Research Journal of Medicinal Plant

ISSN 1819-3455

Academic Journals Inc.

www.academicjournals.com

Research Journal of Medicinal Plant 5 (3): 254-265, 2011
ISSN 1819-3455 / DOI: 10.3923/rjmp.2011.254.265

In vitro Free Radical Scavenging and Antimicrobial Activity of Some Selected Thai Medicinal Plants

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ABSTRACT

The aims of the present study were to determine the free radical scavenging activity and antimicrobial activity against 15 food spoilage and pathogenic microorganisms of 18 selected Thai medicinal plants from 12 different families based on ethnopharmacological and taxonomic information. The methanolic extract of *Allium sativum* L. gave highest yield (34.76% db) following *Rheum officinale* Baill. (24.94% db) and *Curcuma longa* L. (23.39% db), respectively. Sixteen plant materials out of 18 selected Thai medicinal plants showed high effective scavenging capacity (IC_{50}<0.06 mg mL^{-1}) that order: *Mansonia gagei* Drumm., *Pterocarpus santalinus* L., *Cinnamomum iners* Blume., *Kaempferia parviflora* Wall., *Caesalpinia sappan* L., *Rheum officinale* Baill. and *Albizia myriophylla* Benth., respectively. The highest antioxidant activity was detected in *Kaempferia parviflora* Wall. by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. The most active medicinal plant extracts showed broad spectrum antimicrobial activity against 15 food spoilage and pathogenic microorganisms. The *Allium sativum* L., *Caesalpinia sappan* L., *Mansonia gagei* Drumm., *Alpinia galangal* Swartz., *Curcuma longa* L. and *Kaempferia parviflora* Wall. extracts had the greatest inhibitory effect against *B. cereus* ATCC 11778, *E. coli* ATCC 29214, *Staph. aureus* ATCC 13150, *Pr. vulgaris* TISTR 100, *Strep. cremoris* TISTR 058, *Salm. typhi* ATCC 43579 and *C. krusei* TISTR 5256 by using the disc diffusion method. The findings support the view that some Thai medicinal plants are promising sources of potential antioxidants and antimicrobial activity, which may be efficient as preventive agents in the pathogenesis of some diseases.

Key words: *Kaempferia parviflora* Wall., *Curcuma longa* L., *Bacillus cereus*, methanolic extract, phytochemicals, antioxidant activity

INTRODUCTION

For thousands of years, mankind has known about the benefit of drugs from nature. Plant extracts for the treatment of various ailments were highly regarded by the ancient civilizations. Today, plant materials remain an important resource for combating illnesses, including infectious diseases and many of these plants have been investigated for novel drugs or templates for the development of new therapeutic agents (Kumarasamy *et al*., 2002). Searches for medicinal plants that are more potent and efficient antibiotic agents have accelerated in recent years. In Thailand

most medicinal plants are traditionally used in Thai folk medicine to treat several diseases (Taylor and Attaur, 1994). It has been established that oxidative stress is among the major causative factors in more than 100 diseases such as malaria, acquired immunodeficiency syndrome, heart disease, stroke, arteriosclerosis, diabetes and cancer (Alho and Leinonen, 1999). In recent years, many studies evidenced that medicinal plants with high content of antioxidants can be effective in prevention of the free radical formation by scavenging, thus playing an important role in the prevention of these diseases (Farrukh *et al*., 2006; Al-Fatimi *et al*., 2007). Subsequently, a worldwide trend towards the use of natural phytochemicals present in berry crops, tea, herbs, oilseeds, beans, fruits and vegetables has increased (Karaman *et al*., 2003; Butkhup *et al*., 2008). Several herbs and spices have been reported to exhibit antioxidant activity, including rosemary, sage, thyme, nutmeg, turmeric, white pepper, chili pepper, ginger and several Chinese medicinal plants (Lee *et al*., 2003). The natural active compounds found in medicinal plants belong to various chemical structures including terpenes, alkaloids, coumarins, lignans, quinines, flavonoids, tannins, stilbenes, curcuminiods, polysaccharides, etc. and some of these compounds have anti cancer, antioxidant and antimicrobial activity (Kumar *et al*., 2007).

