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In vitro controlling of food borne disease causing bacteria in kachhagolla using ginger rhizome (*Zingiber officinale* Roscoe.) extracts.

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Antimicrobial compound of ginger (*Zingiber officinale* Roscoe) rhizome extract was proven against food borne disease causing bacteria in kachhagolla when compared with the commercially available antibiotic (ciprofloxacin). Among the three extracts, hot water exhibited highest (15.4±0.57mm) antibacterial activity followed by ethanol (14.3±0.6mm) and petroleum ether (11.5±0.28 mm) at 250mg/ml of concentration. Significant variation among the extracts, strains, concentrations and their interactions showed differential effects for the studied factors. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the extracts ranged from 25-175 mg/ml and 50-200 mg/ ml, respectively. These low MIC and MBC values indicated high bacteriostatic and bactericidal activity of zinger rhizome extract and could be used as an effective preservative to control disease causing bacteria in kachhagolla.

Key words: Kachhagolla, ginger rhizome extracts, antibacterial activity

Introduction

Kachhagolla a traditional sweetmeat of Bangladesh is very delicious and nutritious. It contains minerals, especially calcium, phosphorous as well as fat soluble vitamins particularly vitamin A and D (Alam *et al*., 2002; Mannan *et al*., 1994). Due to its high sugar content, kachhagolla is a good carbon source for common food borne disease causing bacteria i.e. *Escherichia coli, Salmonella typhi*, *Listeria monocytogenes*, *Bacillus cereus,* and *Staphylococcus aureus* (Natta *et al*., 2008). After contamenation, bacteria secret their toxic

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metabolites in the product especially during long term preservation (Masud *et al*., 1988)**,** thus causing severe diarrhea, vomiting, listeriosis in human (Fleming *et al*., 1985; Soomro *et al*., 2003). So for long term preservation against bacterial contamination, it is needed to check some natural additives for remedy from these health hazards. Being an aromatic herb, ginger rhizome might be a potential source for controlling of food borne disease causing bacteria (Nakatani, 1994) in kachhagolla. Ginger is added in foods since ancient times, not only as flavoring agents, but also as folk medicine and food preservatives (Buchart, 1994; Cutler, 1995). The major active component of ginger is gingerol [5-hydroxy-1-(4-hydroxy-3-methoxy phenyl) decan-3-one] which has strong inhibitory activity against food borne pathogenic bacteria (Hirasa and Takemasa, 1998). Therefore, the present study was undertaken to determine the antimicrobial activity of ginger rhizome extracts against food borne disease causing bacteria in kachhagolla.

Materials and methods

Plant material

Fresh and disease free ginger (*Zingiber officinale* Roscoe., Fam: Zingiberaceae) was purchased locally from whole sales and retail organic food stores at Binodpur Bazaar, Rajshahi, Bangladesh and authenticated by Dr. A. H. M. Mahbubur Rahman (Plant Taxonomist), Assistant professor, Department of Botany, University of Rajshahi, Bangladesh.

Preparation of rhizome powder

After collection, rhizomes were washed with clean sterile distilled water and cut into small pieces followed by oven dried at 50°C for 24 h to reduce water content. The dried rhizome pieces were ground into powder using electric blender (IR-091, China) and used for extraction.

Preparation of rhizome extracts

Fifty gram (50g) of the ginger powder was soaked in 150 ml of sterile hot water, ethanol and petroleum ether separately to prepare their respective extracts. Then it was shaken vigorously for 72h on orbital shaker (*IKA Labortechnik KS 250***,** Staufen, Germany) to allow for proper extraction and then filtered through sterile teton cloth and Whatman No. 1 filter paper. The resultant juice was evaporated at 80^0C in water bath (HH-S0235, China), after complete evaporation the extracts were preserved aseptically in a screw cap

tube at 4°C until further use. Finally the extracts were dissolved in to their respective solvents and prepared 50, 100, 150, 200 and 250 mg/ml of concentrations superlatively.

Test organisms

Two gram negative viz. *Escherichia coli* BMLRU1031, *Salmonella typhi* BMLRU1033, and three gram positive *Listeria monocytogenes* BMLRU1018, *Bacillus cereus* BMLRU1020*, Staphylococcus aureus* BMLRU1024 bacteria were used as experimental materials which were isolated and identified from kachhagolla sample (food associated) according to Holt *et al* (1994) using their respective standard strain (collected from ICDDRB, Dhaka, Bangladesh) in Biotechnology and Microbiology Laboratory, Department of Botany, University of Rajshahi, Bangladesh. After identification the bacterial strain were listed with accession number as mentioned in the parenthesis.

