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Antibacterial Effects of Viable Antibiotics and Pomegranate (*Punica granatum*) Bark Extracts on *Salmonella typhi* and *Salmonella paratyphi* A Isolates from Asymptomatic Typhoid Carriers in Ongole, Andhrapradesh, India

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***Salmonella typhi* and *Salmonella paratyphi* A were isolated from asymptomatic typhoid carriers in Ongole, India. The antibacterial activity of the isolates was assessed using commercially available antibiotics. *Punica granatum* was used as antibacterial agent in this study in order to come over the problem of antibiotic resistant. *Punica granatum* (Pomegranate) bark was collected from Kolli Hills, Tamil Nadu, India. Pomegranate extract was obtained by Soxhlet method. The bark extract of pomegranate prepared using acetone; isopropanol and hexane possess antibacterial activity against *S. typhi* and *S. paratyphi* A. Among these three solvents, acetone and hexane extracts showed better efficiency than isopropanol extract.**

Key words: Antibiotics; Pomegranate; Antibacterial activity; Bark extracts; Asymptomatic typhoid carriers.

Typhoid fever, a food and water borne disease caused by *S.typhi* and *S. paratyphi A* is a serious public health problem in developing countries. Poor personal hygiene and inadequate food handling can potentiate the transmission of *S.typhi*. Several food products kept at room temperature were found to favor the growth of *Salmonella* species. The food handlers prominently play a role in disseminating typhoid bacilli through different food products and water (Lin *et al.*, 1988; Senthilkumar and Prabakaran, 2005). It was suggested that a periodic survey of samples from food handlers and food stuffs should be made and proper sanitation methods should be followed in hotels and restaurants to avoid food contamination and spread of *Salmonella sp* (Sasikumar *et al.*, 2005). *S. typhi* resistant to multiple antibiotics has now spread all over the world. Currently, the incidence of multidrug resistant *S. typhi* (MDRST) in the Indian subcontinent and China ranges from 50% to 80% (Gupta *et al.*, 1994). Even though pharmacological industries have been produced a number of new antibiotics in the last

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three decades, resistant to these drugs by bacteria have increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs (Alanis *et al.*, 2005).

The problem of microbial resistance is growing and the outlook for the case of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce this problem, for example to develop research to better understand the genetic mechanism of resistant and to develop new drugs, either synthetic or natural. For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade. According to World Health Organization medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine (Nascimento *et al.*, 2000). Therefore such plants should be investigated to better understand their properties. In our study we have used to study pomegranate for their antimicrobial activities against *S. typhi* and *S. paratyphi* A isolated from asymptomatic typhoid carriers.

The pomegranate (*Punica granatum*) is a fruit bearing deciduous shrub or small tree growing to 5-8m tall. It is widely cultivated throughout Algeria, America, Azerbaijan, Iran, India, Turkey and drier parts of South Asia. It has long been cultivated as an ornamental and for its edible fruit. They are either eaten freshly or used for grenadine syrup, in which the juice of the acid fruit pulp is the chief ingredient. The astringent properties of the rind and bark have been valued medicinally for several thousand years, especially as a vermifuge (Morton, 1987). In preliminary laboratory research and human pilot studies, juice of the pomegranate has been found effective in reducing heart disease risk factors (Aviram *et al.*, 2004) and against proliferation of breast cancer cells *in - vitro* (Kim *et al.*, 2002). The juice may also have antiviral and antibacterial effects (Neurath *et al.*, 2004). In the present study, we have suggested that extract of pomegranate bark can be used to treat asymptomatic typhoid carriers.

MATERIAL AND METHODS

Screening of the *Salmonella* isolates

Stool sample from the asymptomatic typhoid carriers were collected and transported to the laboratory using the Cary- Blair transport medium in the screw capped tubes. Samples were transported to the Selenite - F broth (Hi-Media, India) to enhance the growth of organisms and it was incubated at 37 °C for 24 h. The colonies were inoculated into the Mac Conkey agar media and Bismuth Sulphite agar media (Hi-media, India) and incubated at 37 °C for 24 h. Further , the isolates were identified by Gram's staining, motility, catalase, oxidase, sugar fermentation, indole, methyl red, vogues- proskauer, citrate, triple sugar iron and urease test.

Antibiotic susceptibility Test

This test was performed by Kirby-Bauer disc diffusion method. Over night cultures in peptone water were lawned over the Muller Hinton agar and incubated at 37 °C for 24 hrs. The following antibiotics (Hi-Media, India) were placed on inoculated plates, tetracycline (30mcg), norfloxacin (10µg), amikacin (30µg), nalidixic acid (30µg), chloramphenicol (30µg), ciprofloxacin (5µg) and ampicillin (10µg).

