Microbiological Research 167 (2012) 500–506

Contents lists available at SciVerse ScienceDirect

Microbiological Research

journal homepage: www.elsevier.de/micres

Antimicrobial activity of *Sesbania grandiflora* flower polyphenol extracts on some pathogenic bacteria and growth stimulatory effect on the probiotic organism *Lactobacillus acidophilus*

Ratna China[a], Sayani Mukherjee[a], Sauradip Sen[a], Sreedipa Bose[a], Sanjukta Datta[b], Hemanta Koley[c], Santinath Ghosh[b], Pubali Dhar[a,*]

[a] Food and Nutrition Division, Department of Home Science, University of Calcutta, 20B Judges Court Road, Kolkata 700027, India
[b] Department of Chemical Technology, University College of Science & Technology, University of Calcutta, 92 A.P.C. Road, Kolkata 700009, India
[c] Division of Bacteriology, National Institute of Cholera and Enteric Disease (NICED), P-33 CIT Road, Scheme XM, Beliaghata, Kolkata 700010, India

ARTICLE INFO

Article history:
Received 13 January 2012
Received in revised form 29 March 2012
Accepted 16 April 2012

Keywords:
Sesbania grandiflora
Flavonoids
Probiotic
Rutin

ABSTRACT

Polyphenolic extracts (PE) of edible flower of *Sesbania grandiflora* were tested to evaluate its antimicrobial effect against some common pathogenic bacteria and growth promoting property against probiotic organism *Lactobacillus acidophilus*. The antimicrobial activity of *S. grandiflora* flower PE against selected pathogens was evaluated using both *in vitro* and *in situ* methods. *In vitro* studies suggested that PE has inhibitory effect against *Staphylococcus aureus*, *Shigella flexneri* 2a, Salmonella Typhi, *Escherichia coli* and *Vibrio cholerae*. The gram-positive organism *S. aureus* was the most sensitive organism to PE and minimum inhibitory concentration (MIC) was found to be 0.013 mg/mL where as the MIC of PE against *V. cholerae* was the highest (0.25 mg/mL). On the other hand PE showed growth promoting effect on the common probiotic bacterium *L. acidophilus*. The major finding was that *S. grandiflora* PE induced a significant biomass increase of *L. acidophilus* grown in liquid culture media. PE showed reduction of *S. aureus* growth in food (fish) during storage at 10 °C. High performance liquid chromatography analysis showed that rutin, a major flavonoid of the PE diminished in the culture medium MRS broth with the growth of *L. acidophilus*.

Introduction

Health benefits of polyphenols have been widely described in the recent publications, especially the prevention of diseases associated with oxidative stress such as cancer, cardiovascular, inflammatory and neurodegenerative diseases (Lau et al. 2006; Manach et al. 2005; Scalbert and Williamson 2000; Williamson and Manach 2005). Biological activity of polyphenols depends on their bioavailability. A major part of dietary polyphenols is not absorbed in the small intestine and can interact with colonic microbiota (Scalbert and Williamson 2000). The effect of polyphenols on the growth of bacteria depends on the bacterial strain, phenolic structures and the dosage assayed (Almajano et al. 2008; Campos et al. 2003). Extensive studies have been done on the antimicrobial property of polyphenols on the pathogenic intestinal bacteria but very few studies have been carried out on the effect of polyphenols on beneficial intestinal microflora.

Gastrointestinal (GI) microflora is increasingly being recognized as one of the factors that influence human health. This microflora is in a dynamic equilibrium that may be altered by diet (Kajiwara et al. 2002). Normal healthy intestinal microflora contains many strains of lactic acid bacteria (LAB) some of which have been isolated, ascribed several beneficial functions, and termed probiotic strains (Salminen et al. 1998). The growth of the intestinal microflora serves as the barrier that prevents pathogenic bacteria from invading the gastrointestinal tract. The composition of the intestinal microflora together with the gut immune system allows resident bacteria to exert a protective function. Lactobacilli are gram-positive LAB that constitutes a major part of the normal intestinal microflora in animals and humans. Three microflora modulation tools have emerged: the addition of exogenous living microorganisms to foods (*i.e.* probiotics), the selective stimulation of the growth and activity of beneficial microorganisms indigenous to the gut (*i.e.* addition of prebiotics), and a combination of both approaches (*i.e.* synbiotics). All three approaches attempt to increase the number of bacteria positive for human gastrointestinal health, usually the lactobacilli and bifidobacteria.

