (1990) stated that *Bacillus cereus* is a non-fastidious growth requirement and so it is a widely distributed bacteria in nature, which makes it real environmental food contamination organisms. *B. cereus* possesses heat resistance spores, and high hydrophobicity with long appendages making it also of special importance to food industries.

Many wild and domestic plants are used as food,

flavors, and as traditional medicine. Many are used in human diets as health promoters in foods like cereals, confectionary, snack food and beverages. Plants contain aromatic substances like phenolics, tannins, or their derivatives, which are used as defensive barriers against microbial infection or insect infestation (Cowan, 1999). Traditional uses of herbal medicinal plants to elongate shelf life of food are common in oriental countries. The natural antimicrobials and antioxidants derived from fruits, vegetables and most of the edible plants are believed to be of benefit in food preservation, therefore, the use of natural food additives is an attracting scientific field (Negi *et al.*, 2003). Screening tests like chromatography and bioautography have been used to separate the active compounds from pathogens or

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spoilage microorganisms (Nostro *et al.* 2000).

There are many published reports that deal with the antimicrobial activities of plant extracts. It was found that carvacrol (present in the essential oil of oregano, thyme and sumac) was active in reducing the growth of *B. cereus* and inhibiting its toxin production in brain-heart infusion (Ultee *et al.*, 1999). Garlic water extracts completely inhibited the growth of *B. cereus* in pour plate technique (Saleem and Al-Delaimy, 1982). Spices and herbs extracts, using disc diffusion technique, were active against the growth of *Shigella* spp (Bagamboula *et al.*, 2003). Garcia *et al.* (2002) studied the effect of the extracts of 14 plants used in traditional medicine in Mexico on the growth, spore formation and enterotoxin production of *Clostridium perfringens* type A. They found that the most effective plants were *Psidium guaiava* L., *Heamotoxylin brasiletto* and *Euphorbia prostate*. Seuschner and Iclisch (2003) found that Garlic in broth was more active as anti *Listeria monocytogenes* than cloves and red hot chilli. Negi *et al.* (1999) found that turmeric oil (a by- product of turmeric corcomin production process) possess antibacterial action against many types of bacteria. They suggested the possibility of using turmeric oil as a preservative agent in food industry. Ahmed and Beg (2001) found that out of 42 ethanol extracts of Indian medicinal plants, 40 possessed varied levels of antimicrobial action against one or more drug resistant bacteria, while 24 of these extracts possessed antimicrobial action against the yeast. Conner and Beuchat (1984) reported that among the 32 essential acids extracted from plants, garlic extracts were the most potent inhibitor of spoilage and industrial yeast. Onion, oregano and thyme were also strong inhibitors. Friedman *et al.* (2003) reported that anti-bacterial components of plant essential oils and some of their isolated constituents were active against *C. jejuni*, *E. coli*, *L. monocytogenes* and *S. enterica*.

There are limited studies on the antibacterial activities of many domestic and wild edible plants in Jordan and in

the region. It was the task of this investigation to study the effect of 11 water and ethanol plant extracts used as food or spices in the region on *Bacillus cereus*, which is a widely distributed bacteria in the environment and that causes food poisoning.

## 2. MATERIAL AND METHODS

### Isolation and Characterization of *Bacillus cereus*

A total of 49 samples (weighing approximately 500gm each) of foods (ground meat (8), raw and powdered milk (9), cheeses (2), rice (3), flour (3) and egg-shells (10) were randomly selected from Amman city area to be used for isolating *Bacillus cereus*. The procedures of Wong *et al.* (1988) and Shenagawa (1990) were used to isolate the bacteria.