Medicinal plants, which form the backbone of traditional medicine, have in the last few decades been the subject for very intense pharmacological studies; this has been brought about by the acknowledgment of the value of medicinal plants as potential sources of new compounds of therapeutic value and as sources of lead compounds in drug development. In developing countries, it is estimated that about 80% of the population rely on traditional medicine for their primary health care (Taylor and Attaur, 1994). There arises a need therefore to screen medicinal plants for bioactive compounds as a basis for further pharmacological studies. In recent years, multiple drug/chemical resistance in both human and plant pathogenic microorganisms have been developed due to indiscriminate use of commercial antimicrobial drugs/chemical commonly used in the treatment of infectious diseases. This situation has forced scientists to search for new antimicrobial substances from various sources like medicinal plants. However, the intensive search for novel types of antioxidants has been carried out from numerous plant materials (Lee *et al*., 2003; Al-Fatimi *et al*., 2007; Kumar *et al*., 2007). Scientific studies of antioxidant and antimicrobial activities in Thai medicinal plants are limited. In addition, the different botanical varieties and geographical origin may affect the qualitative and quantitative phytochemicals in the interested plant. Therefore, the search for antimicrobial and antioxidant activity of some selected medicinal plants, which can be applied to synthesize new drugs in order to cure infectious disease. Moreover, the determination of potential antioxidant and antimicrobial activities in plant extracts may provide information for further use in food industry. Consequently, the aims of the present study were to determine the free radical scavenging activity and antimicrobial activity against 15 food spoilage and pathogenic microorganisms of 18 selected Thai medicinal plants.

MATERIALS AND METHODS

Plant materials: Eighteen plant species from 12 different families, native to Thailand, were selected on the basis of ethnopharmacological information or taxonomic relationship with medicinally important species. These plants were obtained from the Department of Plant Production Technology, Mahasarakham University, Thailand during October 2009, identified by using Encyclopedia of Plants in Thailand and authenticated by Suttira Khumkratok, Walai Rukhavej Botanical Research Institute, Mahasarakham University, Thailand (Table 1). The plant materials were cleaned, dried and carefully powdered. All samples were kept in tightened light-protected containers.

Table 1: Botanical, traditional or folk uses, extract yield (%, of the dried weight), phytochemicals detected, inhibitory concentration (IC_{50}) and antiradical activity (A_{AR}) of methanolic extracts obtained from 18 Thai medicinal plants

Scientific name	Common name	Family	Part[a] tested	Traditional uses[b]	Phyto	Yield (%, db)[c]	IC_{50} (mg mL^{-1})	A_{AR} ($1/IC_{50}$)
Allium sativum L.	Garlic	Alliaceae	B	Skin diseases, carminative, expectorant, constipation, cough, digestive disorders, influenza, ringworm,	P, T[d]	34.76	0.61	1.64
Saussurea lappa Clarke.	Costus	Compositae	R	Expectorant, bone tonic, digestive disorders, diuretic	F[j]	15.62	0.28	3.57
Cymbopogon citratus Stapf.	Lemon grass	Gramineae	S	Gallstone, carminative, hypertension, diaphoretic	F	17.07	0.13	7.69
Cinnamomum iners Blume.	Cinnamon	Lauraceae	Ri	Antidiarrhoea, diuretic, digestive, constipation,carminative, stomach infection	T	21.56	0.02	50.00
Derris scandens Benth.	Jewel vine	Leguminosae	S	Rheumatism, poultice for muscle pain, fevers, diuretic,influenza, expectorant, leucorrhea	F[k]	14.67	0.63	1.59
Albizia myriophylla Benth.	Cha em	Mimosaceae	R	Influenza, wound, cough, expectorant, mouth wash	Sa[l]	21.35	0.06	16.67
Caesalpinia sappan L.	Sappan tree	Papilionaceae	H	Antidiarrhoea, leucorrhea, skin diseases, expectorant	T	9.94	0.03	33.33
Pterocarpus santalinus L.	Red saunders	Papilionaceae	H	Pock, scurvy, fevers, cough, wound	F[i]	22.90	0.02	50.00
Piper chaba Hunt.	Long pepper	Piperaceae	Se	Expectorant, parasite, leucorrhea, allergy, digestive disorders, poultice for musclepain, carminative, cough, pharyngitis, constipation	A, P, F[d,f]	19.28	0.52	1.92
Piper nigrum L.	Black pepper	Piperaceae	Se	Carminative, skin diseases, dyspepsia, alzheimer	A, G[m]	11.12	0.21	4.76
Rheum officinale Baill.	Rhubarb	Polygonaceae	R	Anticholesterolemic, antiseptic, antispasmodic, tonic, antitumor, aperients, cholagogue, diuretic, stomachic	F, T[e]	24.94	0.06	16.67
Mansonia gagei Drumm.	Kalamet	Sterculiaceae	H	Antiseptic, carminative, leucorrhea, constipation	C[n]	17.34	0.02	50.00
Conioselinum univitatum Turcz.	Selinum	Umbelliferae	R	Carminative, digestive disorders, squeamish	NI	10.23	0.45	2.22
Alpinia galangal Swartz.	Galangal	Zingiberaceae	R	Antidiarrhoea, skin diseases, toothaches, beriberi	P[h]	7.44	0.61	1.64
Curcuma longa L.	Turmeric	Zingiberaceae	R	Skin diseases, wound, leucorrhea, antidiarrhoea,fevers, influenza, anti aging,anticancer, antivirus, alzheimer	P, F[g,h]	23.39	1.47	0.68
Globba laeta K. Larsen	Kra chai klao	Zingiberaceae	R	Carminative, pharyngitis, leucorrhea, allergy, tonic	F	10.66	2.06	0.48
Kaempferia parviflora Wall.	Black ginger	Zingiberaceae	R	Tonic, antigastric ulcer, hypertension, diuretic, diabetic	F, T[f,h]	17.64	0.02	50.00
Zingiber officinale Roscoe.	Ginger	Zingiberaceae	R	Squeamish, carminative, stomachic, expectorant, fevers,cough, diaphoretic, influenza, antidiarrhoea, wounds and burns, toothaches	P, T[f,h]	16.93	2.06	0.48