Flower vegetables are flowers of traditional vegetable plants and have been used for cooking since ancient time. It was believed

* Corresponding author. Tel.: +91 033 24795594/24491840; fax: +91 033 23519755; mobile: +91 9433122560.
E-mail address: pubalighoshdhar@yahoo.co.in (P. Dhar).

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http://dx.doi.org/10.1016/j.micres.2012.04.003

that consumption of these flower vegetables can cure illness and diseases. They also help people who suffer from diarrhea, which indicates the anti-microbial activity of these vegetables (Boonyaprapatsara 2000).

Sesbania grandiflora is a small, loosely branching tree that grows up to 8–15 m tall and 25–30 cm in diameter. Flower clusters hang at leaf base having 2–5 large or giant flowers. The flowers are pink, red or white, pea-like, 5–10 cm in length, curved, about 3 cm wide before opening. Flowering and fruiting is almost through out the year chiefly during winter. The flowers of *S. grandiflora* are consumed in India as vegetables and flowers, leaves, barks and the roots are also used by tribes (Sinha and Lakra 2005).

Large scale evaluation of the local flora exploited in traditional medicine for various biological activities is a necessary first step in the isolation and characterization of the active principle leading to drug development. To date, there is enough evidence that supports antimicrobial activity of polyphenols and flavonoids present in fruits and vegetables; however, research on the possible stimulatory role of phenolic compounds on beneficial intestinal bacterial growth is scarce. *S. grandiflora* flower water extract have been studied for antimicrobial effect against *Escherichia coli*, *Bacillus cereus* and *Staphylococcus aureus* (Krasaekoopt and Kongkarnchanatip 2005). But its effect on different antibiotic resistant enteric bacterial strains is lacking. *S. grandiflora* flowers have ethnic use in prevention of diarrhea so we have chosen *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923), Salmonella Typhi D641, *Shigella flexneri* 2a, *Vibrio cholerae* 569B for our study as these are mostly enteropathogenic organisms except *S. aureus* which is an enterotoxin producing food borne pathogen.

In situ antimicrobial studies were conducted with food borne pathogen to evaluate the efficacy of *S. grandiflora* PE in a modified food model during typical storage conditions of the test food. This is the first study on the effects of *S. grandiflora* flower PE on growth of probiotic *Lactobacillus acidophilus*. Accordingly, the study presents the antimicrobial spectrum (*in vitro* and *in situ*) of PE from *S. grandiflora* flower against intestinal as well as food-borne pathogenic bacteria and simultaneous effect on the growth of a typical intestinal probiotic bacterium, namely *L. acidophilus* in presence of the PE.

Materials and methods

Bacterial strain and culture conditions

The test organisms *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923), S. Typhi D641, *S. flexneri* 2a, *V. cholerae* 569B were supplied from National Institute of Cholera and other Enteric Disease (NICED), Kolkata, India. *L. acidophilus* (MTCC 447) was bought from Microbial Type Culture collection (MTCC) Chandigarh, India. The above cultures except *L. acidophilus* were maintained aerobically in Nutrient broth (HIMEDIA Laboratories Pvt. Ltd., Mumbai, India) and *L. acidophilus* was maintained in De Man, Rogosa, Sharpe (MRS) broth (HIMEDIA Laboratories Pvt. Ltd., Mumbai, India). *L. acidophilus* was cultured in CO_2 enriched condition at 37 °C in MRS broth or MRS agar.

Bacterial media and other reagents used

Trypticase soy broth, agar powder, Folin and Ciocalteau's phenol reagent AR, dimethyl sulfoxide, methanol extrapure AR were purchased from Merck Specialities Pvt. Ltd., Mumbai, India. Antibiotic discs and tetracycline powder were purchased from HIMEDIA and Sigma Chemical Co. (St. Louis, MO, USA), respectively.

Plant material

The flower samples of *S. grandiflora* were collected from local market. The species were identified at the Botanical Survey of India, Kolkata, India and the petals were separated, sun dried, powdered, and stored in a refrigerator at −20 °C for further studies.

Extraction of S. grandiflora flower petals

One gram of dried finely powered flowers were weighed, homogenized and extracted with 80:20 methanol:water at room temperature and filtered. The residue was re-extracted till the filtrate was colorless. The extracts were pooled and evaporated in a freeze drier till free of solvent (China et al. 2011). The lyophilized PE was dissolved in 10% DMSO for further use in the experiments.