Approximately, 25gm of each sample was placed in a sterilized polyethylene bag containing 225 ml of sterile Nutrient Broth (NB), then mixed by Stomacher 400 for 3 min. Each eggshell was rinsed and brushed with 50 ml NB. The broth resulting from rinsing each sample was collected in a 150 ml flask and incubated at 35°C for 24 hr. A loopful from the incubated broth was streaked on petri dishes containing solidified Polymyxin egg Yolk mannitol agar (PMY) and incubated at 35°C for 24 hr. Typical colonies of presumptive *B. cereus* were recorded. The confirmative tests of the pure typical colonies were continued to include Gram staining reaction, spore formation, glucose fermentation, nitrate reduction reaction, acetyl methylcarbinal production, tyrosine decomposition test and lysozyme resistance test. Results were recorded and compared to the typical *B. cereus* characteristics as reported by Shinagawa (1990) (Table 2). The pure confirmed local *B. cereus* isolate was designated as J<sub>u</sub> and kept in slant tubes of NA in the refrigerator for experimentation.

**Table (1): Ethnobotanical data of the studied plants.**

Family name	Botanical name	English name	Arabic name	Used part
Anacardiaceae	<i>Hipiscus sabdariffa</i> L.	Roselle		Flower
Ancardiaceae	<i>Rhus caria</i> L.	Sumac		Fruit
Lauraceae	<i>Laurus nobilis</i> L.	Ghar		Leaves
Rutaceae	<i>Feoniculum vulgare</i> Miller	Fennel		Leaves
Labiatae	<i>Ocimum basilicum</i> L.	Wild mint		Leaves & Stem
Cruciferae	<i>Eruca sativa</i> L.	Garden rocket		Leaves & Stem
Leguminaceae	<i>Tregonella foenum grasem</i> L.	Fenugreek		Seed
Rutaceae	<i>Petroselinum sativum</i> Hoffm.	Parsley		Leaves
Umbelliferae	<i>Cuminum cymnium</i> L.	Cumin		Seed
Umbelliferae (Apiaceae)	<i>Coriandrum sativum</i>	Coriander		Whole
Ranunculaceae	<i>Neigella sativa</i> L.	(Black cumin)		Seed

**Table (2): Source and tests used for the characterization and identification of the local isolate of *Bacillus cereus*.**

Source <sup>1</sup>	Reference isolate	Beef Meat	Baby milk formula	Boiled potato	Soil	Cow's feces	Egg shells
Cell morphology	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Gram reaction	+ve	+	+	+	+	+	+
Spore formation	+ve	+	+	+	+	+	+
Reaction on MYP <sup>2</sup>	Pink(p)	P	P	P	P	P	P
Lecithinase reaction	+ve	+/- (weak)	+/-	+/-	+	+	+
Catalase test	+ve	+	+	+	-	-ve	+
Nitrate reaction	+ve	+/- (weak)	+	+	+	+	+
AMC <sup>3</sup> from glucose (VP test)	+ve	-ve	-	-	-	+	+
Tyrosine decomposition	+/- weak	+/-	+/-	+/-	+/-	+ve	+
Presence of lysozyme	+ve	+	+	+	+	+	+

<sup>1</sup> Shenigawa, 1990, <sup>2</sup>MYP: Mannitol egg Yolk Polymyxin agar, <sup>3</sup>AMC: Acetyl Methyl Carbinol

### Standardization of *B. cereus* Culture

A loopful from the pure slant stock of *B. cereus* ( $J_u$ ) culture was transferred into a tube containing nutrient broth and then incubated at 35°C for 24 hr. Serial dilutions were made to reach an inoculum concentration of about  $10^8$  CFU/ml to be used as a working culture against the effect of plant extracts on the growth of bacteria.

