

Antimicrobial Activity of Jambul (*Syzygium cumini*) Fruit Extract on Enteric Pathogenic Bacteria

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Abstract

Jambul (*Syzygium cumini*) contain several biological activities including anti diabetic, anti-inflammatory, gastroprotective and antibacterial activity. In this study, we investigated antibacterial activity of Jambul juice extract against some common enteric pathogens like *Salmonella typhimurium*, *Shigella flexneri*, *Staphylococcus aureus*, and ETEC (Enterotoxigenic *E. coli*). Growth inhibition of these enteric pathogenic bacteria was measured by growing in Nutrient Broth media supplemented with 0%, 5%, 10% and 25% juice extract and then plating on Nutrient Agar plate for colony count at 0, 24 and 48 hours time points. The effect of Jambul juice on the growth of *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* were also investigated. We observed that the growth of *Salmonella typhimurium*, *Shigella flexneri*, *Staphylococcus aureus*, and ETEC were significantly inhibited by Jambul juice by 1 to 6 logs ($p < 0.001$), and the growth of probiotics (*Lactobacillus acidophilus* and *Lactobacillus bulgaricus*) were not affected significantly. These findings indicate that Jambul juice have selective bactericidal effects against several enteric pathogens while beneficial species remain unaffected. To far our knowledge, this is the first report about the antibacterial activity of fruit juice in Bangladesh.

Keywords

Jambul, Foodborne Pathogens, Fruit Extract, Probiotics, Antimicrobial Activity

1. Introduction

Traditional use of plants and in treating disease has deep roots in the history of human races [1]. Plants are used in curing different infectious diseases like, diarrhea, malaria, burns, stomach disorders, gonorrhoea etc. Scientists' Efforts in

establishing plants with promising antimicrobial activity are being successful and yielding fruitful results [2] [3] [4] [5] [6]. Essential oils of plants and the antimicrobial properties of the low bush blueberry show antimicrobial activity against a wide range of microorganisms including antibiotic-resistant species of bacteria and fungi [7] [8] [9] [10]. Natural antimicrobials contain dynamic combinations of bioactive compounds to combat against the resistance of bacteria and also conserve probiotic species; so, they are attractive alternatives in many disease models [11].

Jambul (*Syzygium cumini*) also known as *Syzygium jambolanum*, *Eugenia jambolanum* and *Eugenia cumini* is an evergreen tropical tree in the flowering plant family *Myrtaceae* and native to Bangladesh, India, Nepal, Pakistan, Sri Lanka, the Philippines, and Indonesia. The plant is also known as, jam/kalojaam, Jambhul/jambu/jambula/jamboola, jamun, jamblang, jambolan, black plum, Damson plum, Duhat plum, Jambolan plum or Portuguese plum and so on.

Phytochemical screening of extracts of *Syzygium cumini* revealed that seed contains alkaloids, amino acids, phytosterols, saponins, steroids, tannins and triterpenoids etc. and leaf contains crude protein 9.1%, fat 4.3%, crude fiber 17%, ash 7%, calcium 1.3% and Phosphorus 0.19%. These phytochemicals probably explain the plants medicinal properties [12]. The bark and seed have anti diabetic activity [13]; seed has anti-inflammatory activity [14], Radioprotective activity [15] [16], and antibacterial activity against *E. coli*, *B subtilis*, *P. aeruginosa* and *S.aureus* [17]; bark has gastroprotective and anti-ulcerogenic effects [18]; and leaf has anti-allergic [19] and anti-*Vibrio cholera* activity [20].

The edible portion of the fruit also contains some essential Phytochemicals: per 100 grams of edible portion contains: Moisture, 85.8 gm; ether extract, 0.15 gm; crude fiber, 0.3 gm; nitrogen, 0.129 gm; ash, 0.32 gm; calcium, 8.3 mg; phosphorus, 16.2 mg; iron, 1.62 mg; carotene, 0.004 mg; thiamine, 0.008 mg; riboflavin, 0.009 mg; niacin, 0.290 mg; total ascorbic acid, 5.7 mg [21]. The composition of the edible portion suggests it to have some antimicrobial properties, and we should investigate the properties against the enteric pathogens as the fruit is taken as drink or directly. But if fruit extract destroys our beneficial normal flora of stomach, it won't be an effective antimicrobial agent, so bactericidal activity should be selective.