Determination of polyphenols

Total polyphenol content of the flower extracts were determined by the method described by Matthaus 2002. Briefly, 0.2 mL of different concentrations of the extracts were taken, to which 1 mL of Folin–Ciocalteau reagent (diluted to 10 folds) and 0.8 mL of 2% Na_2CO_3 were added. The volume was increased to 10 mL with methanol:water (6:4). The mixture was incubated at 37 °C for 30 min before the absorbance was measured at 740 nm. The results were expressed as gallic acid equivalents per gram of extract.

Determination of flavonoids

Total flavonoid content was measured by the aluminum chloride colorimetric assay (Ebrahinzadeh et al. 2008). An aliquot of appropriately diluted (50 and 100 μg/mL) PE or standard solution of quercetin (20–100 μg) mixed with 4 mL of distilled H_2O and subsequently with 0.3 mL 5% $NaNO_2$ solution. After 5 min, 0.3 mL 10% $AlCl_3$ were added and further the solution was appended with 2 mL 1 M NaOH. Final volume was made upto 10 mL with distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm.

Agar well diffusion assay

The agar well-diffusion method (Perez et al. 1990) was conducted to evaluate the inhibitory spectrum of PE against test microorganisms. A freshly grown culture was serially diluted, and 0.1 ml of diluted inoculum (10^6 CFU/mL) of test organism was spread on agar plates. Muller Hinton Agar (Hi media) was used for *E. coli*, *S. aureus*, S. Typhi and *S. flexneri* 2a, and *V. cholerae*. Wells (6 mm in diameter) were made in agar using a sterilized stainless steel borer. Each well was filled with 50 μL of diluted extracts (30 μg/well). The plates were left at room temperature for 30 min to allow diffusion of materials in media. Ampicillin, streptomycin, chloramphenicol, ciprofloxacin, azithromycin, doxycycline, nalidixic acid, ofloxacin, tetracycline were used as positive controls. Negative controls (10% dimethyl sulfoxide DMSO) were also used under the same conditions. Plates were incubated at 30 °C for 24 h, until visible growth of test microorganisms was evident in control plates. Inhibition zones in mm (including well diameter) around wells were measured. The antimicrobial activity was expressed as the diameter of inhibition zones produced by the extracts against test microorganisms. The experiment was repeated for three times. The cultured bacteria with halos equal to or greater than 7 mm were considered susceptible to the PE of *S. grandiflora* flower (Nascimento et al. 2000) where as zone of inhibition formed by commercial antibiotics were compared with the zone size interpretative chart supplied by HIMEDIA (Bauer et al. 1966; CLSI 2008).

Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration (MIC) was determined by the method of successive dilution as described by Mazzola et al. (2009). Twelve numbered screw tubes (10 mm × 100 mm) were taken with 1 mL of TSB (Trypticase soy broth) medium in each of them except, number 1. For the first and the second tubes of the series, 1 mL of PE (0.05–0.55 mg/mL) or antibiotic (0.02–0.05 mg/mL) was added; tube 2 was stirred and 1 mL was withdrawn and transferred to tube 3. This successive transference was repeated until tube 11. All the tubes except tube number 11 were inoculated with 0.1 mL of inoculum (10^6 CFU/mL) at known concentration. The tubes were incubated at optimal temperature (37 °C) for 24 and 48 h and the results were noted. Tube 11 (TSB + PE/antibiotic) and tube 12 (TSB + inoculum) were taken as controls.

In situ effect of extract

The *in situ* efficacy of PE (Al-Zoreky 2009) was evaluated against *S. aureus* ATCC 25923 in a modified food model (fresh chilled fish). A commonly consumed fish (Silver Pomfret, *Pampus agrenteus*) was used to evaluate the antibacterial activity of PE toward the above pathogenic organism. Three fish samples were beheaded and degutted under aseptic conditions in the laboratory. The fish samples were rinsed under running tap water, drained and cut to 5 g pieces (11 cm × 3.5 cm × 1.5 cm). The pieces were put inside plastic bags (3 pieces per bag) followed by cold storage (4 °C) for approximately 3 h. *S. aureus* ATCC 25923 was pre-cultured at 37 °C for 18 h in Mannitol salt broth (MSB) (5 mL). The freshly grown pathogen was serially diluted with sterile phosphate buffer saline (PBS) to 10^6 CFU/mL, as determined by spread plating onto Mannitol salt agar (MSA), the selective media for the growth of *S. aureus*. Each piece was inoculated by spreading 200 μL of *S. aureus*. The inoculated samples were put in bags and stored at 10 °C for overnight to evaporate excess moisture from inoculated slices and also to allow adhesion of cells. After storage, fish pieces were transferred in a pre-sterilized container and 500 μL of PE (0.15 mg polyphenol) was added to the fish pieces and thoroughly minced for uniform mixing of the PE. Pieces were immediately stored at 10 °C. Fish pieces treated with sterile PBS under the same conditions was used as control. 10% salt (W/V) solution, a common preservative used to preserve fish was used as a reference. After predetermined intervals (12 h), fish pieces were aseptically removed and blended with 3 mL PBS. Aliquots (0.1 mL) were spreaded on selective agar medium (MSA) for *S. aureus* followed by incubation at 37 °C. Counts of *S. aureus* from the experiments (each in duplicates) were statistically analyzed (*t*-test) for significant difference between control and treatment employing the SPSS 12.0 (SPSS Inc., USA).