Preparation of standard culture

A loopful colony of 24 h surface growth on a nutrient agar plate of each bacterial strain was transferred individually to 5 ml of nutrient broth (pH 7.0, Hi media). After incubation at 37°C for 24 h, bacterial cells were collected by centrifugation at 3000 rpm for 15 min (Tomos supermini centrifuge,12×1.5ml, 13,400rpm,China) followed by washing twice and resuspended in 0.1% peptone water. Turbidity was adjusted to McFarland turbidity standard approximately 1.5×10^8 (CFU/ml).

Antibacterial assay

The antibacterial activity of the ginger rhizome extracts was carried out by disc diffusion assay (Gulluce *et al*., 2003). Sterile filter paper discs (6 mm) were impregnated in different concentration (50, 100, 150, 200 and 250 mg/ml) of each solvent extract separately then air dried aseptically for 5 minutes. Then the discs were carefully placed on seeded plates and incubated at 37°C for 24 h. For each extract three replicate trails were conducted against the test organisms to confirm the reproducible results. Moreover, blank paper discs were impregnated in same amount of solvents as negative control, and ciprofloxacin (0.03mg/ml) as positive control. The antimicrobial activity of ginger rhizome extracts was determined by measuring the zone of inhibition (in mm) against all studied bacteria comparing with control (ciprofloxacin).

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Minimum inhibitory concentration (MIC) of three extracts was determined for each of the test organism in triplicate in test tubes (Doughari *et al*., 2007). To 0.5 ml of varying concentrations (5, 25, 50, 75, 100, 125, 150, 175 and 200 mg/ml) of the extract in test tubes, Nutrient broth (2 ml) was added, and then a loopful of the test organism, previously diluted to 0.5 McFarland turbidity standard, was introduced. The procedure was repeated on the test organisms using the standard antibiotics (ciprofloxacin). The culture tubes were incubated at 37°C for 24 h. After incubation the tubes were examined for microbial growth by observing for turbidity, and the lowest concentration that did not show any growth visually was considered as Minimum Inhibitory Concentration (MIC). The contains of all tubes that showed no visible growth were streaking on nutrient agar plate and incubated at 37°C for 24 h. The lowest concentration that did not show any growth of bacterial colony was considered as Minimum Bactericidal Concentration (MBC).

Statistical analysis

Statistical analysis (ANOVA) was done using SPSS software version 10.0, (Chicago IL, USA) and MSTAT version 2.10 (Russel, D. Freed, Michigan State University, USA), and expressed as mean ± S.E.M. Least Significant Difference (LSD) test was used to speculate further if there was a significant difference with in means of three ginger extracts, various concentrations and 5 studied bacteria. P values < 0.001 were considered as significant.

Results

The results of antibacterial activity of ginger extracts are represented in Table 1. The studied concentrations of extract exhibited different degrees of antibacterial activity depend on bacterial strains and solvents compared with the reference standard antibiotic (Ciprofloxacin). In hot water extract the zone of inhibition was ranging from 7.00 mm (*B. cereus*) to 15.40 mm (*S.typhi*) followed by ethanol 7.17 mm (*E. coli*) to 14.30 mm (*B. cereus*) and petroleum ether 7.2 mm to (*B. cereus*) to 11.5 mm (*B. cereus*). Comparing among the three types of extract, hot water extract gave the best results against all studied bacteria (Table-1&Figure-1).Statistical analysis shows, significant difference among the bacterial strains, concentrations and extracts, and their interactions S×C, S× E,C×E, and S×C×E (Table 2). According to the LSD test results

(Table 3), mean of hot water extract (10.74) was significantly different from ethanol (8.11) and petroleum ether (5.13). In case of means of concentration, significant difference was observed among them, highest for 250 $mgml^{-1}$ (12.13) followed by 200 $mgml^{-1}$ (10.55), 150 $mgml^{-1}$ (8.76), 100 $mgml^{-1}$ (5.86) and 50 $mgml^{-1}$ (2.66) as expected. Regarding means of strain, *S. typhi* (9.33) in the top followed by *S. aureus* (8.88), *B. cereus* (8.58), *E. coli* (7.54) and *L. monocytogenes* (5.63). *E. coli* and *L. monocytogenes* were significantly different from others and between them. Where as between strain of *S.typhi* and *S. aureus*, and *S.aureus* and *Bacillus cereus* were not significant (Table 3).

The MIC and MBC results of ginger extract are presented in Table 4.The results reveal that MIC values were ranged from 25 to175 $mgml^{-1}$.For hot water extract, it was ranged from 25 to75$mgml^{-1}$, 50 to 125 $mgml^{-1}$ for ethanol, and 100 to 175 $mgml^{-1}$ for petroleum ether. In three types of extract, hot water extract gave lowest MIC value (25 $mgml^{-1}$) followed by ethanol (50 $mgml^{-1}$) and petroleum ether (100 $mgml^{-1}$). In case of MBC values, it was ranged from 50 to 200 $mgml^{-1}$. For hot water extract, it was ranged from 50 to 100 $mgml^{-1}$, 75 to 150 $mgml^{-1}$for ethanol, and 150 to 200 $mgml^{-1}$ for petroleum ether. In three types of extracts, hot water extract gave lowest MBC value (50 $mgml^{-1}$) followed by ethanol (75$mgml^{-1}$) and petroleum ether (150 $mgml^{-1}$).

