

## ***In Vitro* Antibacterial Effects of Pomegranate Fruit Peel Infusion Against Two Diarrheogenic Bacteria**

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### **ABSTRACT**

Pomegranate, which is known as Rumman in Arab region, has been used in traditional medicine in many countries for its beneficial health effects to treatment various illnesses as dysentery, diarrhea, acidosis and many other diseases. Water extract of pomegranate has been used to treatment diarrhea in Syria for long time, and dried peels are kept in almost every house in Damascus for this purpose. However, the contents of water extract of local pomegranate peel, which is used in traditional method to cure diarrhea, and the *in vitro* antibacterial activity are not investigated.

In the current study we tried to investigate the total phenolic contents as indicator of beneficial compounds in the water extract of pomegranate peels obtained from the two varieties (sweet and sour pomegranate), and the antibacterial activity of these extracts against two foodborne pathogenic bacteria namely *Bacillus cereus* and *Salmonella typhi*, which are responsible for diarrhea syndromes.

**Keywords:** Pomegranate, Water Extract, Phenolic Compounds *Bacillus cereus*, *Salmonella typhi*. Minimal Bactericide Concentration (MBC).

### **Introduction**

*Punica granatum* L., commonly known as pomegranate, is a fruit-bearing deciduous shrub or small tree, native to Asia and belongs to the family Lythraceae (Altuner, 2011). *P. granatum* has been extensively used as a traditional medicine in many countries for the treatment of dysentery, helminthiasis, acidosis, hemorrhage and respiratory pathologies (Choi *et al.*, 2011) The pomegranate has long been used in traditional medicine to treat a variety of ailments, including sore throat, inflammation, and rheumatism. Additional traditional uses include treatment of diarrhea and colic and to remove intestinal worms in children. In Ayurvedic medicine, the pomegranate is considered “a pharmacy unto itself,” the bark and roots believed to have anthelmintic and vermifuge properties; the peels a powerful astringent and cure for diarrhea and oral aphthae; and the juice a “refrigerant” and “blood tonic.” Dried pomegranate peels are decocted in water and employed both internally and externally for numerous problems demanding astringents and/or germicides, especially for aphthae, diarrhea, and ulcers (Saad and Said, 2011). Different parts of the plant such as bark, leaves, immature fruits and fruit peel have medicinal significance (Arun and Singh, 2012). Additionally, this plant is reported to have excellent antibacterial, antifungal, antiprotozoal and antioxidant properties (Dahham *et al.*, 2010; Inabo and Fathuddin; 2011; Moussa *et al.*, 2011). Numerous phytochemical constituents have been reported to be present in different parts of the pomegranate plant making it pharmacologically precious (Prakash and Prakash, 2011).

*Bacillus cereus* is Gram positive, spore-forming, motile, aerobic rod, commonly found in soil, water

and food. The organism has been isolated from various foods, including cooked rice, dairy products, eggs, meat and spices (Kramer and Gilbert, 1989; Ombui *et al.*, 2008). *B. cereus* is found in soil, and can be isolated from raw milk and dairy foods (Asano *et al.*, 1997). *B. cereus* occurred ubiquitously in soil and in many raw and processed foods such as rice, milk and dairy products, spices and vegetables (Christiansson *et al.*, 1999; Carlin *et al.*, 2000; Sarrías *et al.*, 2002; Guinebretier *et al.*, 2003). *B. cereus* cause food poisoning, there are two types of food poisoning: diarrheal and emetic.

*Salmonella typhi* has killed over 600,000 people annually all over the world. It is a deadly bacterial disease that causes typhoid fever and is transmitted through food and water. It has become an epidemic in South Asian countries where sanitation is lacking. *S. typhi* usually invades the surface of the intestine in humans, but have developed and adapted to grow into the deeper tissues of the spleen, liver, and the bone marrow. Symptoms most characterized by this disease often include a sudden onset of a high fever, a headache, and nausea. Other common symptoms include loss of appetite, diarrhea, and enlargement of the spleen (depending on where it is located).

