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Evaluation of antimicrobial properties of some local fruits of Bangladesh

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Abstract

The antimicrobial activities of fruit pulp of five plants namely tamarind, Indian gooseberry, star fruit, ambarella and shatkora were tested *in vitro* against nine human pathogenic bacteria viz., *Escherichia coli* 1, *Escherichia coli* 2, *Escherichia coli* 3, *Escherichia coli* 4, *Escherichia coli* 5, *Staphylococcus aureus*, *Salmonella* sp., *Pseudomonas* sp. and *Klebsiella* sp. by disc diffusion method. The highest zone of inhibition for both ethanolic extract (20 mm) and aqueous extract (22.5 mm) was found for tamarind against *Staphylococcus aureus*. Star fruit showed least sensitivity for aqueous and ethanolic extract. Minimum inhibitory concentration (MIC) of the aqueous and ethanolic extracts was determined against all tested bacteria by well diffusion method. In case of ethanolic extract the highest MIC was observed for tamarind (125 mg/ml) and lowest MIC was found for Indian gooseberry (3.91 mg/ml). For aqueous extract highest MIC (125 mg/ml) was recorded for tamarind and ambarella and lowest MIC (7.81 mg/ml) was found for Indian gooseberry. Star fruit showed no MIC. From this research work it is obvious that investigated fruit pulp extract has excellent potential to be used as antimicrobial agent against human pathogen.

Introduction

In today's modern world the primary source of most of the commercial drugs and medicines are the natural products. Approximately 61% of drugs produced worldwide are inspired by natural products (Cseke *et al*., 2004). For this reason, biologically active compounds need to be isolated from natural products. Variation in geographical area or in ecological or climatic condition can cause the variation in the amount of biologically active compounds. Moreover, the presence of undesirable compounds can stimulate undesirable or unpredictable bioactivity. Isolated pure bioactive compounds can overcome these problems and can be used for particular therapeutic purposes (Colegate *et al*., 2007). Fruits contain many macro- and micro-nutrients which help to maintain a sound health and to prevent diseases and these can also be used to cure diseases if we can understand its therapeutic and nutritional value. Risks of cardiovascular diseases and cancers can be reduced with the consumption of fruits and vegetables (Hossain *et al*., 2008). Despite the huge consumption of fruits, scientific data regarding their pharmacological and immunological properties are relatively scarce. Recent researches suggest that external immunostimulants can induce immune responses against microorganisms to prevent infections in immune-compromised conditions, such as aging, cancers, sepsis, diabetes, etc (Goto *et al*., 2010).

However, from the available literature, scientific evaluation of the antimicrobial activity of the local fruits extract has been done in many countries. It has not been done yet for the fruits such as tamarind, Indian gooseberry, star fruit, ambarella and shatkora produced in our country. Since these fruits are easily available in our country, the antimicrobial activity was tested against a range of human pathogen and evaluated minimal inhibitory concentrations. This study is an attempt to contribute to the use of this fruits as an alternative product of medicine to control diseases.

Materials and methods

Sample collection

Five species of Bangladeshi local fruits namely tamarind *(Tamarindas indica)*, star fruit *(Averrhoa carambola)*, Indian gooseberry *(Phyllanthus emblica)*, ambarella *(Spondias dulcis)*, shatkora *(Citrus macroptera)* were collected from local market of Sylhet region in Bangladesh.

Bacterial isolates and their maintenance

Five Strains of *Escherichia coli* namely *E. coli* 1, *E. coli* 2, *E. coli* 3, *E. coli* 4, *E. coli* 5 and

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one strain of *Staphylococcus aureus*, *Salmonella* sp., *Pseudomonas* sp. and *Klebsiella* sp. were collected from Microbiology Laboratory of Sylhet Osmani Medical College, Bangladesh and from the Microbiological Laboratory of Lab Aid Hospital, Sylhet, Bangladesh. All of the bacterial strains are isolated from human. All the bacterial strains were suspended in nutrient broth and incubated at 37°C for 48 h. Nutrient Agar (NA) was used to determine the MIC and the antibacterial activity of the extract.