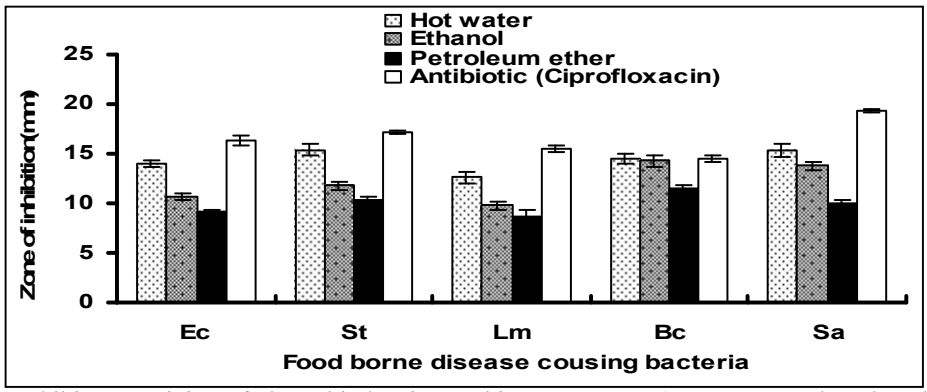

Fig. 1. Inhibitory activity of three kinds ginger rhizome extract (Hot water, Ethanol and petroleum ether extract=250 mg/ml) against food borne bacteria in kachhagolla. Values are represented as mean ± SEM of triplicate experiments. Here, Ec=*Escherichia coli*, St= *Salmonella typhi*, Lm=*Listeria monocytogenes*, Bc=*Bacillus cereus* and Sa=*Staphylococcus aureus.* Antibiotic (Ciprofloxacin) was used as positive control.

Table 1. Antibacterial activity of three solvents extract of ginger rhizome.

	Concentration ($mgml^{-1}$)		Zone of inhibition (mm)				
			E. coli BMLRU1031	***S. typhi*** BMLRU1033	***L. monocytogenes*** BMLRU1018	***B. cereus*** BMLRU1020	***S. aureus*** BMLRU1024
Ginger extracts	**50**	**HW**	7.83 ± 0.44	9.83 ± 0.44	0	7.00 ± 0.28	7.17 ± 0.44
		ET	0	8.00 ± 0.16	0	0	0
		PE	0	0	0	0	0
	100	**H W**	9.23 ± 0.16	11.83 ± 0.28	9.00 ± 0.28	9.20 ± 0.60	8.93 ± 0.43
		ET	7.17 ± 0.16	8.80 ± 0.44	0	8.70 ± 0.47	7.60 ± 0.44
		PE	0	0	0	0	7.50 ± 0.50
	150	**HW**	10.50 ± 0.28	13.00 ± 0.28	10.00 ± 0.28	11.80 ± 0.16	10.67 ± 0.44
		ET	8.33 ± 0.16	10.10 ± 0.16	0	9.73 ± 0.46	10.30 ± 0.44
		PE	7.30 ± 0.16	8.70 ± 0.33	0	7.20 ± 0.16	7.80 ± 0.44
	200	**HW**	11.67 ± 0.44	14.23 ± 0.44	10.83 ± 0.44	13.20 ± 0.16	13.33 ± 0.44
			9.00 ± 0.16	10.80 ± 0.44	8.80 ± 0.28	12.50 ± 0.57	12.20 ± 0.30
		PE	8.30 ± 0.16	10.30 ± 0.60	8.30 ± 0.16	9.80 ± 0.28	8.50 ± 0.28
	250	**H W**	14.00 ± 0.28	15.40 ± 0.57	12.63 ± 0.52	14.50 ± 0.57	15.33 ± 0.72
		ET	10.67 ± 0.44	11.80 ± 0.44	9.80 ± 0.44	14.30 ± 0.60	13.80 ± 0.44
		PE	9.20 ± 0.16	10.3 ± 0.60	8.70 ± 0.44	11.50 ± 0.28	10.00 ± 0.28
Antibiotic	**0.03**	**CF**	16.33±0.44	17.13 ± 0.30	15.53 ± 0.16	18.00 ± 0.28	19.33 ± 0.16

HW=Hot water extract, ET= Ethanol extract, PE=Petroleum ether extract and CF=Ciprofloxacin (Positive control).Values are presented mean zone of inhibition (mm) ±S.E.M of the three replicates.

Table 2. Analysis of mean data of the antibacterial activity of three zinger rhizome extracts.