2. Materials and Methods

2.1. Fruit Collection and Juice Extraction

Fresh Jambul (*Syzygium cumini*) fruits were purchased from local market (Savar Bazar, Savar, Dhaka, Bangladesh). Fruits were checked to exclude the rotten, cracked or unripe ones and washed several times thoroughly to remove unwanted dirt. Then the selected fruits were added to a steam jacketed kettle; stirred continuously (1000 ×g), kept for 3 minutes at 95°C to reduce the bacterial count of the surface. When the fruits were cooled down to 40°C, the seeds were removed by using sterile forceps. These were taken and pressed in a hand operated squeezer to extract the juice; and the resultant juice was filtered through

sterilized double layers of cheesecloth. Juice was subsequently bottled in glass jars and stored at 4°C.

2.2. Bacterial Strains and Growth Conditions

Strains of four potential enteric pathogens, *Salmonella typhimurium*, *Shigella flexneri*, *Staphylococcus aureus*, and ETEC (Enterotoxigenic *E. coli*) and two important probiotic bacteria, *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* were collected from the laboratory stock of dept. of microbiology, Jahangirnagar University, Savar, Dhaka, Bangladesh. Pathogenic bacteria were cultured on Nutrient Agar medium where the probiotic bacteria were cultured on Lactobacillus MRS (de Man, Rogosa, and Sharpe) Agar medium at 37°C.

2.3. Media Preparation

Nutrient agar (Oxoid, UK) and Lactobacillus MRS Agar (Oxoid, UK) plates were used to subculture and to enumerate the bacterial count of pathogenic and probiotic bacteria respectively from the test tubes. Nutrient Broth (Oxoid, UK) and Lactobacillus MRS Broth media were used as the sole media to perform the growth effect test of pathogenic and probiotic bacteria respectively. All media were prepared by following the instructions of manufacturer, and sterilized by autoclaving at 121°C for 15 minutes.

2.4. Preparation of Sole Test Media

- **Control media:** Control media contain only 9 ml of single strength broth media (Nutrient broth or Lactobacillus Broth) but no fruit juice.
- **5% juice media:** 5% juice media are composed of 8.1 ml of single strength, 0.45 ml of double strength broth media and 0.45 ml of fruit juice.
- **10% juice media:** 10% juice media is made by mixing together 7.2 ml of single strength, 0.9 ml of double strength broth media and 0.9 ml of fruit juice.
- **25% juice medium:** 4.5 ml of single strength, 2.25 ml of double strength broth media and 2.25 ml of fruit juice are the ingredients of 25% juice medium.

2.5. Standardization of Inoculum

Pathogenic bacteria were subcultured on fresh plates of Nutrient Agar at 37°C for 24 hours, and Probiotic bacteria were subcultured on fresh plates of Lactobacillus MRS Agar at 37°C for 48 hours. Colonies from these plates were washed out using 1 ml of phosphate buffered saline (PBS) under laminar air flow cabinet. The optical density (OD) of the bacterial suspension was adjusted to an absorbance value of 0.10 at 600 nm on a spectrophotometer (Spectronic 200).

2.6. Growth Effect Test

After standardization, 1 ml of bacterial suspension was added to each of the screw capped test tubes in the set of sole test media; Control medium, 5% juice medium, 10% juice medium, and 25% juice medium. Then the set of sole test media were incubated at 37°C for 48 hours. Growth of bacteria was measured

by counting the number of bacteria present in per 1 ml of media at different time points (0, 24, and 48 hours) by colony count method. In colony count method, serial dilutions were performed in PBS and plated on strain-specific agar to count bacterial colony forming units (CFU). Triplicate plates were prepared for each dilution in each trial and three trials were performed for each strain.

2.7. Statistical Analysis

SPSS version 12.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The chi squared test (χ^2) was used to compare the effectiveness of Jambul fruit extract on enteric pathogenic bacteria. A p value less than 0.05 was considered to be significant.

