In vitro Antimicrobial Screening of Selected Traditional Thai Plants

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Abstract

Four different solvent extracts from 13 indigenous plant species were subjected for preliminary antimicrobial screening against thirteen pathogenic and spoilage microorganisms. Aqueous, ethanolic, ethyl acetate and hexane extracts of each plant were examined for antimicrobial activity using agar disc diffusion method. The result of *in vitro* antimicrobial screening showed that 6 extracts from 3 plant species; *Momordica charantia* Linn., *Litsea glutinosa* (Lour.) C.B. Rob. and *Bidens pilosa* Linn. had antimicrobial activities. The effect of different solvent extract on antimicrobial activity was also pronounced. The crude ethanolic extract exhibited the most inhibitory effect following by ethyl acetate extract while no inhibitory effects were observed in aqueous and hexane extracts. In addition, different part of each plant had different antimicrobial potential. The stem and leave extract of *M. charantia* had better antimicrobial potential than its fruit extract. The plant extracts had antimicrobial potential against gram-positive bacteria while no inhibitory effect on gram-negative bacteria and mold was observed. Only crude alcoholic extract of *B. pilosa* had antimicrobial potential against yeast *Saccharomyces cerevisiae.* Crude ethanolic extracts from *M. charantia* showed the most inhibitory effect and broad spectrum inhibiting *Bacillus subtilis, Staphyloccocus aureus, Lactococcus lactis, Listeria innocua* and *Lactobacillus plantarum*. This extract was selected to determine the minimum inhibitory concentration (MIC) using broth dilution methods. The research showed that MIC values were 0.39-25 mg mL^{-1}.

Keywords: *antimicrobial activity, Momordica charantia, Litsea glutinosa, Bidens pilosa,* crude ethanolic extract

Introduction

Incidences of foodborne illnesses are still a major problem. It has been estimated that approximately 140,000 cases of illnesses were attributed to foodborne pathogens in Thailand (Food Control Division, 2008). In fact, food spoilages are causing the dramatic economic loss in the food industry. Meanwhile, consumers are concerned about the safety of foods containing preservatives. Therefore, there has been growing interest in new and effective techniques in order to decrease those losses.

Thirteen different microbial species were used to screen the possible antimicrobial activity. *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa and Bacillus subtilis* are common foodborne microorganisms. *Salmonella* Anatum and *Listeria innocua* are used as representatives of *Salmonella* spp. and *Listeria monocytogeneses,* respectively. The other microorganisms; *Proteus mirabilis, Lactobacillus plantarum, Lactococcus lactis, Pichia anomala, Saccharomyces cerevisiae, Aspergillus niger* and *Penicillium pinophilum* commonly cause spoilage in food products.

For centuries, indigenous plants, widely used for flavoring foods and for folk medicinal purposes, are numerous and diverse in Thailand. In recent years, the uses of higher plants in processed foods are intensely scientific attention in the food industry as an alternative source of synthetic antimicrobials and other purposes (i.e. antioxidant activity and other food additives etc.). Major phytochemicals known as antimicrobial agents are phenolic and polyphenols, terpenoids, essential oils, alkaloids, lectins and polypeptides which are wildly various in qualitatively and quantitatively active agents among different plant species (Cowan, 1999).

A large number of plants in different locations around the world have been extracted and semi-purified to individually investigate their antimicrobial activity, for example, Asia (Alzoreky and Nakahara, 2003), Taiwan (Hsieh et al., 2001), India (Ahmad and Beg, 2001), Tanzania (de Boer et al., 2005), Rwandan (Cos et al., 2002), Argentina (Muschietti et al., 2005) and Thailand (Supar-in, 2000; Chomnawang et al., 2005; Wannissorn et al., 2005).

Bitter gourd (*M. charantia*) has been used to treat various illnesses (diabetes, abortifacient, anthelmintic, malarial, kidney stone, ulcer and cancers, etc.) (Grover and Yadav, 2004). The fruit extract of bitter gourd has broad antimicrobial activity against pathogenic microorganisms for example *E. coli, Salmonella paratyphi* and *Shigella dysenterae* and *Streptomyces griseus* (Omoregbe et al., 1996; Ogata et al., 1991). The extract of *B. pilosa* is used as folk medicine in several countries and has been tested for antimicrobial activity (Rojas et al., 2006, Deba et al., 2008; Oliveira et al., 2007). Other selected plants are used as various treat for illness and few commonly used as food ingredients.