Determination of growth promoting property of the PE of S. grandiflora flower

The prebiotic property *S. grandiflora* flower PE was studied on the probiotic bacterium *L. acidophilus* by two different ways. The growth-promoting activity of PE on *L. acidophilus* was measured in liquid cultures by plate count method. Fresh MRS broth (4.8 mL) was inoculated with *L. acidophilus* culture (0.1 mL containing 10^6 CFU/mL) containing PE (0.03 mg of polyphenol). In the positive control sets the culture was grown in absence of extract and in the negative control set the culture was grown in presence of DMSO. The cultures were incubated at 37 °C for 24 h under CO_2 enriched condition. After incubation, the samples were diluted in MRS broth and were plated on agar medium. The effect of *S. grandiflora* PE on *L. acidophilus* was determined comparing the number of CFU in the presence of PE against those obtained from controls.

In addition, the *L. acidophilus* culture was grown in MRS broth taken in Nephelometric flasks. In the sample set, bacterial culture with PE was added in the MRS broth whereas in positive control no PE was added and in the negative control set DMSO was added. The growth rate of *L. acidophilus* was measured photometrically at 600 nm.

Polyphenol content present in the broth

Total polyphenol content was measured in the broth before and after 24 h incubation with *L. acidophilus* at 37 °C (Arima et al. 2002).

Extraction and identification of flavonoid by high performance liquid chromatography

L. acidophilus was incubated with the PE of *S. grandiflora* flower in MRS broth. The flavonoids were extracted from the broth (Arima et al. 2002) before and after the growth of the bacterium for 24 h at 37 °C. Same volume of 10% TCA was mixed with the culture and centrifuged at 11,200 × *g* for 20 min. The flavonoids in the supernatant were extracted 3 times with ethylacetate, the resulting extracts were then dried under a stream of nitrogen gas. The flavonoids extracted from the broth were analyzed by HPLC (Siddhuraju and Becker 2003). In brief, a gradient elution was employed for flavonoids other than catechins with a mobile phase consisting of 50 mM H_3PO_4, pH 2.5 (solution A), and acetonitrile (solution B) which is as follows: isocratic elution 95% A/5% B, 0–5 min; linear gradient from 95% A/5% B to 50% A/50% B, 5–55 min; isocratic elution 50% A/50% B, 55–65 min; linear gradient from 50% A/50% B to 95% A/5% B, 65–67 min; 6 min before the next injection. The HPLC system (WATERS 2487) was equipped with a C-18 column (Nova-Pak C_{18}, 3.9 mm × 150 mm). Flavonoids were analyzed at 280 nm, 340 nm and 370 nm wavelengths. The flow rate of the mobile phase was 0.8 mL/min, and the injection volume was 20 μL. The peaks were identified in comparison with standards.

Results

Total phenolic content of the S. grandiflora flower PE

The total polyphenol content of the *S. grandiflora* flower PE was expressed as gallic acid equivalent per gram of dry weight. The edible flower *S. grandiflora* PE was found to be 465.02 ± 7.45 mg of GAE/g of dry wt and the total flavonoid content of the PE was estimated to be 0.71 g of quercetin/g of polyphenol.

Agar well diffusion assay

In this study the PE of *S. grandiflora* flower extract was tested for its antimicrobial effect against pathogens. The antimicrobial potency was initially determined by the agar well-diffusion method. Table 1 presents diameters of inhibition zones (clear zones around wells) exerted by the extracts toward challenged microorganisms. DMSO (negative control) was inactive against tested microorganisms. The marked inhibition was only associated with PE (Table 1). It had also a broad activity since it was antagonistic for both gram positives and gram negatives bacteria. The extract showed antibacterial activity against all the bacteria tested. The PE was more active against S. Typhi and *S. aureus* (16–17 mm).