Extraction methods

An extract is a mixture of phytochemical from any plant which is obtained by extraction of specific parts of the plant (Pandey *et al.*, 2011). Two types of solvents namely water and ethanol was used for extraction. To prepare crude aqueous and ethanolic extracts of five fruits except shatkora were washed and cut into small pieces. The cut slices were air dried and again dried into an oven for 14 days at 40^0 C. Then, 10 g of each dried fruit were soaked in 100 ml of 70% ethanol and water, and kept for 24 h at ambient temperature. Shatkora juice was extracted from by using a blender and the extracted juice with pulp was soaked in water and 70% ethanol.

The mixtures were then filtered twice with Whatman No.1 filter paper. The filtrates were evaporated using vacuum rotary evaporator and air dried at 40^0C with reduced pressure. The solutions of crude aqueous and ethanolic extracts were then stored at 4^0C. (Sarker *et al*., 2012). Stock solution prepare was prepared by dissolving one gram of each extract in 1 ml of the same solvent used for extraction (Ward *et al*., 2007).

Determination of antimicrobial activity

Preparation of discs

The discs of about 6 mm in diameter were cut by punching machine from Whatman No. 1 filter paper. The discs were then taken into petri dish and sterilized by autoclave, followed by drying in oven at 100^0C.

Procedure for performing the disc diffusion test

The required amount of petri dish is prepared and autoclaved at 121^0C for 15 minutes. They were allowed to cool under laminar air flow. About 20 ml of nutrient agar were transferred aseptically into each sterile petri dish and allowed to solidify. Then 100 µl of inoculums suspension was spread uniformly over the agar medium using sterile glass rod. The readily prepared sterile discs were loaded with 40 µl of ethanolic and aqueous fruit extract. The paper diffuse discs were placed on the medium suitably apart and the plate were incubated at 5^0C for 1 hour to permit good diffusion and then transferred to an incubator at 37^0C for 24 hours. In each petri dish antibiotic disc of gentamicin was used as positive control. The antibacterial activity was recorded by measuring the width of the clear inhibition zone around the disc in millimeter (Dhale *et al*., 2011). Reference antibiotic gentamicin was obtained from local pharmaceuticals shop.

Determination of minimum inhibitory concentration (MIC)

The Minimum Inhibitory Concentration (MIC) for each extract and test organism was determined by agar well diffusion method (Nwodo *et al*., 2011). Nutrient agar was prepared, poured into petri dishes and allowed to cool. Then each petri dish was seeded with bacterial inoculums and spreaded with sterile L-shaped glass rod and allowed to dry for ten minutes. After that 6 mm diameter wells were punched over the agar plate with sterile cork borer.

A 125 mg/ml concentration of the reconstituted extract was serially diluted in two fold, down to 3.91 mg/ml. A 100 µL volume of each dilution was introduced into duplicate wells in the NA plates pre-inoculated with test bacterial strain; and incubated at 37°C for 24 h. The MIC was taken as the lowest concentration of the extract showing measurable inhibition zone.

Statistical analysis

Data were analyzed by using analysis of variance (ANOVA) to determine if significant differences ($P < 0.05$) existed between mean values.

Results and Discussion

Determination of the inhibition zones (Table 1 and 2) and minimum inhibitory concentration (Table 2) by means of the disc diffusion method of the pulps extract by ethanol and water exhibited different antimicrobial effect against all tested bacteria. For tamarind, ethanolic extract showed strong sensitivity than aqueous extract against all tested bacterial except *Escherichia coli* 1 and *Escherichia coli* 4 where aqueous extract showed strong sensitivity. But in a study with different concentration (80, 120, 140, 160, 180 mg/ml) of tamarind showed the order of sensitivity as *Staphylococcus aureus*> *Escherichia coli*> *Pseudomonas* with the exception of *Salmonella typhi* (Abukakar *et al.*, 2008). Against *Staphylococcus aureus* the highest zone of inhibition was found as 22.6 mm for ethanolic extract and it was the highest