However, sufficient scientific studies of antimicrobial activities in Thai indigenous plants are limited. In addition, the different botanical varieties and geographical origins may affect the qualitative and quantitative phytochemicals in the interested plants. Otherwise, antimicrobial activities in selected microbial strains have not been reported. This study is a part of larger research project which is aimed to develop some indigenous plant species for their economic importance. The determination of potential antimicrobial activities for plant extracts will provide information for further use in food industry. Therefore, the aim of this study was to determine the antimicrobial activity of 13 selected indigenous plant extracts against 13 food spoilage and pathogenic microorganisms.

Materials and Methods

Plant Materials

The plants used in the work were collected in different places as described in Table 1. All fresh plants were washed with deionized water. The samples were cut into small pieces and then dried at 50^{o}C for 24 hours. The dried plants were ground and then sieved through 80 mesh siever.

The ethnobotanical data including botanical name, local name, location and part used of selected plant species are summarized in Table 1.

Extraction

The method of Dupont et al. (2006) was adopted for extraction with little modification. Each sample was subjected to four different solvents. For water extraction, each sample (1:5 w/v) was soaked at ambient temperature for 24 hours under shaking condition at 130 rpm. For 80% ethanol, hexane and ethyl acetate were used under the same conditions in the ratio of 1:4, 1:2 and 1:2 respectively. Each extract was filtered through Whatman filter paper No. 1 and then re-filtered using 0.22 µm filter membrane (Sartorius, Germany). The filtrate was stored at –20^{o}C.

Microorganisms

The microorganisms used included nine bacteria (*Escherichia coli* TISTR 1034, *Salmonella* Anatum, *Staphylococcus aureus* ATCC12600, *Listeria innocua* ATCC33090, *Pseudomonas aeruginosa* TISTR 781, *Bacillus subtilis* TISTR008, *Proteus mirabilis* TISTR100, *Lactobacillus plantarum* ATCC14917 and *Lactococcus lactis* JCM7638), two yeasts (*Pichia anomala* TISTR5285 and *Saccharomyces cerevisiae* TISTR5051) and two molds (*Aspergillus niger* TISTR3245 and *Penicillium pinophilum* TISTR3386) were purchased from Thailand Institute of Scientific and Technological Research, Bangkok. Bacterial, lactic acid bacterial, yeast and fungi cultures were maintained on TSA-YE (Merck), MRS (Merck),

Table 1 List of plants used in this study.

Botanical name	Family	Common name	Location	Part used
Azadirachta indica A. Juss.	Meliaceae	Siamese neem tree	Local market, Bangkok	seed
Bidens pilosa Linn.	Compositae	Peun nok sai	Angkhang, Chaingmai	stem and leaf
Budleja asiatica Lour.	Buddlejaceae	Rachawadi pa	Angkhang, Chaingmai	stem and leaf
Centella asiatica Linn.	Umbelliferae	Asiatic pennywort	Local market, Bangkok	stem and leaf
Commelina diffusa Burm.f.	Commelinaceae	Phak plap, Bi reau	Angkhang, Chaingmai	stem and leaf
Embelia ribes Burm.f.	Myrsinaceae	Som Jee	Angkhang, Chaingmai	leaf
Embelia sessiliflora Kurtz	Myrsinaceae	Som Kui	Angkhang, Chaingmai	leaf
Foeniculum vulgare Mill.	Umbelliferae	Fennel	Local market, Bangkok	stem and leaf
Justicia adhatoda Linn.	Acanthaceae	Sa niat	Angkhang, Chaingmai	leaf
Litsea cubeba (Lour.) Pers.	Lauraceae	Ta khai tom	Angkhang, Chaingmai	leaf
Litsea glutinosa (Lour.) C.B. Rob.	Lauraceae	Mi men	Angkhang, Chaingmai	leaf
Litsea salicifolia Neex ex Roxb.	Lauraceae	Mi bang	Angkhang, Chaingmai	leaf
Momordica charantia Linn.	Cucurbitaceae	Bitter cucumber	Local market, Bangkok	fruit, stem and leaf

Source: The plants were identified by Prof. Dr. Sutharm Areekul (Faculty of Agriculture, Kasetsart University, Thailand).

