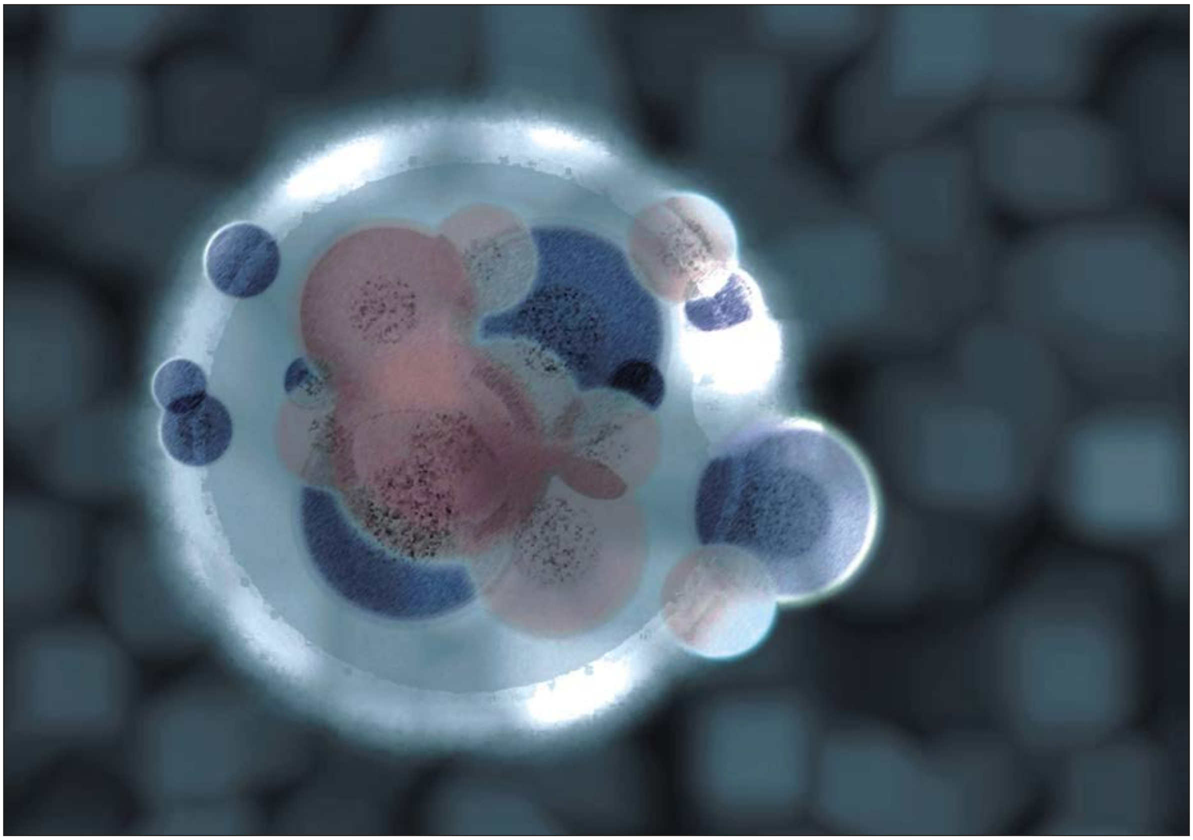

Journal of Biological Sciences

ISSN 1727-3048

Journal of Biological Sciences 8 (8): 1280-1288, 2008
ISSN 1727-3048

Antibacterial Activities of Fermented Plant Beverages Collected in Southern Thailand

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Abstract: Total of 19 samples of household Fermented Plant Beverages (FPBs) were investigated for their ability to inhibit the growth of some pathogenic bacteria. Increasing amounts, 50<75<125 μL, of beverages caused increased inhibition of *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* sp., *Shigella* sp., *Vibrio parahaemolyticus* and *Pseudomonas aeruginosa*. The FPBs from banana (*Musa sapientum* L.) and wild forest noni (*Morinda coreia* Ham) produced the best inhibitions. In both these cases the total acidity and potassium levels exceeded the average value of all FPBs by >500 mg L^{-1}. Although both these FPBs significantly inhibited all Gram positive bacteria their effects on Gram negative bacteria were more variable. *E. coli* was the most resistant bacterium followed by *Salmonella* sp. whereas *P. aeruginosa*, *Shigella* sp. and *V. parahaemolyticus* were more sensitive. The banana FPB had higher amounts of acetic acid, lactic acid, ethanol, methanol and acetaldehyde than did the wild forest noni beverage. This may cause its higher inhibitory effect on *Salmonella*, *Shigella* and *Pseudomonas*. Some chemicals with antibacterial activity were also extracted from some plants. Consequently the antibacterial activities of FPBs were mainly attributed to their total acidity including their organic acids and some bioactive compounds derived from the plants themselves.