3. Result

3.1. Growth Effect Test of *Shigella flexneri*

Antimicrobial activity of Jambul juice against *Shigella flexneri* was measured in liquid cultures by plate count method. Growth of the bacteria was reduced significantly after 24 hours and 48 hours of incubation in liquid bacterial culture media supplemented with different concentration of Jambul juice in each of the three trials. At 24 hours time point 5% juice reduced the growth by about 2 logs, 10% juice reduced the growth by 2.65 logs, and 25% juice reduced the growth by 4.75 logs. And the inhibitory effect remained active even after 48 hours, though reduced slightly (at 5% juice, 1.73 logs; at 10% juice, 2.5 logs; and at 25% juice, 4 logs) (Table 1).

3.2. Growth Effect Test of *Staphylococcus aureus*

Growth of *Staphylococcus aureus* was reduced significantly after 24 hours and 48 hours of incubation in liquid bacterial culture media supplemented with different concentration of Jambul juice in each of the three trials. At 24 hours time point 5% juice reduced the growth by about 1 log, 10% juice reduced the growth by 1 log, and 25% juice reduced the growth by 3 logs. And the inhibitory effect remained active after 48 hours, though reduced slightly at 25% juice (2.3 logs) (Table 2).

3.3. Growth Effect Test of ETEC

In each of the three trials, growth of the bacteria was inhibited significantly after 24 hours incubation in liquid bacterial culture media supplemented with different concentration of Jambul juice in each of the three trials. At 24 hours time point 5% juice reduced the growth by about 1.5 logs, 10% juice reduced the growth by 1.6 logs, and 25% juice reduced the growth by 2 logs. And the inhibitory effect remained active even after 48 hours (Table 3).

3.4. Growth Effect Test of *Salmonella typhi*

Growth of *Salmonella typhi* was reduced most significantly after incubation in

liquid bacterial culture media supplemented with different concentration of Jambul juice in each of the three trials. At 24 hours time point 5% juice reduced the growth by about 2.3 logs, 10% juice reduced the growth by 6.5 logs, and 25% juice reduced the growth 7 logs. At 48 hours of incubation, inhibitory effect of 5% juice was reduced by about 1 log, where 10% juice and 25% juice showed >7 logs reduction (Table 4).

Table 1. Effect of jambul juice on the growth of *Shigella flexneri* (cfu/ml).

A	B	C	D	E	F	G	H
Type of media	Average count after 0 hour (log10)	Average count after 24 hours (log10)	Average count after 48 hours (log10)	Effect after 24 hours (C-B)	Effect after 48 hours (D-B)	Growth reduction after 24 hours	Growth reduction after 48 hours
1. Control	6.8	8.17	7.89	+1.37 Log	+1.09 Log		
2. 5% JP	7.12	6.15	6.48	-0.62 Log	-0.64 Log	E1 - E2 = 1.99 Log	F1 - F2 = 1.73 Log
3. 10% JP	7.14	5.86	5.72	-1.28 Log	-1.42 Log	E1 - E3 = 2.65 Log	F1 - F3 = 2.51 Log
4. 25% JP	6.87	3.49	3.92	-3.38 log	-2.95 Log	E1 - E4 = 4.75 Log	F1 - F4 = 4.04 Log

Table 2. Effect of jambul juice on the growth of *Staphylococcus aureus* (cfu/ml).

A	B	C	D	E	F	G	H
Type of media	Average count after 0 hour (log10)	Average count after 24 hours (log10)	Average count after 48 hours (log10)	Effect after 24 hours (C-B)	Effect after 48 hours (D-B)	Growth reduction after 24 hours	Growth reduction after 48 hours
1. Control	6.89	8.51	8.53	+1.62 Log	+1.64 Log		
2. 5% JP	6.92	7.53	7.56	+0.61 Log	+0.64 Log	E1 - E2 = 1.01 Log	F1 - F2 = 1.0 Log
3. 10% JP	6.84	7.33	7.24	+0.49 Log	+0.4 Log	E1 - E3 = 1.13 Log	F1 - F3 = 1.24 Log
4. 25% JP	6.73	5.44	6.02	-1.29 Log	-0.71 Log	E1 - E4 = 2.91 Log	F1 - F4 = 2.35 Log

Table 3. Effect of jambul juice on the growth of ETEC (cfu/ml).