SDB (Scharlau) and SDA (Scharlau) slants, respectively. All cultures were sub-cultured monthly and subsequently stored at 4^{o}C.

Culture Preparation

A loopful of each tested bacterial/yeast strain was aseptically transferred into 5 mL of their maintained media and incubated at 37^{o}C for 18-24 hours before use. The optical density at 600 nm of each active culture was adjusted using fresh broth to obtain approximately 10^6 CFU mL^{-1}. Bacterial counts were confirmed by planting out on their suitable media and incubated for 48 hours.

For fungi, they were cultured on SDA for 4-7 days at 30^{o}C. The spore suspension was prepared by washing the slant culture with 0.1% sterile Tween 80. The spore suspension was counted by haemacytometer and diluted with the same diluents to obtain the final concentration of each strain of 10^6 spore mL^{-1}.

Screening for Antimicrobial Activities

Antimicrobial tests were carried out by a little modification from double layer procedure of Oonmetta-aree et al. (2006). The concentration of cell suspension uniformly yielded 10^6 CFU mL^{-1} in the TSA-YE plates for bacteria, MRS for lactic acid bacteria and SDA plates for yeast and fungi. The discs (diameter, 6 mm) were each impregnated with 20 µL of crude extracts, dried and then placed on the inoculated agar.

The plates were incubated at 37^{o}C for 24 hours for bacteria whereas the incubation of 30^{o}C for 24-48 hours was used for yeast and fungi. On each plate, an appropriate reference antibiotic assay disc was applied depending on tested microorganisms. The positive control for bacteria and for yeast and fungi were chloramphenical and nystatin, respectively, whereas the negative controls were extracted solvents. Diameter of inhibition zone was measured. Each experiment was carried out in triplicate and the means of the diameter of the incubation zones were calculated.

Determination of Minimal Inhibitory Concentration (MIC)

The determination of MIC using broth dilution method was applied on extracts that already proved for their high efficacy against tested microorganisms. The filtrate was evaporated under the vacuum at 45^{o}C and then freeze-dried to complete dryness. The residue re-dissolved in sterile water to give the stock concentration of 1,000 mg mL^{-1}. The stock concentration was

serially diluted to TSB-YE broth in order to observe their activities at lower concentrations. Bacteria inoculum was added into the broth at the concentration of 10^6 CFU mL^{-1} and then cultured at appropriated temperature. Every 4 hours, the samples were taken for microbial count to evaluate mode of actions, bacteriostatic and bactericidal action.

Statistical Analysis

The triplicate data were subjected to an analysis of variance for a completely random design using statistical program. The Duncan test was used to compare the differences among means at the level of 0.05.

Results

The total of thirteen selected plants were prepared in extracts of four different forms, namely aqueous, ethanolic, ethyl acetate and hexane extracts. Among them, the aqueous extract gave the highest yield (% concentration w/v) followed by ethanolic and hexane extracts in each plant sample (the result not shown). All extracts were tested against nine bacteria, two yeasts and two fungi. Antimicrobial activities were observed in ethanolic and ethyl acetate extracts while no inhibition in aqueous and hexane extracts was found. The results of the antimicrobial screening of various ethanolic plant extracts are shown in Table 2. Antimicrobial activities were found in three ethanolic plant extracts of different plant parts: *B. pilosa* (leave), *L. glutinosa* (leave) and *M. charantia* (stem and leave) which had antimicrobial potential against gram-positive bacteria, *Staph. aureus, Lis. innocua* and *B. subtilis.* The inhibition of *Lc. lactis* and *Lb. plantarum* were only observed from *M. charantia* (stem and leave) extract. The fruit of *M. charantia* showed inhibitory effect against *Lis. innocua* and *B. subtilis.* From the results, *M. charantia* produced outstanding antimicrobial effect with inhibition zone greater than 8.79 mm indicating the gram-positive bacteria to be more susceptible than gram-negatives. No inhibitory effects on gram-negative bacteria, yeast and mold were observed except the inhibition of *Sc. cerevisiae* from *B. pilosa* (Table 3).