Key words: Antibacterial activity, fermented plant beverages, Gram positive bacteria, Gram negative bacteria, total acidity, potassium

INTRODUCTION

Fermented plant beverages (FPB or FPBs) are non-alcoholic beverages produced from different kinds of plants such as cereals, fruits and vegetables. In a previous study we found that FPBs have a sour flavor that develops during fermentation for at least 3 months and a little sweetness from residual sugar (Kantachote *et al.*, 2005). There is no information available on when local Thai people began to produce FPBs and it is still a household product made throughout the country. People who consume these FPBs believe it is a healthy beverage and helps to cure some diseases. They have a high nutritional value and contain bioactive compounds derived from the raw materials, particularly the plants, as well as products from the fermentation reactions. However, unlike wine, there is little information available about the properties of FPB's or their microflora, therefore there are no criteria or standards set for FPB products. Because of the above reasons the National Science and Technology Development Agency (NSTDA), Thailand has allocated a budget to a number of universities to obtain scientific information about FPBs. All the scientific information collected on the effects of FBP's on human health whether positive or negative will be evaluated and this information will be provided to the public to help them make their own decisions on whether to consume them or not. In parallel, the scientific information could be used by Government agencies to design quality guidelines for FPBs.

The FPB product is derived by lactic acid fermentation catalyzed by Lactic Acid Bacteria (LAB) (Kantachote *et al.*, 2005). The lactic acid fermentation has long been recognized and used by humans for producing a variety of foodstuffs (Battcock and Azam-Ali, 1998; Jay, 2000; Ray, 2003). LAB produce a variety of compounds such as organic acids, diacetyl, hydrogen peroxide and bacteriocins (bactericidal proteins) during lactic fermentations and these compounds extend the shelf life of the fermented foods by controlling spoilage organisms and also potential pathogens (Adams and Moss, 2000; Oyetayo *et al.*, 2003; Savadogo *et al.*, 2004; Montville and Matthews, 2005). There are many reports on the beneficial effects of LAB on human health and one of these is the presence of antimicrobial activity (Cleveland *et al.*, 2001; Messens and De Vuyst, 2002; Silva *et al.*, 2002; Soomro *et al.*, 2002; Şimşek *et al.*, 2006). Here we report one of our studies on FPBs with the aim to investigate the antibacterial action of FPBs on pathogenic bacteria, particularly enteropathogenic bacteria.

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MATERIALS AND METHODS

Samples used: Nineteen samples of household Fermented Plant Beverages (FPBs) were collected from various sources in southern Thailand and investigations carried out during 2004-2005. In general, the main components of FPBs are fruit or vegetables, sugar or honey and potable water in the proportions of 3:1:10 (w/w/v) and all raw materials are incubated in a nearly full container for at least 3 months. However, the proportions of raw materials and fermentation time are varied depending on the producers and more details are shown in Table 1. None of the collected samples received any treatment except perhaps for a brief storage in a cold room (4±1°C) until being used.

Determination of physico-chemical properties of FPBs: In this study we focus on the antibacterial activity of the FPBs and some of their physico-chemical properties that may be concerned with their antibacterial actions. The following physico-chemical properties were examined using standard analytical methods (AOAC, 2000), pH, titratable acidity and EC (Electrical conductivity: A measure of the total concentration of dissolved salts in water or solution). Elements (Cu, Zn, K and Na) were determined using inductively coupled plasma-atomic emission spectroscopy (ICP-AES) according to the instructions for the instrument.

Determination of the antibacterial activity of FPBs: The antibacterial activity of FPBs was investigated using the agar disc/well diffusion method (Schillinger and Lucke, 1989). Test organisms, their growth media and the high potency antibiotic discs used as controls following Seeley *et al.* (1991) are shown in Table 2. The organisms used (Table 2) were obtained from the Department of Microbiology, Faculty of Science, Prince of Songkla University. The test organisms were maintained by routine culture on Nutrient Agar (NA) slants except for *Vibrio parahaemolyticus* for which NA plus 3% NaCl was used. Slants were stored at 4°C (FDA, 2001). At least two additional subcultures (24 h, 37°C) were made on fresh (NA or NA plus 3% NaCl) plates prior to use in the experiment. For testing 3-4 single colonies were transferred into 4 mL Tryptic Soy Broth (TSB) and incubated at 37°C for 3 h. The turbidity of each actively growing culture was adjusted to 0.5 (McFarland standard) and then swabbed over the surface of a TSA (Tryptic soy agar) plate and then swabbing was repeated to ensure an even distribution of inoculum. Ten minutes was then allowed for excess surface moisture to be absorbed. The antibiotic disc used as a positive control and 3 metal cup wells were then added to the plate surface with sterile forceps. Either 50, 75, or 125 μL of the FPB was added to the metal cup well and the final volume in each case was adjusted to 125 μL with distilled water. Plates were incubated at 37°C for 18 h and then the diameters of the

Table 1: Information of fermented plant beverages collected from various sources in southern Thailand