*Salmonella typhi* involves colonization of the Reticuloendothelial system. Some individuals who are infected with *S. typhi* become life-long carriers that serve as the reservoir for these pathogens. *S. typhi* has an endotoxin (which is typical of Gram negative organisms), as well as the Vi antigen, which increases virulence. It also produces a protein called invasins that allows non-phagocytic cells to take up the bacterium and allows it to live intracellularly. *Salmonella typhi* is a strong pathogen for humans due to its resistance to the innate immune response system (Monack *et al.*, 2004).

**Materials and methods**

**Plant material**

Pomegranate fruits (two varieties: sweet and sour pomegranate) were obtained from the local market in Damascus, Syria. The fruits were washed by tap water to remove impurities, dried and peel was removed manually.

**Preparation of pomegranate peel Water Extracts (WE)**

The peels were dried at 50°C for 24 h. A stock solution of peel extract was prepared by adding 10 g of the ground dried peels into 100 mL of distilled water and refluxing for 1 h at 100°C (Turgut *et al.*, 2016). The water extracts were cooled and filtered through filter paper. The resultant clear extracts were used to prepare different concentrations ranged from 1 to 50 mg.mL<sup>-1</sup>.

**Preparation of bacterial suspensions**

Two strains of foodborne pathogenic bacteria that cause diarrhea namely *Bacillus cereus* and *Salmonella typhi* were obtained from The National Commission for Biotechnology (NCBT), Damascus, Syria. A concentration of 10<sup>7</sup> (0.5 McFarland) of each strain was prepared using sterile saline (0.85% NaCl).

**Minimal Bactericide Concentration (MBC) assay**

Eight concentrations of pomegranate WE (1, 2.5, 5, 10, 15, 20, 25 and 50 mg.mL<sup>-1</sup>) were prepared by mixing the suitable amounts of stock solution and Mueller Hinton Broth (MHB). One mL of each concentration was distributed aseptically into sterile 2 mL eppendorf tube. Each tube was inoculated with

10 µL of the inoculum prepared as described in the section "Inoculum preparation". The tubes were incubated at 37°C for 24 h. Then 10 µL of each tube was subcultured on plates of Luria Bertani Agar (LBA), and the plates were incubated at 37°C for 24 h. The MBCs values were determined when no growth appeared on the LBA plates after 24 h of incubation.

**Determination of total phenolics compounds**

The concentration of total phenolics was measured according to the Folin-Ciocalteu (FC) method. This method is based on colorimetric oxidation/reduction reaction where Folin-Ciocalteu reagent is used as oxidising agent. The pomegranate WE stock solution (0.25 mL) was transferred to a 25 mL volumetric flask, containing 15 mL of distilled water and 1.25 mL of Folin-Ciocalteu reagent. The solution was neutralized by adding 20% sodium carbonate (3.75 mL). The volume was made up with distilled water to 25 mL and after 2 hours the absorbance was measured at 765 nm (Mekinić *et al.*, 2014). Total phenolics was expressed as Gallic Acid Equivalent (GAE).

**Results and Discussion**

The antibacterial activity of two local pomegranate varieties (sweet and sour pomegranate) against two foodborne pathogenic bacteria responsible of diarrhea (*Bacillus cereus* and *Salmonella typhi*) was evaluated, using the peels water extracts to determine the MBC values. Table 1 shows the ability/inability of the two bacterial strains to growth on LBA plates after the treatment with the water extracts of pomegranate peels at different concentrations (see Fig. 1).