Table 2 Antibacterial activity of ethanolic plant extracts using disc diffusion assay.

Plant	Conc. (% w/v)	Inhibition zone diameter[1/] (mm)								
		Gram positive bacteria					Gram negative bacteria			
		Staph. aureus	*Lis. innocua*	*B. subtilis*	*Lb. plantarum*	*Lc. lactis*	*P. mirabilis*	*Ps. aeruginosa*	*E. coli*	*S.* Anatum
A. indica	7.24	-	-	-	-	-	-	-	-	
B. asiatica	7.37	-	-	-	-	-	-	-	-	-
B. pilosa	5.91	9.98 ± 0.52a	7.62 ± 0.18c	7.12 ± 0.56c	-	-	-	-	-	-
C. asiatica	7.62	-	-	-	-	-	-	-	-	-
C. diffusa	2.35	-	-	-	-	-	-	-	-	-
E. ribes	6.08	-	-	-	-	-	-	-	-	-
E. sessiliflora	7.26	-	-	-	-	-	-	-	-	-
F. vulgare	7.26	-	-	-	-	-	-	-	-	-
J. adhatoda	5.34	-	-	-	-	-	-	-	-	-
L. cubeba	8.19	-	-	-	-	-	-	-	-	-
L. glutinosa	6.97	8.78 ± 0.83b	8.40 ± 0.26b	9.73 ± 0.53b	-	-	-	-	-	-
L. semecarpifolia	4.44	-	-	-	-	-	-	-	-	-
M. charantia (Stem and leaves)	5.89	9.99 ± 0.52a	9.33 ± 0.54a	10.77±0.63a	8.79 ±0.23	9.41 ± 0.24	-	-	-	-
M. charantia (fruit)	7.61	-	7.44 ± 0.43c	9.30 ± 0.58b	-	-	-	-	-	-
Chloramphenicol	-	21.34±0.31	28.90±0.49	19.40±0.78	23.81±0.68	20.50±0.36	21.37±0.59	18.93±0.71	19.96±0.72	22.33±0.70

[1/] Diameter of disc (6 mm), (-) no inhibition, Chloramphenical (positive control), the difference in letter on column represented different statistic at 95%.

Table 3 Antifungal activity of alcoholic plant extracts using disc diffusion assay.

Plant extract	Inhibition zone diameter[1/] (mm)			
	Pi. anomala	*Sc. cerevisiae*	*A. niger*	*Pen. pinophilum*
A. indica	-	-	-	-
B. asiatica	-	-	-	-
B. pilosa	-	8.79±0.52	-	-
C. asiatica	-	-	-	-
C. diffusa	-	-	-	-
E. ribes	-	-	-	-
E. sessiliflora	-	-	-	-
F. vulgare	-	-	-	-
J. adhatoda	-	-	-	-
L. cubeba	-	-	-	-
L. glutinasa	-	-	-	-
L. semecarpifolia	-	-	-	-
M. charantia (Stem and leaves)	-	-	-	-
M. charantia (fruit)	-	-	-	-
Nystatin	9.84 ±0.40	19.84±0.49	19.49±0.66	12.44 ±0.52

[1/] Diameter of disc (6 mm), (-) no inhibition, Chloramphenical (positive control), the difference in letter on column represented different statistic at 95%.

The results of the antimicrobial screening of various ethyl acetate plant extracts are shown in Table 4. Of thirteen indigenous plants, the extracts from 2 plant species, *L. glutinosa* and *M. charantia* (stem and leaves), inhibited *B. subtilis* and *Lis. innocua* while only *L. glutinosa* inhibited *Staph. aureus*. None of the negative control used had antimicrobial activity. No inhibitory effects on gram-negative bacteria, yeasts and molds were observed. The result indicated the effect of solvent extract on the antimicrobial potential.

The minimum inhibitory concentrations (MICs) using broth dilution methods of *M. charantia* were also investigated (Table 5). The MIC is defined as the lowest concentration to inhibit initial inoculum. *Staph. aureus, Lis. innocua, B. subtilis* and *Lc. lactis* had MIC values of 25,3.52, 0.39 and 2.35 mg mL^{-1}, respectively.