Sample	Source	Composition[1]	Container	Fermentation time
1: Gotu kola: *Centella asiatica* Urban 2: Cherry: *Malpighia glabra* L. 3: Mince: *Mentha cordifolia* Opiz 4: Passion fruit: *Passiflora* sp. 5: Aloe: *Aloe vera* (L.) Burm.f	Thaksinasolk Muang district Trang province (samples 1-5)	Plant 2.5/5, honey1/5 and starter 1/5 (v/v of bottle)	PET drinking water bottle (2 L)	2 years from start of fermentation. Every 3 months remove sample and replace with a fresh sample of raw materials
6: Banana: *Musa sapientum* L.	Muang district Trang province	Sliced banana 2.5/5, honey 1/5 and starter 1/5 (v/v of container)	Plastic cylinder (1 L)	3 years with the same condition as above
7: Noni: *Morinda citrifolia* L.	Muang district Phangnga province	Fruit 3 kg, brown sugar 1 kg and clean water 10 L and 10% starter (v/v)	Sale in glass bottle (750 mL)	12 months
8: Wild forest noni: *Morinda coreia* Ham. 9: Mixed fruit[2]	Hat-Yai district Songkhla province (samples 8-9)	Same as the sample 7	Big plastic bucket (50 L)	6 months
10: Eucalyptus: *Eucalyptus citriodora* Hook. 11: Soybean: *Glycine max* L. 12: Lemon glass: *Cymbopogon citratus* Stapf 13: Green seaweed[3] 14: Chamuang: *Garcinia cova* Roxb. 15: Cordifolia: *Tinospora crispa* (L.) Mers ex Hook.f. 16: Half-milled rice: *Oryza sativa* L. 17: Nutmeg: *Diospyros decandra* Lour. 18: Thammang: *Litsea elliptica* Boerl 19: Mangosteen shell: *Garcinia mangostana* L.	Huay-yoi district, Trang province (samples 10-19)[4]	Fruit 3 kg, honey and brown sugar 1 kg and clean water 10 L and 10% starter (v/v)	Big size (20 L) of drinking water bottle	3-6 months except a few sample (14 and 17) <3 months

[1]: Previously fermented plant beverage was used as a starter for each sample, [2]: Mixed fruit: Pineapple, noni, pumpkin, star apple and fig (*Ficus racemosa* Linn.), [3]: As finished product could not identify scientific name, [4]: Samples 10-19 were collected from the same place and their composition, containers used and fermentation times were similar

clear zones (inhibition zones) were measured using a vernier caliper. The results are given in mm, with the disc/well diameter having been subtracted. Each sample was tested 3 times and the mean values are presented.

Antibacterial activity of FPBs was ranked by size of the inhibition zones resulting from diffusion of the antibacterial compounds in FPB/antibiotic and the growth rate of the indicator organisms. Bacteria with zones of inhibition of less than 12 mm in diameter are considered to be not sensitive to the FPB. When the inhibition zones are from 12-18 mm, bacteria are considered to be sensitive and the FPB may have some potential as an inhibitory agent. When the zones are larger than 18 mm the bacteria are considered very sensitive and perhaps the FPB producing such a zone should be examined in more detail at some later stage.

Selection of samples for further study: FPBs producing an inhibition zone similar to that given by the control antibiotic or with a high acidity (>2.44 g/100 mL) or higher than average K, Na, Cu and Zn contents were selected for further investigation. Organic acids (acetic and lactic), alcohols (ethanol and methanol) and acetaldehyde were assayed using gas chromatography following the methods of Yang and Choong (2001) with HP 6850 Gas chromatograph and a Flame Ionization Detector (FID). The conditions used for lactic and acetic acids were as follows: inlet temperature 240°C, oven temperature 75°C /1 min, Ramp to 180°C at 5°C/min, ramp to 230°C at 15°C/min. For alcohols (ethanol and methanol) and acetaldehyde, inlet temperature was 240°C, oven temperature 35°C/5 min, Ramp to 100°C at 5°C/min, ramp to 230°C (5 min) at 15°C/min. The column used was Rtx-5MS, length 30 m, film thickness 0.25 µm, ID 0.25 mm. Samples with higher than average levels of K and Na were also considered because these ions could contribute to any antimicrobial activity (Shelef, 1994; Del Campo *et al*., 2000). FPBs with safe levels of Cu and Zn can lead to the production of a copper-zinc superoxide dismutase, a key antioxidant enzyme (Woodruff *et al*., 2004; Choi *et al*., 2005) that may act as an inhibitor. One fermented chamuang beverage was also selected because it showed some antibacterial activity even though it contained the lowest level of total acidity.

Table 2: Test organisms, their growth media and antibiotic discs used as controls for inhibition studies of FPB's

Test organism	Test medium	Antibiotic disc	Dose
Staphylococcus aureus	Tryptic soy agar (TSA)	Vancomycin	30 (µg)
Bacillus cereus	TSA	Penicillin G	10 (U)
Escherichia coli	TSA	Cephalothin	30 (µg)
Pseudomonas aeruginosa	TSA	Tetracycline	30 (µg)
Salmonella sp.	TSA	Cephalothin	30 (µg)
Shigella sp.	TSA	Chloramphenicol	30 (µg)
Vibrio parahaemolyticus	TSA plus 3% NaCl	Cephalothin	30 (µg)

Data presentation: Descriptive analysis was used to present the data as minimum, maximum, mean, range and Standard Deviation (SD). The means of three replicates and SD are presented.