A	B	C	D	E	F	G	H
Type of media	Average count after 0 hour (log10)	Average count after 24 hours (log10)	Average count after 48 hours (log10)	Effect after 24 hours (C-B)	Effect after 48 hours (D-B)	Growth reduction after 24 hours	Growth reduction after 48 hours
1. Control	6.89	8.43	8.12	+1.54 Log	+1.23 Log		
2. 5% JP	6.89	7.02	6.90	+0.13 Log	+0.01 Log	E1 - E2 = 1.51 Log	F1 - F2 = 1.22 Log
3. 10% JP	6.86	6.83	6.59	-0.03 Log	-0.27 Log	E1 - E3 = 1.57 Log	F1 - F3 = 1.5 Log
4. 25% JP	6.9	6.47	6.15	-0.43 Log	-0.54 Log	E1 - E4 = 2.0 Log	F1 - F4 = 1.77 Log

Table 4. Effect of jambul juice on the growth of *Salmonella typhi* (cfu/ml).

A	B	C	D	E	F	G	H
Type of media	Average count after 0 hour (log10)	Average count after 24 hours (log10)	Average count after 48 hours (log10)	Effect after 24 hours (C-B)	Effect after 48 hours (D-B)	Growth reduction after 24 hours	Growth reduction after 48 hours
1. Control	6.48	7.39	7.71	+0.91 Log	+1.23 Log		
2. 5% JP	6.68	5.28	5.75	-1.4 Log	-0.93 Log	E1 - E2 = 2.31 Log	F1 - F2 = 1.22 Log
3. 10% JP	6.66	1.06	0*	-5.6 Log	<-6 Log	E1 - E3 = 6.51 Log	F1 - F3 = >7 Log
4. 25% JP	6.66	0*	0*	-6.36 Log	<-6 Log	E1 - E4 = 7.27 Log	F1 - F4 = >7 Log

* = Below the detection level (<100 cfu/ml).

3.5. Growth Effect Test of *Lactobacillus acidophilus*

Effect of Jambul juice on the growth of *Lactobacillus acidophilus* was measured in liquid cultures by plate count method. Growth of the bacteria was not reduced but stimulated slightly (about 0.2 log), which is not also significant after 24 hours and 48 hours of incubation (Table 5).

3.6. Growth Effect Test of *Lactobacillus bulgaricus*

Effect of Jambul juice on the growth of *Lactobacillus bulgaricus* was also measured in liquid cultures by plate count method. Growth of the bacteria was not reduced nor stimulated by significantly after 24 hours and 48 hours of incubation in liquid culture media supplemented with different concentration of Jambul juice in each of the three trials. The inhibition or stimulation rate was negligible (between +0.1 log and -0.1 log) in each of the three trials at both 24 hours and 48 hours time point (Table 6).

4. Discussion

In recent years, food borne illness like gastroenteritis, diarrhoea etc. are increasing day by day as our food habit and livelihood has changed a lot [22] [23]. *Salmonella typhimurium*, *Shigella flexneri*, *Staphylococcus aureus*, and ETEC (Enterotoxigenic *E. coli*) are the common pathogens those cause food borne intestinal disease. It's a major challenge to control those organisms and their infection. All natural organic antimicrobial agents have the potential to be an alternative way to control infection with food borne bacteria [24] [25].

No significant study has been performed yet to evaluate the role of jambul juice on the growth of pathogenic bacteria and beneficial microflora. Our study

Table 5. Effect of jambul juice on the growth of *Lactobacillus acidophilus* (cfu/ml).

	A	B	C	D	E	F	G	H
Type of media	Average count after 0 hour (log10)	Average count after 24 hours (log10)	Average count after 48 hours (log10)	Effect after 24 hours (C-B)	Effect after 48 hours (D-B)	Growth reduction after 24 hours	Growth reduction after 48 hours	
1. Control	5.82	8.92	9.06	+3.1 Log	+3.24 Log			
2. 5% JP	6.05	8.87	9.1	+2.82 Log	+3.05 Log	E1 - E2 = 0.28 Log	F1 - F2 = 0.19 Log	
3. 10% JP	5.97	8.84	9.09	+2.87 Log	+3.12 Log	E1 - E3 = 0.23 Log	F1 - F3 = 0.12 Log	
4. 25% JP	6.03	8.81	9.1	+2.78 Log	+3.07 Log	E1 - E4 = 0.32 Log	F1 - F4 = 0.17 Log	

Table 6. Effect of jambul juice on the growth of *Lactobacillus bulgaricus* (cfu/ml).