Minimum inhibitory concentration (MIC)

Quantitative evaluation of the antimicrobial activity of PE was carried out against selected microorganisms. The MICs, of the PE and tetracycline were presented in Table 2. Of the bacteria tested,

Table 1
Antimicrobial activity of polyphenol extract of *Sesbania grandiflora* flowers using agar well diffusion method.

Microorganisms	Diameter of inhibition zone (mm)[a]										
	PE	A[b]	S[c]	C[d]	Cf[e]	At[f]	Do[g]	Na[h]	Of[i]	T[j]	DMSO
E. coli	13 ± 0.3	15I ± 0.5	17S ± 0.5	21S ± 1	25S ± 0.5	22S ± 0	18S ± 0	20S ± 0	27S ± 0.5	20S ± 0.5	0
Staphylococcus aureus	17S ± 0.2	19R ± 0	14S ± 0	20S ± 0.5	24S ± 1	25S ± 0	23S ± 0	10R ± 0.5	24S ± 0.5	25S ± 1	0
Salmonella Typhi	16S ± 0.2	23S ± 0	14I ± 0	26S ± 0.5	28S ± 0	22S ± 0	25S ± 0.5	10R ± 0	26S ± 1	24S ± 0.5	0
Shigella flexneri	13 ± 1.0	10R ± 0	12I ± 0	18S ± 1	18I ± 0.5	18S ± 0	18S ± 0.5	13R ± 1.2	20S ± 0.5	20S ± 0	0
Vibrio cholerae	12 ± 0.1	10R ± 0	10S ± 0.5	12R ± 0	22S ± 0.5	20S ± 0.5	0R ± 0	10R ± 0	0R ± 0	25I ± 0.5	0

S – sensitive; R – resistant; I – intermediate.
[a] Values are expressed as mean ± SEM, $n = 3$.
[b] Ampicillin.
[c] Streptomycin.
[d] Chloramphenicol.
[e] Ciprofloxacin.
[f] Azithromycin.
[g] Doxycycline.
[h] Nalidixic acid.
[i] Ofloxacin.
[j] Tetracycline.

the MICs of *S. aureus* ATCC 25923 (0.013 mg/mL) and *Shigella flexineri* (0.02 8 mg/mL) showed the highest susceptibility to the PE of *S. grandiflora* flower compared with S. Typhi (0.065 mg/mL), *E. coli* (0.2 mg/mL), and *V. cholerae* (0.250 mg/mL), The MIC was found to be in the order of *S. aureus* < *S. flexineri* < S. Typhi < *E. coli* < *V. cholerae*.

Antagonistic activity to S. aureus in fish

PE of *S. grandiflora* demonstrated highest zone of inhibition and lowest MIC against *S. aureus*. *S. aureus* being a well known food poisoning pathogen producing "enterotoxin" has been used for *in situ* antimicrobial experiment. In the present study, *Staphylococcus*-contaminated fish slices were treated separately with PBS (control), 10% salt (w/v) solution (Ravesi and Krzynowek, 1991) and PE and stored at 10 °C for two days. During the cold storage trials, *S. aureus* were not detected in non-inoculated fish or PBS (controls) used in the experiments. PE showed inhibition against *S. aureus* in *in situ* food model (Fig. 1). *S. aureus* gradually increased at 10 °C in control slices, reaching more than 7.61 $\log_{10}$/g and 9.23 $\log_{10}$/g after 1 day and 2 day storage, respectively. Their growth rate in PE is significantly lower reaching 5.13 $\log_{10}$/g and 5.32 $\log_{10}$/g compared to control and 10% salt solution in 1 day and 2 day storage, respectively. PE significantly prevented ($p < 0.05$) the proliferation of the pathogen in fish pieces from 12th hour onwards.

Growth promoting property

The growth curve pattern of three sets was shown in Fig. 2. In the control set only the *L. acidophilus* culture was grown in MRS broth. In the sample set the bacterial culture was grown in MRS broth in presence of the PE and a negative control set containing DMSO was also maintained. The set containing the PE showed higher rate of growth compared to the control as measured by spectrophotometric analysis at 600 nm. This shows that the extract is having a growth promoting effect on *L. acidophilus.*

Table 2
Minimum inhibitory concentration (MIC) of polyphenol extract of flowers of *Sesbania grandiflora.*

Organism	MIC (mg/mL)[a]	Tetracycline (mg/mL)[a]
Escherichia coli	0.2 ± 0.01	0.02 ± 0.001
Staphylococcus aureus	0.013 ± 0.001	<0.001 ± 0.001
Salmonella Typhi	0.065 ± 0.003	0.005 ± 0.005
Shigella flexneri	0.028 ± 0.006	0.025 ± 0.002
Vibrio cholerae	0.250 ± 0.007	0.05 ± 0.001

[a] Values are expressed as mean ± SEM, $n = 3$.