RESULTS AND DISCUSSION

Physico-chemical property of FPBs: A high variation of Electrical Conductivity (EC) was found in FPBs as the average EC for all 19 samples was 2529±1367 µS. The EC value of each FPB can be shown in Table 3. Only 2 samples, those from green seaweed and lemon grass had EC higher than 5000 µS. EC values between 2500-5000 µS were found for banana, wild forest noni, soybean, thammang and mangosteen shell. All other values were less than 2500 µS with the lowest from cherry and mince being 896 and 989 µS, respectively. Although Na and K would be expected to make a major contribution to the total EC values and the levels of Cu and Zn were too small less than 0.15 and 7.00 mg L^{-1}, respectively to make a significant contribution there is no direct correlation between the total amounts of Na and K and the measured EC values. What may be surprising is that the levels of K were always far higher (except as expected that of green seaweed) than the Na levels. Values of K of 1000 mg L^{-1} or more were found in the fermented beverages produced from banana, wild forest noni, soybean, lemon grass and mangosteen shell. The average value for K was 788 mg L^{-1} with a lowest value of 204 mg L^{-1} for the noni

Table 3: Physico-chemical properties of fermented plant beverages collected in southern Thailand

Sample code/ Common name	EC[1] (µS)	K (mg L^{-1})	Na (mg L^{-1})	Total acidity (g/100 mL)	pH
1: Gotu kola	1339	847	36	2.44	3.17
2: Cherry	896	751	16	1.92	3.37
3: Mince	989	581	43	1.92	3.38
4: Passion fruit	1183	811	18	3.20	3.21
5: Aloe	2105	423	25	2.41	3.5
6: Banana	3305	1350	11	7.13	2.83
7: Noni	1238	204	9	2.85	3.06
8: Wild forest noni	4640	1310	52	5.76	3.27
9: Mixed fruit[2]	2195	503	11	3.39	3.28
10: Eucalyptus	1950	519	15	2.85	3.28
11: Soybean	3520	1335	16	5.39	3.39
12: Lemon grass	5145	1290	24	3.17	3.72
13: Green seaweed	5060	385	971	2.10	3.06
14: Chamuang	1987	432	20	0.98	3.48
15: Cordifolia	1591	451	5	3.65	3.17
16: Half milled rice	2235	710	41	4.55	3.24
17: Nutmeg	1705	479	61	4.01	2.63
18: Thammang	3405	987	503	1.07	3.60
19: Mangosteen shell	3570	1600	22	4.01	3.28
Mean	2529	788	100	3.31	3.26
Standard deviation	1367	410	238	1.59	0.25

[1]EC: Electrical conductivity, [2]Mixed fruit: Pineapple, noni, pumpkin, star apple and fig (*Ficus racemosa* Linn.)

Table 4: Amounts of organic acids, alcohols and acetaldehyde in fermented plant beverages in southern Thailand

Sample's common name	Concentration (g/100 mL) ±standard deviation				
	Lactic acid	Acetic acid	Ethanol	Acetaldehyde	Methanol
Gotu kola	4.30±0.13	1.21±0.02	1.85±0.03	0.048±0	0.038±0.001
Passion fruit	0.34±0.06	0.04±0.003	3.32±0.14	0.088±0.01	0.084±0.006
Banana	2.72±0.05	1.95±0.01	0.57±0.09	0.027±0.004	0.04±0.0050
Noni	2.78±0.15	1.42±0.03	0.03±0	0.022±0	0.033±0.001
Wild forest noni	1.94±0.13	1.91±0.01	0.05±0	0.007±0.001	0.019±0
Soybean	0.76±0.07	0.68±0.02	2.87±0.13	0.024±0.001	0.049±0.004
Green seaweed	0.32±0.003	0.55±0.02	2.27±0.09	0.023±0.001	0.035±0.001
Chamuang	0.09±0.007	0.09±0.004	1.35±0.05	0.02±0.001	0.039±0.002
Half-milled rice	0.62±0.03	1.61±0.02	3.00±0.02	0.025±0	0.041±0.001
Mangosteen shell	1.95±0.02	1.66±0.08	0.49±0.02	0.025±0.001	0.048±0.001
mean	1.58	1.11	1.57	0.031	0.043
Standard deviation	1.39	0.72	1.27	0.022	0.017

fermentation. The highest amount of sodium (971 mg L^{-1}) in the green seaweed FPB was followed by thammang at 503 mg L^{-1}. Excluding these 2 values the average Na levels of the remaining 17 samples was 24 mg L^{-1}, whereas with them included the average level was 100 mg L^{-1}. In general, the levels of Na are not likely to contribute to any microbial inhibitory activity.