Plant extract preparation

Pomegranate bark (Fig.1) was collected from Kolli Hills, Tamil Nadu, India. The plant bark was shade dried at room temperature for 7 days. It was powdered and extracted using Soxhelt apparatus with acetone, isopropanol and hexane solvents. To ascertain the bactericidal activity of bark extract, different concentrations were made by dissolving the appropriate amount of extract with sterile distilled water. The inhibition of growth was determined by disk diffusion method.

Antimicrobial activity of pomegranate against *Salmonella* isolates

Sterile discs were prepared by cutting 6 mm diameter of Whatman No. 1 filter paper. The concentrated discs were made by impregnating the discs with 0.1ml of the selected concentrated solutions (50, 100, 150, 200, 250 µg/ml) of the extract and kept for 5 min for uniform diffusion of the extract. All the discs were dried at room temperature. Over night cultures of *S. typhi* and *S. paratyphi* A were seeded separately on the plates containing Muller-Hinton agar. The discs with different concentrations were placed over the seeded plates and incubated at 37 °C for 24 h. Then the diameter of the zones was measured to find out the bactericidal activity of bark extract of *P. granatum*.

RESULTS

S. typhi and *S. paratyphi A* were isolated from the asymptomatic typhoid carriers. They were identified by Gram's staining, biochemical characters (Table 1), growth characters on selective medium and differential medium. *S. typhi* from the asymptomatic typhoid carriers showed sensitive to norfloxacin, amikacin, nalidixicacid, and ciproflaxin and resistant to tetracycline, ciprofloxacin and ampicillin and intermediate sensitive to chloramphenicol (Table 2). *S. paratyphi A* showed sensitive to norfloxacin, amikacin, and chloramphenicol and resistant to tetracycline, nalidixic acid, ciprofloxacin and ampicillin (Table 2). The diameters of the inhibition zone produced by acetone, isopropanol and hexane extracts of pomegranate against *S.typhi* and *S. paratyphi* A are given in Table 3, 4 and 5 respectively and Fig. 2, 3 and 4.

Table 1. Biochemical characteristics of *S. typhi* and *S. paratyphi* A

Isolates	Preliminary	Biochemical Test												
	test	Sugar fermentation										IMVIC		
S. typhi	G	M	C	O	G	L	Mal	S	I	MR	VP	C	TSI	U
	-ve rod	+	+	-	+	-	+	-	-	+	-	-	AK / AG H_2S	-
S. paratyphiA	-ve rod	+	+	-	+	-	+	-	-	+	-	-	AK / AG	-

G – Gram staining ; M – Motility ; C-catalsae ; O-oxidase test ; G-glucose; L-lactose ; Mal-maltose ; S-sucrose ; I-Indole ; MR-methyl red ; VP-voges proskauer ; C-citrate ; TSI-triple sugar Iron ; U-urease

Table 2. Antibiotic sensitivity test of *S.typhi* and *S. paratyphi* A

Antibiotics	Zone of Inhibition (mm)	
	S .typhi	*S.paratyphi A*
Amikacin	22(S)	26(S)
Ampicillin	12 (R)	13(R)
Chloramphenicol	16(I)	20(S)
Ciproflaxin	14(R)	18 (R)
Nalidixicacid	19(S)	12(R)
Norflaxacin	20(S)	17(S)
Tetracyclin	12(R)	14(R)

(S- sensitive; R-resistant; I-intermediate)

Table 3. Antimicrobial Activity of Acetone Extract of Pomegranate against *S. typhi* and *S. paratyphi A*

Extract	Inhibition zone of extract (mm)	
(µg / ml)	*S. typhi*	*S.paratyphi A*
50	15	15
100	16	17
150	16	19
200	17	23
250	18	24

Table 4. Antimicrobial Activity of Isopropanol Extract of Pomegranate against *S. typhi* and *S. paratyphi* A

Extract	Inhibition zone of extract (mm)	
(µg / ml)	*S. typhi*	*S.paratyphi A*
50	12	10
100	13	12
150	14	13
200	16	15
250	19	16

Table 5. Antimicrobial Activity of Hexane Extract of Pomegranate against S. typhi and S. paratyphi A

Extract	Inhibition zone of extract (mm)	
(µg / ml)	*S. typhi*	*S.paratyphi A*
50	15	16
100	16	17
150	16	17
200	17	18
250	18	19

Fig.1. Pomogranate with fruits and bark

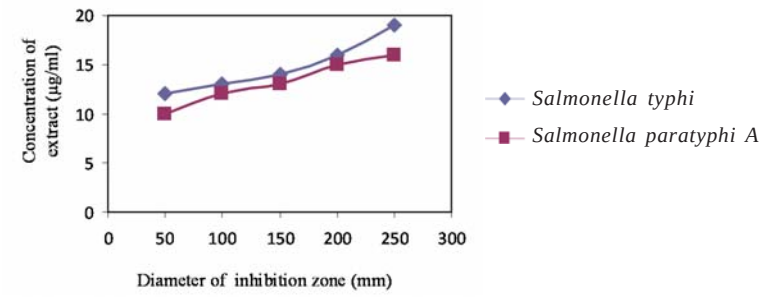

Fig. 2. Antimicrobial Activity of Acetone Extract of Pomegranate against *S. typhi* and *S. paratyphi* A