### Plant Extracts Preparation

Eleven edible plants (leaves, flowers, fruits and seeds)

(Table 1) were collected from their natural habitats or purchased from local market and then used to examine their antibacterial activity against the wild isolate of *B. cereus* ( $J_u$ ). The fresh plants were dried in an oven for 24 hr at 40°C. The dried plants and seeds were ground using home mixer and the coarse pieces of plant materials were reground. Each ground plant sample was extracted by water or ethanol according to the procedure of Garcia *et al.* (2002): Twenty grams were soaked with 100ml of any

of distilled sterilized water or 96% ethanol for 20 min and blended in sterilized blender for 3 min. Extracts were filtered by Whatman filter paper # 4 and refiltered through microfilter 0.45µm. Extracts then were concentrated in a Rotavapor (Heidolph Instruments, Schwarbach, Germany) at 50°C and stored thereafter at 4°C. The extracts were screened for their antibacterial activity by Disk Inhibition Zone Technique used by Sokmen *et al.* (1999). Filter paper discs of 6 mm diameter were impregnated with 3 different concentrations (1, 2 and 4 mg/ml) of each extract and placed on petri dishes containing nutrient agar with about  $10^8$  CFU of *Bacillus cereus* ( $J_u$ ) against control. After screening all extracts; rossle flowers and sumac fruits were found to contain the highest antibacterial activity and were adopted to be used in the progressive study. Tables (1) and (3).

**Table (3): Screening tests of water and ethanol extracts of 11 edible plants for their anti- *Bacillus cereus* ( $J_u$ ) growth using disc inhibition zone technique.**

Plant	Diameter (mm) Inhibition Zone					
	Water Extracts			Ethanol Extracts		
	Added extract (mg)			Added extract (mg)		
	1*	2*	4*	1*	2*	4*
Roselle	2c**	6b**	16a**	4c**	9b**	12a**
Sumac	3c	7b	15a	3c	8b	14a
Coriander	2b	4ab	6a	0c	2b	5a
Parsley	0b	2b	7a	2b	4b	7a
Fenugreek	0b	3ab	6a	2a	4a	5a
Wild mint	0b	2ab	5a	0b	2ab	4a
Fennel	1c	2b	4a	0b	2ab	4a
Black cumin	0b	1ab	4a	0b	2ab	5a
Garden rocket	0a	2ab	3a	3a	5ab	8a
Ghar	0	0	0	3b	6ab	8a
Cumin	0	0	0	3a	5ab	8a

\*Each value was the average of triplicate.

\*\* Means with the same letter at rows are not significantly different ( $p \leq 0.05$ ) according to LSD test.

#### Anti-*Bacillus cereus* ( $J_u$ ) Activity of the Prepared Extracts

The general procedures of Kim *et al.* (1995) and

Saleem and Al- Delaimy (1982) were used. Different concentrations of water or ethanol extract of sumac fruits (0.0, 0.36, 0.6, 0.9, 1.2, 1.5 mg/ml nutrient agar) and of rossle flowers (0.0, 0.82, 1.6, 2.5, 3.45 and 4.12 mg/ml nutrient agar) were experimented against the growth of *B. cereus* ( $J_u$ ). An inoculum of 1.0 ml of *B. cereus* ( $J_u$ ) culture dilution of  $10^{-5}$ , containing approximately 200 cells was placed in each Petri dish. Volumes from the extract solutions, equivalent to each of the above concentrations, were added to petri dishes. Between 12-15 ml of nutrient agar was pour plated. Then, the plate contents were thoroughly mixed so that the extract and bacterial cells were well mixed with the media. Plates were incubated at 35°C for 24 hr. Results were expressed as CFU/plate. The average CFU of duplicate plates, the % inhibition compared with the controls and minimum inhibitory concentrations (Which is the lowest concentration of the extracts to completely inhibit the growth of *B. cereus* ( $J_u$ )) for each concentration of the extracts were determined.