Total acidity varied from 0.98-7.13 g/100 mL with an average value of 3.31±1.59 while the pH values varied from 2.63-3.72 with an average value of 3.26±0.25. The highest level of acid (7.13 g/100 mL) was found in the fermented banana beverage with the pH of 2.83 followed by wild forest noni 5.76 g/100 mL and a pH of 3.27 (Table 3). The lowest amount of total acidity about 1 g/100 mL was detected in a fermented chamuang and thammang beverage. There was no direct correlation between total acidity and pH values. In most cases, where significant amounts of acid were detected and organic acids were quantified, lactic acid was the predominant organic acid. Amounts of lactic acid in the 10 samples tested varied in the range of 0.09-4.30 g/100 mL with an average of 1.58±1.39 g/100 mL. The average amounts of acetic acid in all 10 samples tested was 1.11±0.72 g/100 mL. Only the FPB's from half milled rice and green seaweed had more acetic than lactic acid with lactic/acetic acid ratios of 0.39 and 0.58, respectively (Table 4). The lactic/acetic acid ratio in the FPB from Goto kola that had the highest level of lactic acid was 3.55. Ethanol was detected in almost all samples of FPBs but only in the range of 0.03-3.32 g/100 mL. The highest amounts were found in the fermented passion fruit beverage (3.32 g/100 mL) which also had the lowest amount of acetic acid. The FPB from half milled rice had 3.00 g ethanol/100 mL and the lowest amounts of 0.03 and 0.05 g/100 mL were found in fermented beverages of noni and wild forest noni. Two potentially toxic chemicals methanol and acetaldehyde were present in minute non toxic amounts in all samples with values ranging from 0.007 to 0.088 g/100 mL for acetaldehyde and 0.019 to 0.084 g/100 mL for methanol.

Antibacterial activity of FPBs: It must be remembered that every FPB sample used in this study had a different time of fermentation and also used a different production process (Table 1). In spite of this the inhibitory effect of each FPB on potentially pathogenic bacteria was investigated because it has been claimed that one of the benefits of FPBs was their ability to act as an antimicrobial agent. The antibacterial activity of all FPBs against the bacteria increased with increasing volume used. In each case the amounts used were 50, 75 and 125 μL, but only the results of the 125 μL amounts are presented. It must be noted that in all cases the SD's of the 3 aliquots of each sample tested were large. However, in general the Gram positive bacteria, *Bacillus cereus* and *Staphylococcus aureus* (Fig. 1A-B), were less sensitive than the Gram negative bacteria *Pseudomonas aeruginosa* and *Vibrio parahaemolyticus*. However, two of the Gram negative enteric pathogens *Escherichia coli* and *Salmonella* sp. (Fig. 2A-B) known to be resistant to acid and bile salts (FDA, 2001) were not sensitive to most of the FPB's.

Most FPBs gave zones of growth inhibition on the Gram positive bacteria, *Bacillus cereus* and *Staphylococcus aureus*, particularly *B. cereus* that were greater than 11 mm (Fig. 1A-B). The best inhibition was found with the FPBs from eucalyptus and mangosteen shell giving zones of 21 and 20 mm, respectively and followed by FPB's from banana, wild forest noni, lemon grass and nutmeg with inhibition zones of between 17-19 mm (Fig. 1A). Beverages produced from eucalyptus and lemon grass were not further discussed because eucalyptus had the lowest amount of Cu and the lemon grass FPB was heavily contaminated by fungi. FPB's from banana and wild forest noni showed no signs of contamination but the mangosteen shell did have

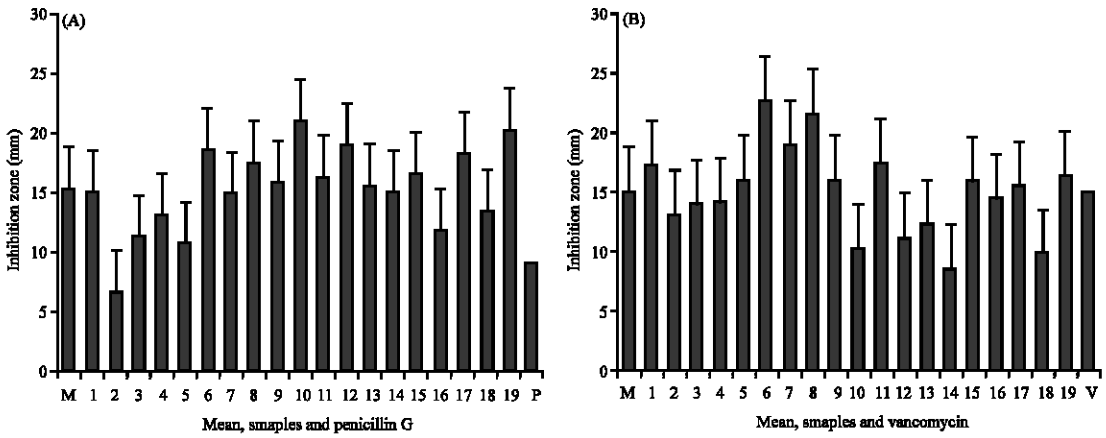

Fig. 1: Inhibitory activities of 125 µL fermented plant beverages against Gram positive bacteria. (A) *Bacillus cereus* and (B) *Staphylococcus aureus*, Each point represents the mean of three replicates±SD (standard deviation)

low amounts (≈ 2 log cfu mL^{-1}) of LAB and yeast (Kantachote *et al*., 2005). Although *Staphylococcus aureus* was sensitive to almost all FPB's and the control antibiotic of 30 µg vancomycin gave a 14.8 mm zone of inhibition, the FPB's from banana and wild forest noni gave even bigger zones of inhibition (22.5 and 21.4 mm), respectively (Fig. 1B).