Mode of action of *M. charantia* was determined by studying the growth of *Staph. aureus, Lis. innocua, B. subtilis* and *Lc. lactis* at three different concentrations (Figure 1). The bactericidal effect is defined as the lowest concentration needed to kill 99.9% of the initial inoculum after 24 hour incubation (NCCLS, 1999). The bacteriostatic effects on *Staph. aureus, Lis. innocua* and *Lc. lactis* were observed whereas the bactericidal effect was found in *B. subtilis.* The results indicated that the minimum bactericidal concentration (MBC) value was 0.48 mg mL^{-1} of crude ethanolic extract for *B. subtilis* while MBCs were not observed in other microorganisms at the tested concentrations. The MIC values of 0.39, 2.35, 3.52 and 25 mg mL^{-1} were determined against *B. subtilis, Lc. lactis, Lis. innocua* and *Staph. aureus*, respectively.

Discussion

Depending on the ability to dissolve in different polarity, each solvent extracted different phytochemicals resulting in different quantitative and quantitative active agents (Cowan, 1999). The effect of solvent extraction found that the aqueous extract provided the highest yield but no inhibitory effect was observed in this solvent. The similar result was also observed by Rosa et al. (1993). The aqueous extract obtained the highest yield (%) followed by less polarity solvents. Cowan (1999) indicated that aqueous extraction could be ineffective because water soluble compounds might interrupt the antimicrobial effect. In addition, antimicrobial phytochemicals are soluble in

Table 4 Antibacterial activity of ethyl acetate plant extracts using disc diffusion assay.

Plant	Conc. (% w/v)	Inhibition zone diameter[1/] (mm)								
		Gram positive bacteria					Gram negative bacteria			
		Staph. aureus	*Lis. innocua*	*B. subtilis*	*Lb. plantarum*	*Lc. lactis*	*P. mirabilis*	*Ps. aeruginosa*	*E. coli*	*S.* Anatum
A. indica	1.17	-	-	-	-	-	-	-	-	-
B. asiatica	1.21	-	-	-	-	-	-	-	-	-
B. pilosa	2.80	-	-	-	-	-	-	-	-	-
C. asiatica	1.15	-	-	-	-	-	-	-	-	-
C. diffusa	0.83	-	-	-	-	-	-	-	-	-
E. ribes	6.01	-	-	-	-	-	-	-	-	-
E. sessiliflora	7.66	-	-	-	-	-	-	-	-	-
F. vulgare	4.90	-	-	-	-	-	-	-	-	-
J. adhatoda	4.08	-	-	-	-	-	-	-	-	-
L. cubeba	6.08	-	-	-	-	-	-	-	-	-
L. glutinosa	2.89	7.21 ± 0.48	8.58 ± 0.39a	7.79 ± 048a	-	-	-	-	-	-
L. semecarpifolia	1.96	-	-	-	-	-	-	-	-	-
M. charantia (Stem and leaves)	2.91	-	7.65 ± 0.33b	7.61±0.43a	-	-	-	-	-	-
M. charantia (fruit)	4.83	-	-	-	-	-	-	-	-	-
Chloramphenicol	-	21.34±0.31	28.90±0.49	19.40±0.78	23.81±0.68	20.50±0.36	21.37±0.59	18.93±0.71	19.96±0.72	22.33±0.70

[1/] Diameter of disc (6 mm), (-) no inhibition, Chloramphenical (positive control), the difference in letter on column represented different statistic at 95%.