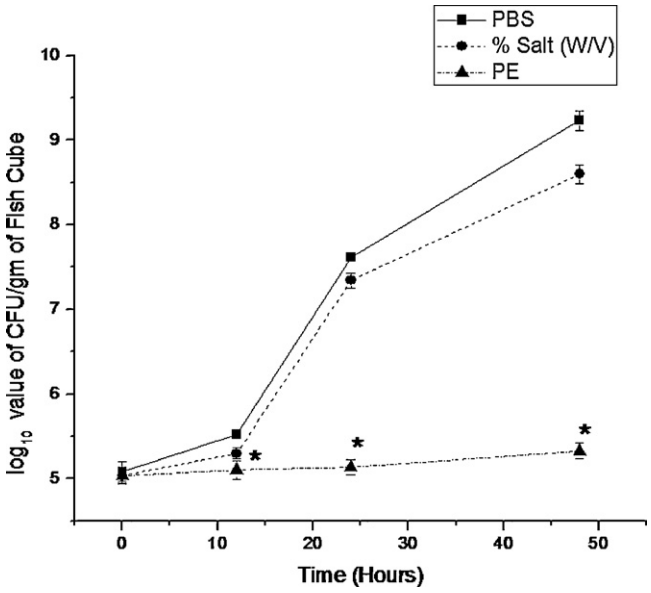

Fig. 1. Effect of PE of *Sesbania grandiflora* to fish pieces on the growth of *S. aureus* ATCC 25923 during storage at 10 °C.

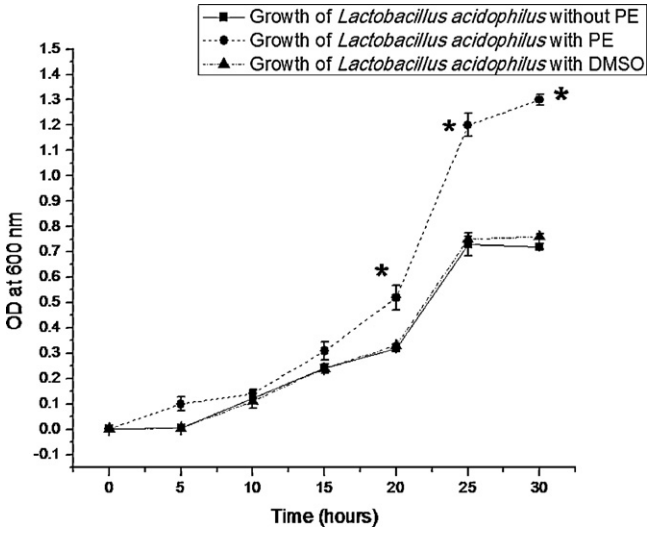

Fig. 2. *Lactobacillus acidophilus* grown in MRS broth with and without polyphenol extract.

Table 3
Growth stimulatory effect of *Sesbania grandiflora* flower PE on *L. acidophilus*.

Initial polyphenol content in MRS broth before incubation (mg)	0.03 ± 0.002
Polyphenol content after incubation for 24 h with *L. acidophilus* (mg)	$0.017 \pm 0001^{*}$
Initial CFU count/mL	$1 \times 10^{6} \pm 0.001$
Control CFU count/mL	$3.10 \times 10^{9} \pm 0.001$
DMSO CFU count /mL	$3.00 \times 10^{9} \pm 0.03$
Sample CFU count/mL	$5.53 \times 10^{9} \pm 0.004^{*}$

* Control *vs.* sample ($p < 0.05$).

For determining the quantitative growth promoting activity of the PE, plate count method was used. It was observed that after 24 h incubation, the addition of PE, in culture media, induced a statistically significant increase ($p < 0.05$) of *L. acidophilus* growth, in comparison with the cell count obtained from control culture (*L. acidophilus* grown only in MRS broth) and the negative control. No statistically significant difference was observed between plate count results of control growth media and DMSO-supplemented culture media. The CFU count/mL of three sets in triplicate is shown in Table 3.