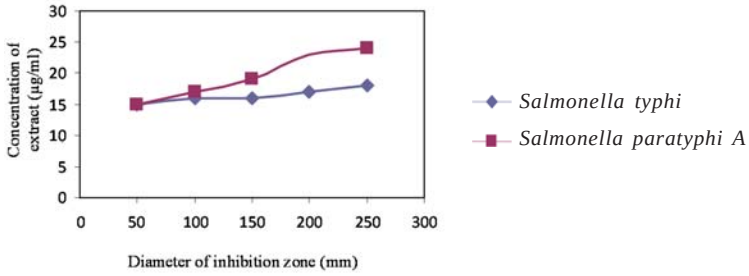

Fig. 3. Antimicrobial Activity of Isopropanol Extract of Pomegranate against *S. typhi* and *S. paratyphi* A

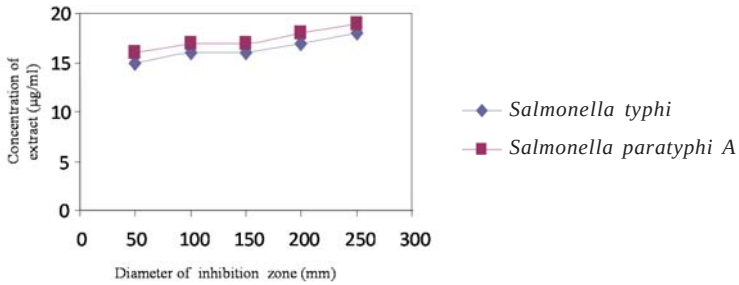

Fig. 4. Antimicrobial Activity of Hexane Extract of Pomegranate against *S. typhi* and *S. paratyphi* A

DISCUSSION

Typhoid fever is wide spread in all parts of India. Transmission of typhoid fever is primarily through the contaminated water and food. In the present study, the isolated *S. typhi* and *S. paratyphi A* from the asymptomatic typhoid carriers showed resistant to tetracycline, ciprofloxacin and ampicillin and tetracycline, nalidixic acid, ciprofloxacin and ampicillin respectively and *S. typhi* was intermediate sensitive to chloramphenicol. Malickbhasa *et al.,* (2010) have reported the presence of two typhoid asymptomatic carriers from food handles in Namakkal District, Tamil Nadu, India. Jesudasan (1996) also suggested that MDRST has been prevalent in India since 1989 and they are resistant to chloramphenicol, ampicillin, trimethoprin and sulfamethoxazole.

Antibacterial activity of the bark extract of *P. granutum* was tested against *S. typhi* and *S. paratyphi A* isolated from asymptomatic typhoid carriers. These isolates shown the inhibition zone against acetone, isopropanal and hexane extract of pomegranate at the concentration of 50 µg, 100 µg, 150µg, 200 µg and 250 µg than the antibiotics used in our study. Likewise Mathabe *et al.,* (2006) studied 21 plant species of 14 families for screening of antibacterial activity against *E.coli, Staphylococcus aureus, Shigella sp and S. typhi*. Of which, the most active extracts were those obtained from *Punica granatum* and *Indigofera daleoides*. They found that all the extracts (methanol, ethanol and acetone) from two plants, namely, *P. granutum* and *Ozora insignis* were active against all the tested bacteria.

In our study we have used acetone, isopropanol and hexane as solvents for the preparation of pomegranate extract. Among these three, acetone and hexane extracts showed higher efficiency than isopropanol extract. Negi and Jayaprakasha (2003) also used four solvents (ethyl acetate, acetone, methanol and water) to study the antibacterial activity of the extract of pomegranate peels. And they found that acetone extract showed the highest antibacterial activity followed by methanol and water extract. Alanis *et al.,* (2005) suggested that methanolic extract of some plants (*Caesaipinia pulcherria, Chiranthodendrom pentadatctylon, Cocos nuclfera, Geranium mexicanum, Hippocratea excelsa and Punica granutum*) possess strong antibacterial activity against most of the pathogens tested (2 *Escherichia* sp, 2 *Shigella* sp and 2 *Salmonella* sp) and Prashanth *et al.,* (2001) also found that methanolic extract of *P. granutum* was found to be active against all the tested bacteria than petroleum ether, chloroform and water extracts. Machando *et al.,* (2002) found that ellagitannin is the substance responsible for the antimicrobial activity of the pomegranate and it was found to be active against methicillin resistant *Staphylococcus aureus* strains.

This present study revealed that the antibacterial potential of the *P. granutum* investigated; thereby extract can be used to treat asymptomatic typhoid carriers and drug resistant among the typhoid bacilli also overwhelmed.

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