Table 1. Zone of inhibition (mm) in ethanolic extract

Name of Bacteria	Zone of inhibition(mm) in Ethanolic extract					
	Tamarind	Indian gooseberry	Ambarella	Star fruit	Shatkora	Standard Gentamycin
Escherichia coli 1	11.75 ± 0.75*	14.65±0.78	13.90±0.36	0.00±0.00	8.38±0.82	34.00±1.50
Escherichia coli 2	14.00±0.66	11.92±0.51	13.45±0.67	0.00±0.00	11.16±0.62	16.00±0.50
Escherichia coli 3	11.25±0.90	15.87±0.62	13.00±1.00	0.00±0.00	10.00±1.39	34.41±0.87
Escherichia coli 4	15.00±0.37	12.33±0.85	12.80±0.05	0.00±0.00	13.50±0.65	21.66±0.38
Escherichia coli 5	15.33±0.99	10.12±0.24	12.00±0.25	0.00±0.00	0.00±0.00	24.41±0.62
Staphylococcus aureus	22.60±1.10	13.40±0.90	11.35±1.21	7.92±0.16	15.36±0.96	20.00±0.50
Salmonella sp.	10.15±0.95	0.00±0.00	0.00±0.00	0.00±0.00	14.00±0.38	21.16±0.76
Pseudomonas sp.	12.00±0.38	8.85±0.35	8.12±1.11	8.43±1.30	20.70±0.10	19.66±0.62
Klebsiella sp.	13.26±0.37	13.70±0.30	12.13±1.15	7.63±0.05	15.52±1.01	32.00±0.50

*Mean ± standard deviation

Table 2. Zone of inhibition (mm) in aqueous extract

Name of Bacteria	Zone of inhibition (mm) in *water* extract					
	Tamarind	Indian gooseberry	Ambarella	Star fruit	Shatkora	Standard Gentamycin
Escherichia coli 1	16.00±0.50	8.50±1.00	14.22±2.12	0.00±0.00	8.22±0.98	34.00±1.50
Escherichia coli 2	12.50±0.75	10.16±0.37	8.00±0.66	0.00±0.00	8.00±0.50	16.00±0.50
Escherichia coli 3	9.35±0.62	13.00±0.25	12.18±0.30	0.00±0.00	0.00±0.00	34.41±0.87
Escherichia coli 4	19.08±0.38	9.91±0.37	11.53±0.45	0.00±0.00	0.00±0.00	21.66±0.38
Escherichia coli 5	12.08±0.62	13.00±0.20	11.75±0.47	0.00±0.00	0.00±0.00	24.41±0.62
Staphylococcus aureus	20.00±0.25	10.65±0.37	12.00±0.50	0.00±0.00	0.00±0.00	20.00±0.50
Salmonella sp.	8.41±.0.14	0.00±.0.00	0.00±0.00	0.00±0.00	0.00±0.00	21.16±0.76
Pseudomonas sp.	9.36±0.34	8.47±0.25	7.86±0.35	0.00±0.00	0.00±0.00	19.66±0.62
Klebsiella sp.	10.33±1.04	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	32.00±0.50

*Mean ± standard deviation

zone for any ethanolic extract in the this study. For aqueous extract the highest zone of inhibition was 20 mm against Staphylococcus aureus and lowest zone of inhibition was 9.35 mm against *Escherichia coli* 3. Aqueous extract of tamarind was the only one that showed any sensitivity against *Klebsiella* sp., amongst all other aqueous extract samples. It was the only tested bacteria that showed sensitivity against all the bacterial strains for both aqueous and ethanolic extract. The highest minimum inhibitory concentration was found against *Salmonella* sp. and *Pseudomonas* sp.

Ethanolic extract of Indian gooseberry showed sensitivity against all tested bacteria except for *Klebsiella* sp. The aqueous extract showed less sensitivity than ethanolic extract and showed no sensitivity against two tested organisms namely *Salmonella* sp. and *Klebsiella* sp. Ethanolic extract showed strong sensitivity than aqueous extract against all tested bacterial except for *Escherichia coli* 5. Both ethanolic and aqueous extract were showed highest zone of inhibition against *Escherichia coli* 3. But Dhale *et al.* (2011) found highest inhibition zone in case of *Staphylococcus aureus*. Against *Escherichia coli* 1 the ethanolic extract of Indian gooseberry showed highest zone of inhibition (14.60 mm) than other ethanolic extracts of samples.