#### Heat Stability of Sumac and Rossle Water Extracts Against *Bacillus cereus* ( $J_u$ )

Two samples of each water extract were used; one was heated at 70°C for 3 min, while the other was used as a control. The concentrations used from sumac as mg/ml nutrient agar were 0.35, 0.72, 1.08, 1.44, 1.8, and 2.16, while for rossle extracts they were 4.0, 6.0, 8.0, 10.0, 12.0, and 14.0 ml. Pour plate technique was used as follows: For each heat-treated and non-heat-treated extracts, the above given concentrations were transferred into petri dishes in duplicate. An inoculum of approximately  $10^8$  CFU/ml media was added to each petri dish including the control. Nutrient agar (tempered at 45°C) was pour-plated, mixed thoroughly with the extracts and inocula and incubated at 35°C for 24 hr. Results were expressed as the number of CFU/plate and compared to the concentrations tested with the control using the equation:

% Inhibition =  $C - I / C \times 100$

C = count in control plate

I = Count in sample

### 3. STATISTICAL ANALYSIS

Data were analyzed using the Statistical Analysis System (1998) program (SAS). Significant differences between the means were determined using LSD tests.

### 4. RESULTS AND DISCUSSION

#### *Bacillus cereus* Isolation and Characterization

Among the 49 samples of food and other sources examined, 6 isolates were found to be presumptive *B. cereus* according to Shinagawa (1990) procedures for characterizing and identifying bacteria. After running the confirmation, an isolate from egg-shells was confirmed to be *Bacillus cereus* (Table 2). This isolate therefore was used for the experimentation of antibacterial activities of water and ethanol extracts of sumac fruits and rossle flowers.

#### The Antibacterial Activity of Water and Ethanol Extracts of the Selected Plants

Table (3) shows the screening test, using the disc inhibition zone technique of the 11 water and ethanol extracts at concentrations of 2, 4 and 6 mg/ml media against a wild isolate of *B. cereus* ( $J_u$ ). No significant difference of the inhibition activity ( $P \leq 0.05$ ) was found between water and ethanol as extracting agents irrespective to the type of the plant.

Roselle flowers and sumac fruits showed the highest inhibition activity in both water and ethanol extracts ( $p \leq 0.05$ ). Both plants were selected to be used in the progressing study. Other plant extracts contained less and variable or nil activities against the growth of *B. cereus* ( $J_u$ ). Further research is needed for investigating the possibility of these extracts to be active against other types of bacteria or fungi.

#### The Anti-*B. cereus* ( $J_u$ ) Activity of Sumac and Roselle Extracts

The effect of water and ethanol extracts of sumac and roselle flowers on the growth of the bacteria *B. cereus* is presented in Table (4). It was found that as the concentration of water and ethanol extracts increased from 0.36-1.5 mg/ml media (0.3mg/ml segments increase) for sumac and from 0.82-4.120 mg/ml (0.8mg/ml segments increase) for roselle, the inhibition of the growth of the bacteria increased. The Minimum Inhibitory Concentration (MIC) of water and ethanol extracts of sumac against *B. cereus* ( $J_u$ ) was therefore 1.5mg/ml media and of roselle was 3.45 g/ml. No significant difference ( $p \leq 0.05$ ) between the inhibition effects of the water and ethanol extracts of the two plants was observed. However, sumac extract was significantly ( $p \leq 0.05$ ) more effective against *B. cereus* ( $J_u$ ) than roselle extract. This might be attributed to their phenolics content, since in a previous study on these extracts (Al-Ismail *et al.*, (2006) sumac showed higher content of phenolics (250 mg/g extract) than roselle (60 mg/g extract).

Nimri *et al.* (1990) also found that sumac was active for the inhibition of the growth of local isolate of *B. cereus* and found that the MIC ranged between 1.95-31.25 mg/ml, which is much higher than the results of this study. They pointed out that the inhibition was due to tannin, the active component in all plants tested. On the other hand, Negi *et al.* (1990) reported that the active antibacterial component against Gram positive (including *B. cereus*) and Gram negative bacteria was tumeric oil (a by-product of corcomin). Lin *et al.* (2000) reported that the mode of action of isothiocyanate (in cabbage) against bacteria appears to be the oxidative cleavage of disulfide bonds leading to the inactivation of extracellular enzymes. Aliin in garlic and onion is hydrolyzed to yields allicin, pyrovate and ammonia. Allicin is an antimicrobial substance due to its inhibitory action of sulphydryle enzymes in a wide variety of types of bacteria. Rico-Munz and Davidson (1983) reported that phenolic antioxidant compounds have antimicrobial activities

against a variety of types of bacteria, molds and viruses. These compounds were found to be much less active in the food system than in broth or agar systems.