**Table 1.** The MBC values of water extracts of pomegranate peels

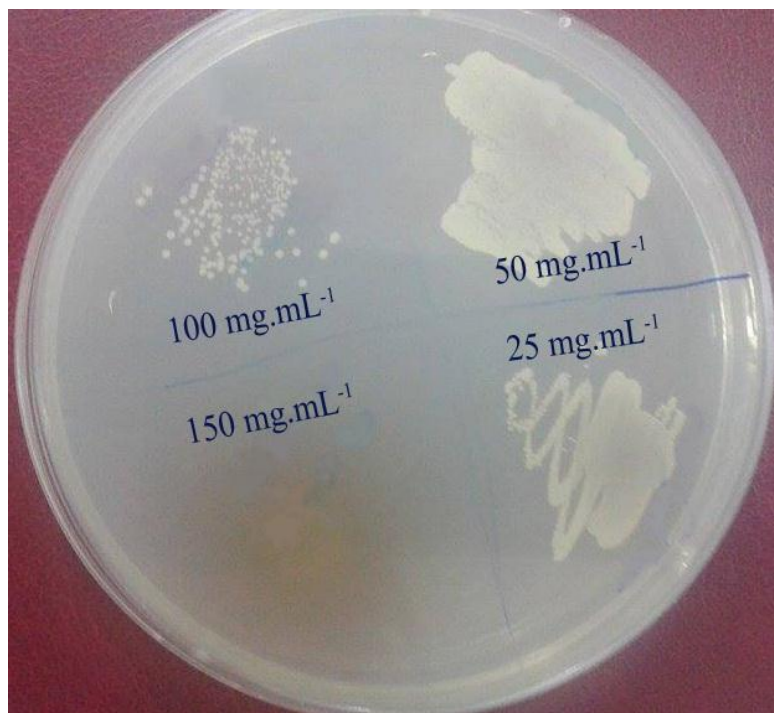
Concentration (mg.mL <sup>-1</sup> )	WE of sweet pomegranate peel		WE of sour pomegranate peel	
	<i>Bacillus cereus</i>	<i>Salmonella typhi</i>	<i>Bacillus cereus</i>	<i>Salmonella typhi</i>
50	-*	-	-	-
25	-	-	-	-
20	-	-	-	-
15	-	+	-	+
10	+**	+	+	+
5	+	+	+	+
2.5	+	+	+	+
1	+	+	+	+

\*-, No Growth on LBA plate after treatment with pomegranate WE at corresponding concentration for 24 h.

\*\*+, Growth on LBA plate after treatment with pomegranate WE at corresponding concentration for 24 h.

Table 1 shows that the MBC values of both sweet and sour pomegranate peel water extracts were 20 and 15 mg.mL<sup>-1</sup> against *Salmonella typhi* and *Bacillus cereus*, respectively. The same MBC values of water extracts of sweet and sour pomegranate peel against bacterial strains are due to the almost same values of

total phenolics in sweet and sour pomegranates: 100 and 105 mg GAE per 1 g of dried peels, respectively. The phenolic contents of water pomegranate extract in this study was somewhat higher than that recorded by Kanatt *et al.*, (2009), 94.51 mg GAE per 1 g of dried peels, although the same extract method was applied.



**Fig. 1.** The antibacterial activity of the water extracts of sour pomegranate peels against *Bacillus cereus*. The MBC value is 150 mg.mL<sup>-1</sup>.

The antibacterial activity recorded in this study against *Bacillus cereus* resemble to that found by Abbas *et al* (2012), who noticed that hot water extract of pomegranate peel showed an inhibitory zone of 14.8 mm at a concentration of 100 mg.mL<sup>-1</sup>.

In one study (Mathabe *et al.*, 2006), the water extract (100 mg.mL<sup>-1</sup>) of pomegranate was superior to methanol, ethanol and acetone extracts at the same concentrations against *Salmonella typhi*, with inhibitory zone of 16.6 mm of water extract, while the inhibitory zones were 13.3, 13.3 and 11.7 mm of methanol, ethanol and acetone extracts, respectively. The value of MBC found in this study for *Salmonella typhi* was higher than the MIC recorded by Tanveer *et al* (2014), 25 mg.mL<sup>-1</sup>.

### Conclusion

In the current study we investigated the antibacterial activity of the peel water extract obtained from two local varieties of pomegranate against two pathogenic bacteria: *Bacillus cereus* and *Salmonella typhi*, which are known as principle causes of diarrhea worldwide. Pomegranate peels have a high content of phenolic compounds (100-105 mg gallic acid equivalent per 1 g of dried peels), and high antibacterial activity against the two pathogenic bacteria known as causative agents of diarrhea (MBC values were 15 and 20 mg.mL<sup>-1</sup> for *Bacillus cereus* and *Salmonella typhi*, respectively). So we suggest to use the hot water infusion of pomegranate peels as a safe, low cost and effective traditional treatment of diarrhea.

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