Variables	Growth inhibition Diameter (mm)
Strains	
Salmonella yphi BMLRU1033	9.33 A
Staphylococcus aureus BMLRU1024	8.88 AB
Bacillus cereus BMLRU1020	8.58 B
Escherichia coli BMLRU1031	7.54 C
Listeria monocytogenes BMLRU1018	5.63 D
LSD	0.6334
Concentrations	
50 $mgml^{-1}$	2.66 E
100 $mgml^{-1}$	5.86 D
150 $mgml^{-1}$	8.76 C
200 $mgml^{-1}$	10.55 B
250 $mgml^{-1}$	12.13 A
LSD	0.6334
Extracts	
Hot water	10.74 A
Ethanol	8.11 B
Petroleum ether	5.13 C
LSD	0.4906

Means followed by different letter (S) down the column are significantly different among the strains, concentration as well as extracts at $p<0.001$. Here any two means having a common letter are not significantly different at the 5% level of significance.

Table 3. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of ginger rhizome extracts compare with the antibiotic ciprofloxacin**.**

Organisms	Antibiotic(mg/ml)		Ginger extracts					
	MIC	MBC	MIC(mg/ml)			MBC(mg/ml)		
	Ciprofloxacin		HW	ET	PE	HW	ET	PE
E.coli BMLRU1031	0.003	0.006	25	75	125	50	100	150
Salmonella typhi BMLRU1033	0.012	0.024	25	50	125	75	125	150
L. monocytogenes BMLRU1018	0.096	0.192	75	125	175	100	150	200
Bacillus cereus BMLRU1020	0.024	0.096	50	75	125	75	75	175
S. aureus BMLRU1024	0.030	0.060	50	75	100	75	100	175

HW=Hot water extract, **ET**=Ethanol extract, **PE**=Petroleum ether extract.

Discussion

Results of *in vitro* antibacterial properties of zinger rhizome extracts against the growth of Gram negative *E.coli, Salmonella typhi* A , and Gram positive *L. monocytogenes , Bacillus cereus* and *S. aureus* bacteria, endorse the presence broad spectrum antibiotic compounds in it. The antimicrobial potency of ginger is attributed to its ability to inhibit toxin production and expression of enzymes for pathogenesis supported by the others (Dewitt *et al*., 1979; Parekh and Chanda, 2007). Moreover, comparing among the three extracts, hot water showed best zone of inhibition significantly ($p<0.001$) different from ethanol and petroleum ether against all the studied bacteria, thus showing its high soluble ability in hot water (Boer *et al*., 2005). It has been reported that different phytoconstituents have different degrees of solubility in different types of solvents depending on their polarity (El-Mahmood and Doughari, 2008). Among the different strains, *L. monocytogenes* was least sensitive which suggesting that mechanisms of resistance are developing in this organism (Indu *et al.,* 2006), where as *Salmonella typhi* AMB0807A was more sensitive because extract exhibited highest zone of inhibition against it. This supports the presence of some active compounds in ginger rhizome extract. The major pungent component is gingerol [5-hydroxy-1-(4-hydroxy- 3-methoxy phenyl) decan-3-one] which has strong inhibitory activity against food borne pathogenic bacteria (Hirasa and Takemasa, 1998). This compound is an antimicrobial compound having wide spectra of antimicrobial effect which may contribute to growth inhibition of enterobacteria (Beuchat and Golden, 1989). In our experiment, extracts displayed different growth of inhibition, depending upon bacterial strains, these variations might be due to genetical difference among the strains and this is due to chemical compositions and cell wall types of the bacteria (Kaushik and Goyal, 2008). In this study the low MIC value observed for *S. typhi* is a good indication of high efficacy against these bacteria, and high MIC and MBC values are indication of low activity (El-Mahmood and Doughari, 2008).Thus suggesting their differential bacteriostatic and bactericidal effect against the studied crude extracts (Sakagami *et al*., 2000; George *et al*., 2002; Leucherner *et al*., 2002; Ijeh *et al*., 2005; Natta *et al*., 2008). In all of the experiments conducted, only solvents used as negative control did not show any appreciable activity, but the standard antibiotic (ciprofloxacin) consistently displayed superior potency that was different from crude extracts. These differences may be attributed to the fact that ciprofloxacin as a commercial antibiotic is a refined and purified product, while extracts are a mixture of various plant ingredients (El-Mahmood and Doughari, 2008). However, comparing among the three extracts hot water extract showed

relatively better activity against food borne disease causing bacteria which can be put the following order hot water >ethanol>petroleum ether, so it could be used as microbial growth inhibitors in the foods.

According to these results and the best of our knowledge accomplished that though ginger extracts are not refine and purified but it has strong inhibitory activity against studied food borne disease causing bacteria. So it could be used as alternative natural food preservatives.

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