NI: No information. Phyto: Phytochemicals detected key: A: Alkaloids; C: Coumarin; P: Phenols; F: Flavonoids; G: Glycosides, Sa: Saponins; T: Tannins. [a]R: Roots or rhizomes, S: Stems, B: Bulb, Ri: Rind, Se: Seeds, H: Heartwood. [b]Information of traditional use has been taken from native people. [c]Dry weight basis of the original sample of plant parts. [d]Farrukh *et al.* (2006), [e]Cai *et al.* (2003), [f]Chanwitheesuk *et al.* (2005), [g]Jang *et al.*. (2007), [h]Chan *et al.* (2008), [i]Krishnaveni and Rao (2000), [j]Damre *et al.* in 2003, [k]Mahabusarakama *et al.* (2004), [l]Yoshikawa *et al.* (2002), [m]Chatterjee *et al.* (2007), [n]Tiew *et al.* (2002)

Extraction and hydrolysis: Ground samples (5 g) were extracted and hydrolysed with 50 mL of 60% aqueous methanol containing 1.2 M HCl. The mixture was refluxed at 85°C for 2 h to ensure complete extraction (Butkhup *et al*., 2008). Next, the extracts were filtered through Whatman No. 1 paper under vacuum and the residue was repeatedly extracted with the same solvent until it was colorless and centrifuged (10 min, 5000x g). Methanol was evaporated from the supernatants on a rotary evaporator at 50 mmHg pressure and 50°C. The extract was kept in a freezer at -20°C for further study.

Determination of plant extract yield: The yield of evaporated dried extracts based on dry weight basis was calculated from equation:

$$\text{Yield (\%)} = \left(\frac{W_1 \times 100}{W_2} \right)$$

where, W_1 was the weight of extract after evaporation of methanol and W_2 was the dry weight of the fresh plant sample.

Free radical scavenging activity (DPPH assay): A 0.1 mL aliquot of the methanol extract prepared above was mixed with 3.9 mL of an 80% ethanolic 0.6 mM DPPH solution. The tubes were vortexed for 15 sec and allowed to stand for 180 min, as described by Cai *et al.* (2003), after which the absorbance of the mixture was measured at 517 nm using the Hewlett Packard UV-Vis spectrophotometer (UV-vis model 1601, Shimadzu, Kyoto, Japan). Most tested compounds should be reacted completely within 180 min in this condition and reaction time for vitamin C is less than 1 min due to its fast oxidation. Ethanol (80%) was used as a blank solution and DPPH solution without test samples (3.9 mL of DPPH+0.1 mL of 80% ethanol) served as the control. All tests were performed in triplicate. The antioxidant activity of the test samples was expressed as (1) the median inhibitory concentration for radical-scavenging activity (IC_{50}): total phenolics (mg) of antioxidant (test sample) required for a 50% decrease in absorbance of DPPH radicals and (2) inhibition (%) of DPPH absorbance:

$$\text{DPPH absorbance (\%)} = \left(\frac{A_{control} - A_{test}}{A_{control}} \right) \times 100$$

A plot of the absorbance of DPPH vs. concentration of antioxidant was made to establish a standard curves (dose-response curves) and to calculate IC_{50}. $A_{control}$ is the absorbance of the control (DPPH solution without the test sample) and A_{test} is the absorbance of the test sample (DPPH solution plus 0.1 mL of 5 μM test compound). Ascorbic acid served as a standard and the results of the assay were expressed relative to ascorbic acid equivalent.