Determination of the inhibition zones of ambarella pulp extract by ethanol exhibited antimicrobial effect against all tested bacteria except for *Salmonella* sp. and aqueous extract exhibited antimicrobial effect for all tested organisms other than *Salmonella* sp. and *Klebsiella* sp. In case of ethanolic extract of ambarella the highest zone of inhibition (13.90 mm) was found against *Escherichia coli* 1 and lowest zone of inhibition (8.12) was found against *Pseudomonas* sp. Notably, ethanolic extract of ambarella showed the lowest zone of inhibition (8.12mm) against *Pseudomonas* sp. than other samples of ethanolic extracts.

Star fruit showed that the fruit pulp extract by ethanol exhibited an antimicrobial effect against for *Staphylococcus aureus*, *Pseudomonas* sp. and *Klebsiella* sp. But aqueous extract showed no

Table 3. MIC of ethanolic extract and aqueous extract of five fruits

Name of Bacteria	Minimum Inhibitory Concentration (MIC) mg/ml in Ethanolic extract					Minimum Inhibitory Concentration (MIC) mg/ml in Aqueous extract				
	Tamarind	Indian gooseberry	Ambarella	Star fruit	Shatkora	Tamarind	Indian gooseberry	Ambarella	Star fruit	Shatkora
Escherichia coli 1	62.5	15.63	31.25	0	62.50	31.25	62.5	31.25	0	62.50
Escherichia coli 2	31.25	31.25	15.63	0	125	62.5	31.25	125	0	0
Escherichia coli 3	62.5	15.63	31.25	0	62.50	125	31.63	62.5	0	0
Escherichia coli 4	31.25	3.91	15.25	0	7.81	15.63	15.63	31.63	0	0
Escherichia coli 5	31.25	7.81	15.25	0	0	31.25	7.81	15.25	0	0
Staphylococcus aureus	62.5	15.63	7.81	0	31.25	125	31.63	7.81	0	0
Salmonella sp.	125	0	0	0	0	0	0	0	0	0
Pseudomonas sp.	125	31.25	31.25	0	31.25	125	62.5	62.5	0	0
Klebsiella sp.	62.5	15.63	31.25	0	31.25	125	0	62.5	0	0

sensitivity against any of the tested bacteria. Ethanolic extract of Star fruit showed highest zone of inhibition against *Pseudomonas* sp. and that is 8.1 mm. The lowest zone of inhibition for aqueous extract against *Klebsiella* sp. was 7.63 mm. This findings correlate with Wakte *et al.* (2011), who found no sensitivity in case of aqueous extract and found sensitivity against *Staphylococcus aureus* and *Pseudomonas* sp. in case of methanolic extract.

Ethanolic extract of Shatkora exhibited an antimicrobial effect against all the tested bacteria except for *Escherichia coli* 5. The aqueous extract only showed sensitivity against *Escherichia coli* 1 and *Escherichia coli* 2. The highest zone of inhibition of ethanolic extract was found against *Pseudomonas* sp. (20.7 mm) and for aqueous extract the highest zone of inhibition (8.22 mm) was found against *Escherichia coli* 1. Against *Pseudomonas* sp. and *Klebsiella* sp. ethanolic extract of shatkora gave the highest zones of inhibition which were 20.70 mm and 15.52 mm respectively than all other ethanolic extracts of taken fruits. Both aqueous and ethanolic extract showed no sensitivity against *Escherichia coli* 5.

Conclusion

This preliminary study reports that four out of five screened fruits possess good antimicrobial activity against human pathogen. The demonstration of broad spectrum of antimicrobial activity of these fruits may help to discover new chemical classes of antibiotic substances that could serve as selective agents for infectious disease and control. Further research will be helpful if the compounds which are responsible for the antimicrobial activity can be isolated. The effect of these fruits on more pathogenic organisms and toxicological investigations and further purification however, needs to be carried out.

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