	A	B	C	D	E	F	G	H
Type of media	Average count after 0 hour (log10)	Average count after 24 hours (log10)	Average count after 48 hours (log10)	Effect after 24 hours (C-B)	Effect after 48 hours (D-B)	Growth reduction after 24 hours	Growth reduction after 48 hours	
1. Control	6.23	8.59	8.85	+2.36 Log	+2.62 Log			
2. 5% JP	6.23	8.66	8.81	+2.43 Log	+2.58 Log	E1 - E2 = -0.07 Log	F1 - F2 = 0.04 Log	
3. 10% JP	6.24	8.58	8.86	+2.34 Log	+2.62 Log	E1 - E3 = -0.02 Log	F1 - F3 = 0 Log	
4. 25% JP	6.16	8.52	8.91	+2.36 Log	+2.75 Log	E1 - E4 = 0 Log	F1 - F4 = -0.13 Log	

demonstrates clear evidence that jambul juice has selective bactericidal effects against several foodborne pathogens while *Lactobacillus* species remained unaffected. In the present study, we have shown that, different concentrations of jambul juice have strong antimicrobial effect on the growth of *Salmonella typhimurium*, *Shigella flexneri*, *Staphylococcus aureus*, and ETEC (reduced the growth by 1 log to 7 logs). This result supports the study of Biswas *et al.* [26], who reported blueberry juice to inhibit the growth of *S. typhimurium*, *Campylobacter jejuni*, *L. monocytogenes*, and *E. coli* O157:H7; the study of Puupponen-Pimiä *et al.* [27] who reported that phenolic compounds of berries, especially ellagitannins inhibited the growth of gram negative bacteria like *Staphylococcus*, *Salmonella*, and *Listeria*; and Park *et al.* [28] who demonstrated that both ethanol and water extracts of blueberry and muscadine significantly inhibited the growth of four *Salmonella* strains and one *Listeria* strain [26] [27] [28] [29]. The possible reason of this bactericidal activity of jambul juice could be the membrane interface interaction of phenolics, anthocyanins, and proanthocyanidins, due to their partial hydrophobicity which allow them to bind to the outer membrane causing changes in fluidity [24] [30]. Many recent studies showed that the growth of probiotic bacteria, like *Lactobacillus* and *Bifidobacterium* remained unaffected by the presence of phenolic compounds found in the berries [24] [26] [27]. Similarly, our study showed that growth of *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* remained unaffected in each concentrations of juice.

Moreover, ensuring the safety of the food supply chain has become more difficult due to the resurgence of multidrug resistant strains of foodborne pathogens [31] [32]. Natural antimicrobial agents like jambul juice may be an effective alternative choice against the bacterial pathogens that are resistant to available drugs. So, further study may be designed to evaluate the antimicrobial activity of jambul juice against the multi-drug resistant strains of food borne pathogens. Food borne and enteric pathogens moreover the resurgence of drug resistant strains has made it a great challenge to control the food safety. Jambul juice can be a good source of alternative natural drugs against these pathogens. Jambul is very cheap and available in this region (Indian subcontinent). Considering the antimicrobial and nutritional activity of Jambul juice, large scale industrial production of Jambul juice will be possible. By following this study method, investigation of antimicrobial activity of other natural foods and fruits will be possible. On the other hand people's consciousness about enteric diseases and benefit of natural fruit consumption in preventing these types of disease will be increased day by day in developing countries like Bangladesh. The result of this study is just a pointer to new sources of novel drugs and natural antibiotics. Studies should also be done on understanding which of the phytochemicals are responsible for the observed beneficial effects and their mechanism of action. In vivo tests in animal or human model may also be done for better understanding of the test result.

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