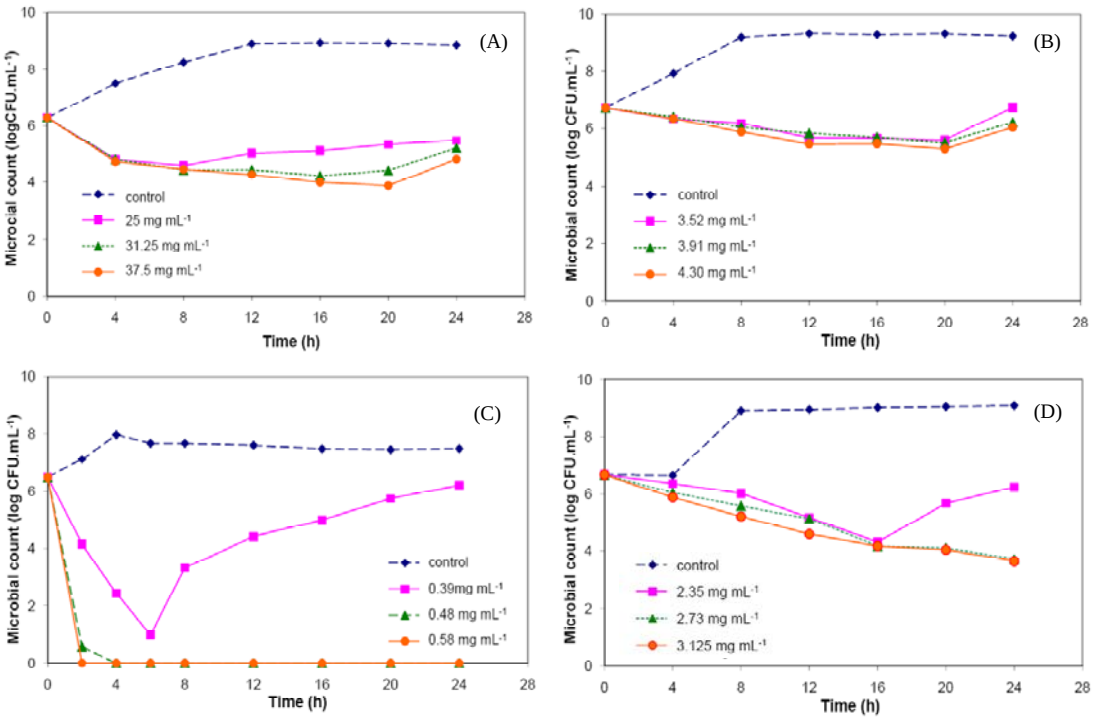

Figure 1 Effect of ethanolic extract of *M. charantia* on the growth of *Staph. aureus* (A), *Lis. innocua* (B), *B. subtilis* (C) and *Lc. lactis* (D) (Source: Jiapiyasakul and Areekul, 2007).

Table 5 MICs of ethanolic extract of *M. charantia* against tested bacteria.

Microorganism	MIC (mg mL^{-1})
Staph. aureus	25.0
Lis. innocua	3.52
B. subtilis	0.39
Lc. lactis	2.35

moderate polar solvents. From our results, only the crude ethanolic and ethyl acetate extracts showed the inhibitory effect (Tables 2 and 4).

The stem-leave extract of *M. charantia* showed higher inhibitory effect than the fruit parts (Table 2). These differences could be due to the qualitative and quantitative antimicrobial agents presented in the plant parts and their mode of actions. Our results agreed with the observations in previous studies where the ethanolic *M. charantia* extract inhibited gram-positive *Staph. aureus* with no inhibitory effect on gram-negative bacteria *E. coli* and yeast *S. cerevisiae* (Supar-in, 2000; Voravuthikunchai et al. 2004). The active phytochemicals in *M. charantia* are reported to be momorcharins, momordenol, momordicilin, momordicins, momordicinin, momordin, momordolol, charantin, charine, cryptoxanthin, cucurbitins, cucurbitacins, cucurbitanes, cycloartenols, diosgenin, elaeostearic acids, erythrodiol, galacturonic acids, gentisic acid, goyaglycosides, goyasaponins and multiflorenol (Grover and Yadav, 2004).

The ethanolic extracts from *B. pilosa* showed inhibitory effect on *Staph. aureus, B. subtilis, Lis. innocua* and *S. cerevisiae.* The results were similar to that reported by Rabe and Staden (1997) who informed no inhibitory effect from aqueous extract (100 mg mL^{-1}) while methanolic extract inhibited *Staph. aureus* and *B. subtilis*. Rojas et al. (2006) also reported that crude extract (root and stem) showed inhibitory effect against *Staph. aureus, B. cereus* and *S. cerevisiae*. However, in this study, no inhibitory effect was observed in *E. coli* which was different from the previous studies (Khan et al., 2001; Cos et al., et al., 2002; Rojas et al., 2006), *B. subtilis* (Oliveira et al., 2007), *Sal.* Anatum (Khan et al., 2001; Cos et al., 2002), *Staph. aureus* (Oliveira et al., 2007), *Ps. aeruginosa* (Cos et al., 2002; Oliveira et al., 2007) and *P. mirabili* (Cos et al., 2002). Ethanolic extract of the leaves showed antimicrobial activity against *E. coli, Salmonella paratyphi* and *Shigella dysenterae* (de Padua et al., 1999). In addition, the petroleum extract of leaves was reported to have inhibitory effect on *E. coli* (Chariandy et al., 1999) while our result showed negative effect.