For the Gram negative bacteria only the FPB's from aloe, banana and wild forest noni showed promise to control *E. coli* growth producing inhibition zones of from 14-17 mm (Fig. 2A). For *Salmonella* sp. only the FPB's from banana (22.6 mm) and wild forest noni (15.7 mm) has a significant inhibitory effect (Fig. 2B). Although *Pseudomonas aeruginosa* is normally considered to be fairly resistant to antimicrobials most FPB's gave zones of inhibition between 15-18 mm but for the FPB's from banana and wild forest noni the zones were 28.9 and 25.7 mm, respectively (Fig. 2C). These were much better than the 16 mm zone produced by the 30 µg tetracycline control so can be considered to be very significant and worthy of further study. For the *Shigella* sp. 12 FPBs produced zones greater than 15 mm but banana and wild forest noni FPB's produced inhibition zones of 26 and 22.8 mm, respectively (Fig. 2D). A 30 µg chloramphenicol control disc produced a zone of inhibition of 32 mm. *Vibrio parahaemolyticus* was sensitive to most FPB's with 15 samples giving zones of inhibition greater than 15 mm (Fig. 2E). Once again the most effective of FPBs against *V. parahaemolyticus* were the fermented beverages produced from banana (clear zone: 28 mm) and wild forest noni (clear zone: 28.6 mm). Based on the average zone size for the FPB's for each organism *V. parahaemolyticus* was the most sensitive to FPBs, whereas *E. coli* was the most resistant bacterium.

The beverage produced from chamuang, which contained the lowest amount of organic acids (Table 3) had a very variable effect on the test organisms. No inhibition zone was detected with *Escherichia coli* and *Salmonella* sp. whereas with *Shigella* sp. the zone of inhibition was 17 mm and for *B. cereus* 15 mm.

DISCUSSION

Inhibition of Gram positive bacteria by FPBs: This study has evaluated the antibacterial activity of FPBs by comparing their effects on the growth of a range of different potential pathogens. The FPB's that were able to inhibit a range of organisms or were particularly effective against a specific organism were considered suitable candidates for further investigations. Some note was also taken of their effect on the bacterium compared to the effect of a 'standard' antibiotic taking into consideration that the solubilities and diffusion characteristics of the FPB's and standard antibiotics might be very different. According to the results most of FPBs had some inhibitory effect on the Gram positive bacteria with the FPBs from banana and wild forest noni strongly inhibiting *S. aureus* (Fig. 1A-B). It has been established that factors contributing to microbial inhibition by fermentation products from lactic acid bacteria are low pH, organic acids, ethanol, diacetyl, hydrogen peroxide and bacteriocins (Parente and Ricciardi, 1999; Adams and Moss, 2000; Soomro *et al*., 2002). As the FPBs are products of lactic acid fermentation and lactic fermentation is essentially an anaerobic process any hydrogen peroxide produced will be limited by the amount of oxygen dissolved in the substrates at the start of fermentation (Adams and Moss, 2000). Acetaldehyde