Polyphenol content present in the broth

The polyphenol content of the broth was reduced after incubation for 24 h with *L. acidophilus*. It was found to be 0.017 mg/mL where as the initial polyphenol content of the broth was 0.03 mg/mL (Table 3).

Extraction and identification of flavonoid by high performance liquid chromatography

The HPLC analysis of flavonoids (Fig. 3) extracted from the broth before and after the incubation with *L. acidophilus* showed that there is a reduction of rutin content in the broth after incubation of bacterium for 24 h.

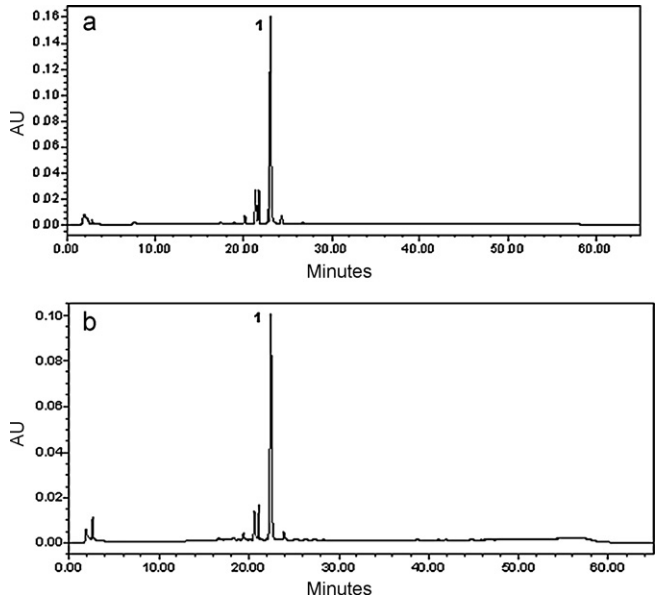

Fig. 3. HPLC chromatograms of *Sesbania grandiflora* polyphenol extract at 370 nm showing rutin (labeled 1). (a) *Sesbania grandiflora* polyphenol extract from broth without microorganisms and (b) *Sesbania grandiflora* polyphenol extract from broth after 24 h growth of *L. acidophilus*.

Discussion

Anti-bacterial screening of traditional medicinal plants has been the source of innumerable therapeutic agents. Among the bacteria used in the present study, *S. aureus*, a gram-positive organism, showed the highest sensitivity to PE of *S. grandiflora* flower. This was similar to the previous reports which suggested higher susceptibility of food-borne pathogenic gram positive bacteria to tea flavonoids in comparison with gram-negative bacteria (Ikigai et al. 1993; Toda et al. 1989). The PE of *S. grandiflora* flower showed MIC activity in the range (concentrations) from 0.013 mg/mL to 0.250 mg/mL against all the pathogenic strains among which *V. cholerae* was a multi-drug resistant organism. Saxena et al. (1994) documented a MIC varying from 12.5 μg to 1000 μg when testing with different concentration of *Rhus glaba* extracts against gram-positive and gram-negative bacteria. The sensitivity of *S. aureus*, and S. Typhi to our PE of *S. grandiflora* flower showed similar result in comparison with the result obtained by an aqueous extract of garlic (Arora et al. 1999). It was previously reported that green tea extract showed MICs at 0.800 mg/mL against *E. coli* ATCC 25922 (Su et al. 2008) where as the inhibition was observed with 0.200 mg/mL of PE of *S. grandiflora* flower.