The antimicrobial effect of *B. pilosa* extract on various gram positive in the study might be due to the contribution of several compounds such as quercetin 3-*O*-rabinobioside, quercetin 3-*O*-rutinoside, chlorogenic acid, 3,4-di-*O*-caffeoylquinic acid, jacein and centaurein (Chiang et al., 2004). Moreover, major active compounds in leave of *B. pilosa* var. *radiata* were -caryophyllene and τ-cadinene. (Deba et al., 2008).

The leave extract of *L. glutinosa* inhibited *Staph. aureus, B. subtilis* and *Lis. innocua* in both solvent extracts (ethanol and ethyl acetate). Our results were similar to those of Mandal et al. (2000) which reported the antibacterial activities by methanolic extract of *L. glutinosa* (bark) against gram-positive and gram-negative strains. It may be due to different parts of this plant containing different active compounds (Chowdhury et al., 2008). However, the leave extract in this experiment ineffectively inhibited only gram-negative bacteria. Grosvenor et al. (1995) also showed positive antimicrobial activity in *Staph. aureus* of *L. elliptica* (leaves) and *L. robusta* (leaves) with no observation in *S. cerevisiae*. Even in different species, those plants may contain the same antimicrobial agents. Chowdhury et al. (2008) reported that chemical constituents of leaf oil in *L. glutinosa* were identified as a-muurolene, β-myrcenem, caryophyllene, cinnamyl acetate, phytol and other constituents that might be contributed to its antimicrobial activity.

The other tested plants showed no inhibitory effect. The results were in disagreement with the previous studies concerning the inhibitory effect of *F. vulgare* extract on *B. subtilis* (Kwon et al., 2002). However, numerous reports informed that some plants showed inhibition to different microorganisms for example, the inhibitory effect of *A. indica* and *C. asiatica* on *Propionibacterium*

acnes and *Staphylococcus epidermidis* (Chomnawang et al., 2005), aqueous extract of *M. charantia* (whole plant) on *Candida albicans* and *Cryptococcus neoformans* (Schmourlo et al., 2005), ethanolic extract of *M. charantia* (fruit) on *Aspergillus flavus* (Thanaboripat et al., 2006). In addition, embelin isolated from *E. ribes* exhibited the inhibition on *Staph. aureus*, *Sal. Typhi*, *P. mirabilis* and *Ps. aeruginosa* (Chitra et al., 2003). The difference in extraction procedure, plant part, susceptibility of tested microbial strain, botanical varieties, geographical origin and cultivation sites may contribute to different level antimicrobial agents resulting in significantly different experimental results (Cowan, 1999).

MICs of *M. charantia* in the tested bacterial strain indicated that *B. subtilis* was the most susceptible (0.39 mg mL^{-1}) whereas the most resistant was *Staph. aureus* (25 mg mL^{-1}). This result might be contributed to different mode of action. The MBC value (0.48 mg mL^{-1}) was only observed in *B. subtilis.* The results indicated that *B. subtilis* was the most susceptible microorganism.

In conclusion, of all the sample extracts from plant parts, only a few ethanolic extracts showed antimicrobial activity. In this study, the ethanolic extract from *M. charantia* had the highest broad spectrum and antimicrobial property. *M. charantia* broadly inhibited gram-positive bacteria, followed by *B. pilosa* whereas the other plants had no effect. The plant sample could not inhibit yeast and mold. The study also indicated that solvents affected the inhibitory effect. *M. charantia* extract showed MBC value of 0.39 mg mL^{-1} on *B. subtilis*.

This study was a preliminary evaluation of antimicrobial activity of the plants. It indicated *M. charantia* to have the potential antimicrobial activity that could be applied in human food. More development of extraction methods and fractionations of extracts should be carried out in order to further investigate the active compounds.

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