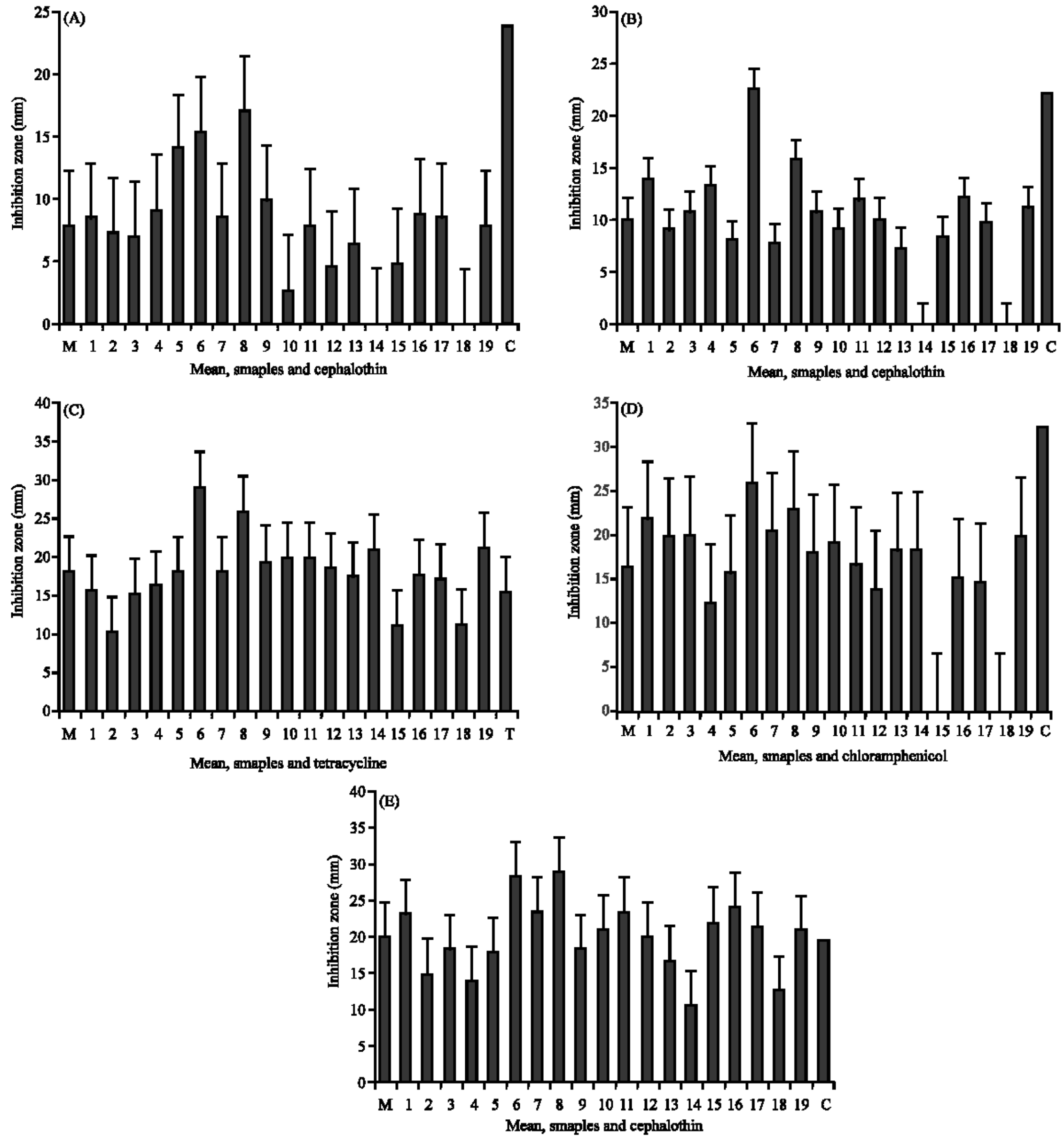

Fig. 2: Inhibitory activities of 125 μL fermented plant beverages against Gram negative bacteria. (A) *Escherichia coli*, (B) *Salmonella* sp. (C) *Pseudomonas aeruginosa*, (D) *Shigella* sp. and (E) *Vibrio parahaemolyticus*, Each point represents the mean of three replicates±SD (standard deviation)

(Moat and Foster, 2007) and diacetyl (Gill and Halley, 2003; Vignolo and Castellano, 2004) also have antimicrobial activity (Keenan, 1968). However the amounts of acetaldehyde detected were too small to cause significant inhibition (Table 4) and diacetyl was not measured. Methanol a potentially toxic compound to humans was produced in the FPBs during fermentation due to the degradation of pectin (Kantachote *et al*., 2005); however, again the amounts were very small. Also the samples containing the highest amounts of ethanol were no more effective than those with lower amounts. Bacteriocins are unlikely to be general control agents as they normally inhibit only closely related organisms (Attaie *et al*., 1987; Adams and Moss, 2000). This is also supported by Ivanova *et al*. (2000) who reported bacteriocins in a traditional Bulgarian cereal drink prepared with LAB had strongly inhibited some lactic acid bacteria. This means that the factors previously recognized as contributing to bacterial inhibition by FPBs were most likely due to a combination of organic acids, (acetic and lactic acids), low pH, diacetyl, or some unidentified compounds or factors.

The FPB's from banana and wild forest noni were the most effective in controlling the growth of the potential pathogens and these had the highest values for total acidity (Table 3). In addition, each had a comparatively high value for K and low value for Na (Table 3). Normal culture media generally contain less than 1000 mg K L^{-1} but a standard medium for bacteria such as TSB contains 1122 mg K L^{-1} (FDA, 2001). Hence the levels of K found in the most active FPB's (about 1300 mg L^{-1}) might be considered to be at the higher end of a normal value. It is possible that these 2 parameters i.e. total acidity and K in the presence of low Na are key factors in inhibiting the growth of *B. cereus* and *S. aureus* (Fig. 1A-B). The FPB from mangosteen shell also with high K and total acidity and low Na was a very effective inhibitor of *B. cereus*. Lactic acid is one of the most widely distributed acids in nature and together with acetic acid is the one most widely employed as a biopreservative compound (Attaie *et al*., 1987; Soomro *et al*., 2002; Ozdemir *et al*., 2006). The potassium, sodium and calcium salts of lactic acid at a level up to 4.8% are effective in controlling the growth of aerobes and anaerobes, so in general they are used as anti-microbial agents in meat and poultry products (Shelef, 1994; Ozdemir *et al*., 2006). Stella and Burgos (2001) have reported that potassium ion increased the susceptibility of *Saccharomyces cerevisiae* strain S288c to fluconazole.