Polyphenols showed antimicrobial activity *in vitro* but they became less active in complex matrices (Lin et al. 2004; Cornu et al. 2006). Therefore, it was appropriate to conduct *in situ* experiments for evaluating the efficacy of PE in a modified food model during typical storage conditions of the test food (chilled fish). We also considered the shelf life of fish as a function of temperature (Doyle 1989). Our PE of *S. grandiflora* flower was found to contain rutin as a major flavonoids. Rutin present is a glycosylated flavonoids that retards the growth of *Pseudomonas* and *Staphylococcus* reported to be the associated microbes of *A. foveicollis* (Sami and Shakoori 2007). When screening natural products for type II topoisomerase inhibitors, Bernard et al. (1997) found that the glycosylated flavonol rutin was very effective. This compound exhibited antibacterial activity against a permeable *E. coli* strain. Using enzyme assays and a technique known as the SOS chromotest, it was shown that rutin selectively promoted *E. coli* topoisomerase IV-dependent DNA cleavage, inhibited topoisomerase IV-dependent decatenation activity and induced the SOS response of the *E. coli* strain. The group suggested that since topoisomerase IV is essential for cell survival, the rutin-induced topoisomerase IV-mediated DNA cleavage that leads to an SOS response and growth inhibition of *E. coli* cells. It had been reported that glycosylated flavonoids act as selective inhibitors for topoisomerases thus interfering with the replication and transcription mechanics (Winter et al. 1989). The mechanism of growth inhibition of quercetin derivatives seems to be different from that of ampicillin. Flavonoids whose mechanisms of antibacterial activity have been investigated include robinetin, myricetin, apigenin, rutin, galangin, 2,4,2′-trihydroxy-5′-methylchalcone (Cushnie and Lamb 2005). The antimicrobial activity of rutin was tested against all pathogenic bacterial flora of the GI tract and compared with erythromycin to ascertain whether the active principle involved is rutin or some other component. The studies showed that rutin exhibited potent activity against *B. cereus*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* with the MIC values of 0.03 mg/mL (Singh et al. 2008). All the studies support the antimicrobial activity of the extract containing rutin as its major flavonoid. The inhibition of *S. aureus* in fish could be attributed to the impact of the flower phenolics on the pathogen. Polyphenols played an important role in protein precipitation and enzyme inhibition of microorganisms (Naz et al. 2007; Fan et al. 2008). Fan et al. (2008) indicated that dipping in tea polyphenols (0.2% total phenolics) accompanied by sub-freezing temperature (−3 °C) delayed the spoilage of a silver carp. It was indicated that mixed extract of oregano and cranberry was more active against

Listeria monocytogenes Scott A in cod fish than either one alone (Lin et al. 2004). Black pepper, papaya and grape polyphenol were found to act as fish preservative against fish spoilage bacteria including *S. aureus* (George et al. 2004).

In the human intestine the extensive metabolism of flavonoids has been attributed to the action of intestinal microflora (Kim et al. 1998). So it was appropriate to conduct an experiment for evaluating the efficacy of PE of *S. grandiflora* flower in promoting growth of a probiotic strain *L. acidophilus*.

The HPLC analysis of flavonoid in the broth before and after the growth of the *L. acidophilus* as depicted in Fig. 3 clearly showed that there is a reduction of rutin content in the broth after incubation of bacterium for 24 h. Hydrolytic enzymes of intestinal microflora (Macdonald et al. 1983) could convert certain flavonoid glycosides (*e.g.*, rutin) to their corresponding aglycones (*e.g.*, quercetin). When human intestinal bacteria were cultured in presence of rutin, at first the quercetin content increased with decrease in rutin. Gradually the quercetin level decreased with the increase in unidentified compounds (Bokkenheuser et al. 1987). In another study it was showed that human intestinal bacteria metabolizing flavonoids cleaves C-ring of rutin and the half-life of aglycone is short in the intestine (Winter et al. 1989). These observations support our results that show *L. acidophilus* metabolizes rutin which is major component of sesbania flower PE. The mechanism by which PE increased the growth of probiotic bacteria is not known. Molan et al. (2009) presented a possible partial explanation for this growth enhancing effect. Polyphenols act as antioxidant and antiradical agent to modulate the oxidative stress in the medium generated by the metabolic activities and consequently provides a better environment for the growth and multiplication of these bacteria.

Conclusion

In conclusion we can say that few gram-negative enteric pathogens are susceptible to PE of *S. grandiflora* flower and it also effective against a gram-positive food borne bacteria *S. aureus*. Human pathogens such as *S. aureus* which are ubiquitous in food showed sensitivity to PE of *S. grandiflora* in food matrices (*in situ* fish preservation). Further studies with different types of strains are required before it can be used as a food preservative. The present work provides new findings about the growth promoting activity of PE of *S. grandiflora* on probiotic organism like *L. acidophilus* and significant increase of *L. acidophilus* MTCC 447 biomass. The behavior of the bacteria in the presence of extract may not reflect their reaction in the intestine, but the *in vitro* growth stimulatory effect on probiotic bacteria and growth inhibitory effect on pathogenic bacteria might indicate the *in vivo* interaction of the PE and those pathogenic as well as probiotic organisms. Added-value items from edible flowers *S. grandiflora* could provide health benefits to humans and may be employed in food preservation and pharmaceutical purposes.

Acknowledgements

We acknowledge University Grants Commission for funding. We also acknowledge Dr. Mahua Ghosh, Assistant Professor, Department of Chemical Technology, University of Calcutta, for her remarkable support and help during the entire research.

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