#### Heat Stability of the Anti-*B. cereus* ( $J_u$ ) Activity of Water Extracts of Sumac and Rossle

The results of testing the anti-*B. cereus* ( $J_u$ ) activities of different concentrations of water extracts of sumac and rossle after and before heat treatment at 70°C for 3 min showed (Tables 5) that heating the extracts did not significantly ( $p \leq 0.05$ ) affect the inhibition activity of *B. cereus* as it was similar to that of unheated extracts. It is evident that as the concentrations of the extracts increased, the inhibition of the bacteria increased and complete inhibition was found to be at any concentration between 1.8-2.16 mg/ml media for sumac and at 14 mg/ml media for rossle. These results probably indicate that the active antibacterial components in the two plants extracts remain stable under heat treatments. Saleem and Al-Delaimy (1982) found that boiling garlic water extract completely inactivated the anti *B. cereus* activity of heat labile garlic extracts. The results also indicate that the MIC of the active ingredients of rossle extract against *B.*

*cereus* is substantially higher than that of sumac. Al-gayar *et al.* (2001) reported that the MIC of essential oils extracted from several plants against selected microorganisms are variable according to the kind of microbe and the essential oil examined.

#### 5. CONCLUSION

From a total of 49 samples of food, soil, manure and eggshells, 6 isolate were identified as presumptive *B. cereus* ( $J_u$ ). One isolate ( $J_u$ ) was identified according to Shenagawa (1999) as *B. cereus*. Among the eleven water and ethanol plant extracts used against the growth of the locally isolated *B. cereus*, sumac fruits and rossle flowers were found to have the highest antibacterial activity. The antibacterial substances in both plants extracts were found to remain stable under a heat treatment at 70°C for 3 min. These two edible plants may have the potential to be used as food preservatives after further investigation on their safety and effectiveness against wider spectra of spoilage microorganisms in food.

Table (4): Antibacterial activity of water and ethanol extracts of sumac and rossle against the growth of *Bacillus Cereus* ( $J_u$ ).

Extract concentration (mg/ml)	Growth Inhibition%	
	Water*	Ethanol*
Sumac		
0.0	0.0**	0.0**
0.36	56a	33b
0.6	72a	75a
0.9	83a	82a
1.2	91b	98a
1.5	100a	100a
Rossle		
0.0	0.0	0.0
0.82	3.0a	3.0a
1.6	14.0b	34.0a
2.5	91.0a	68.0b
3.45	100a	95.0b
4.12	100a	100

\*Each value is the mean of three replicates.

\*\*Means with the same letter at rows are not significantly different ( $p \leq 0.05$ ) according to LSD test.

**Table (5): Effect of heat treatment at 70°C for 3 min of water extract of sumac and rossle on their activities against the growth of *Bacillus Cereus* (J<sub>u</sub>).**

Extract concentration (mg/ml)	Growth Inhibition%	
	Unheated*	Heated*
<b>Sumac</b>		
0.0	0.0**	0.0**
0.36	43b	72a
0.72	83a	82a
1.08	83a	82a
1.44	98b	91a
1.8	100a	97a
2.16	100a	100
<b>Roselle</b>		
0.0	0.0	0.0
2.0	0.0	0.0
4.0	20a	20a
6.0	40b	60a
8.0	62b	70a
10	84a	75b
12.0	88a	75b
14.0	100a	100a

\*Each value is the mean of three replicates.

\*\*Means with the same letter at rows are not significantly different (p ≤ 0.05) according to LSD test.

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/ 1.5 (%100)

/ 4.12 3.45

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