There is the possibility that, compounds extracted from the plant itself may have antibacterial activity. Many researchers have stated that noni fruit has bioactive compounds able to control microorganisms (Duncan *et al*., 1998; Limyati and Juniar, 1998; Solomon, 1999; Wang *et al*., 2002; Solomon, 2006). Compounds extracted from eucalyptus and mangosteen shell may also have contributed to their inhibitory activity of Gram positive bacteria. An ethanolic extract of *Garcinia mangostana* strongly inhibited methicillin-resistant *Staphylococcus aureus* (MRSA) and *S. aureus* (Voravuthikunchai *et al*., 2004a). The active agent was thought to be tannin present in the plant at 11% g g^{-1} (Djipa *et al*., 2000). We presume that in some cases ethanol produced during the fermentation could facilitate the extraction of bioactive compounds from the plant material used and contribute to the inhibitory action of the FPB's. However, in this study there is no evidence that ethanol levels make any contribution to the inhibitory activity.

Inhibition of Gram negative bacteria by FPBs: It has long been recognized that a greater number of medicinal plants contained chemicals that were less active against Gram negative bacteria than Gram positive bacteria (Mccutcheon *et al*., 1992; Del Compo *et al*., 2000). *Escherichia coli* was particularly resistant to extracts from medicinal plants. Voravuthikunchai *et al*. (2004b) as only 8 plant species, 21.05% of those tested, showed an inhibitory effect on *E. coli* O157:H7. In this study we also found that for the FPBs only those produced from banana and wild forest noni, (10.5% of the total) exhibited some inhibition against *E. coli* which was the most resistant microorganism to the inhibitory action of all the FPBs tested (Fig. 2A). However, there was considerable variability of inhibitory effects of these two FPBs on the other 3 Gram negative bacteria used in this study. The FPB from banana was a better inhibitor than that from wild forest noni for *Salmonella* sp., *Shigella* sp. and *P. aeruginosa* (Fig. 2B-D). However, both beverages had similar inhibitory activity for *V. parahaemolyticus* (Fig. 2E). The results indicate that organic acids and low pH in both beverages were mainly responsible for inhibiting those cultures (Table 3, 4). These results agree with those of Gonzales *et al*. (1993) who reported that inhibition of some enteropathogens was mainly dependent on organic acids and antibiotic-like substances.

The presence of lipopolysaccharide in the Outer Membrane, (OM) (Sawer *et al*., 1997; Gao *et al*., 1999) of Gram negative bacteria may be responsible for any differences of sensitivity that occur between different bacteria. Ethanol and lactic acid can cause damage to the OM (Pelczar *et al*., 1986; Alakomi *et al*., 2000). The banana FPB, which, except for *E. coli*, has a greater inhibitory effect than the wild forest noni contains more lactic acid and ethanol and inhibits the Gram negative bacteria more than the Gram positive (Fig. 1-2). In the case of *E. coli* where the wild forest noni FPB is more active than the banana this could indicate that *E. coli* is more sensitive to other bioactive compounds than to organic acids and alcohols. The wild forest noni FPB may contain compounds such as scopoletin previously extracted from ripened noni fruit and shown to have antibacterial properties against *Pseudomonas*, *Escherichia coli*, *Salmonella* and *Shigella* (Wang *et al*., 2002). Although levels of diacetyl were not measured in this work it can be used as a food additive during meat fermentation to control *E. coli* O157:H7 and *Salmonella* sp. (Motlagh *et al*., 1991; Kang and Fung, 1999). Inhibition of enteropathogens by lactobacilli strains used in fermented milk was caused by a combination of organic acids and other extracellular inhibitory substance(s). Gonzales *et al*. (1993) has suggested that some of these compounds could be bacteriocins. The fermented chamuang beverage may also have other antibacterial compounds present because it had the lowest amount of total acidity but strongly inhibited *Shigella* sp. (Fig. 2D).

CONCLUSION

The results indicate that the antibacterial actions found in the FPBs are derived from both lactic acid fermentation and plant extraction during their production. Lactic acid bacteria produced antibacterial substances such as organic acids, alcohols and perhaps bacteriocins; whereas bioactive compounds from plants were also extracted by water, changing pH and maybe ethanol produced during the fermentation. This confirms that FPBs maybe a healthy beverage in view of their antibacterial activities against a range of pathogenic bacteria.

ACKNOWLEDGMENTS

The authors are grateful to National Science and Technology Development Agency (NSTDA) for financial support of this project (CO-B-22-2C-18-601). We thank Scientific Equipment Center, Prince of Songkla University for Gas chromatography analysis. We also thank Dr. Brian Hodgson for critical reading of the manuscript and his valuable suggestions.

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