Preparation of test microorganisms: The microbial strains were obtained from the American Type Culture Collection (ATCC; Rockville, MD, USA). They included the Gram-positive bacteria: *Bacillus cereus* ATCC 11778, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 13150,*Streptococcus thermophilus* ATCC 19258, the Gram-negative bacteria: *Escherichia coli* ATCC 29214, *Salmonella typhi* ATCC 43579, *Vibrio chlolerea* ATCC 14033, *Shigella dysenteriae*

ATCC 13313 and the fungi: *Candida tropicalis* ATCC 9968, *Saccharomyces cerevisiae* ATCC 18824 and *Kluyveromyces marxianus* ATCC 8554 whereas the Gram-positive bacteria: *Streptococcus faecalis* TISTR 459, *Streptococcus cremoris* TISTR 058, the Gram-negative bacteria: *Proteus vulgaris* TISTR 100 and the fungi *Candida krusei* TISTR 5256 were obtained from the culture collection at the Thailand Institute of Scientific and Technological Research (TISTR, Thailand) and were employed for determination of antimicrobial activity.

The bacterial and fungal stock cultures were stored frozen in 40% (v/v) glycerol-either nutrient or yeast malt broth. Working bacterial culture and fungal culture were grown at 37°C for 24 h on nutrient agar and at 30°C for 48 h in yeast malt agar, respectively. To obtain cells in the stationary growth phase, bacterial culture and fungal culture were subcultured twice at 37°C for 24 h on nutrient broth and at 30°C for 48 h in yeast malt broth, respectively. Cells were harvested by centrifugation at 6,000x g for 2 min and washed once with a 5 mM NaCl solution. The supernatant was discarded and the cells were washed again. Bacterial cells and fungal cells were re-harvested and suspended in fresh nutrient broth and yeast malt broth, respectively. The concentration of cultures was to 10^6 colony forming units (1×10^6 CFU mL^{-1}).

Antimicrobial assay: The antimicrobial activity of medicinal plant extracts were separately determined using the disc diffusion method as described by Mackeen *et al.* (1997). Two hundred microlitres of prepared culture was spread on surfaces of Mueller Hinton Agar (MHA). Ten microlitres (1 mg mL^{-1}) of each plant extract was applied to a sterile filter paper disc (Whatman No.1; 6 mm in diameter) and allowed to dry for 15 min. The discs were then placed on the surface of inoculated medium. The plates were inverted and incubated for 24 h at 37°C. Each test was carried out in triplicate with controls. Antibiotic susceptibility discs including ampicillin (10 μg $disc^{-1}$), ciprofloxacin (10 μg $disc^{-1}$) and ketoconazole (10 μg $disc^{-1}$) were used as a positive control. The solvent of each extract was used as a negative control. The results were recorded by measuring the zones of growth inhibition surrounding the disc. Clear inhibition zones around the discs indicate the presence of antimicrobial activity.

Minimum Inhibitory Concentration (MIC): The Minimum Inhibitory Concentrations (MIC) were determined for medicinal plant extracts showing antibacterial activity in the disc diffusion assay. Concentrate extracts of plants were added at two-fold serial dilution (0 to 2640 μg mL^{-1}) to sterilize MHA. Ten microlitres of an overnight culture of each microbial strain, containing approximately 10^6 CFU, was applied on agar surfaces. MIC values were taken as the lowest concentration of extract that completely inhibited microbial growth after 24 h of incubation at 37°C.

Statistical analysis: The data were analyzed by the Windows SPSS program (Version 11.01). Data were expressed as mean using ANOVA, if justified by the statistical probability ($p<0.05$), by Duncan's new multiple range test. IC_{50} values for all the above experiments were determined by linear regression.

RESULTS

From 18 medicinal plant extracts, the methanolic extract of *Allium sativum* L. gave highest yield (34.76% db) following *Rheum officinale* Baill. (24.94% db) and *Curcuma longa* L. (23.39% db), respectively. Among these, the methanolic extract of *Caesalpinia sappan* L. and *Alpinia galangal* Swartz. Gave lowest yield with average of 9.94 and 7.44% db, respectively

(Table 1). Considering the large variation of IC_{50} values, ranging from 0.02 mg mL^{-1} in *Mansonia gagei* Drumm., *Pterocarpus santalinus* L., *Cinnamomum iners* Blume. and *Kaempferia parviflora* Wall. to 2.06 mg mL^{-1} in *Globba laeta* K. Larsen and *Zingiber officinale* Roscoe (Table 1).

The results show a significant ($p<0.05$) scavenging activity of medicinal plant extracts and standards. The scavenging effect on the DPPH radical that order: *Kaempferia parviflora* Wall. > *Rheum officinale* Baill.>Piper chaba Hunt.>*Cinnamomum iners*Blume.>*Saussurea lappa* Clarke.>*Caesalpinia sappan* L.>*Mansonia gagei* Drumm.>*Allium sativum* L.>*Piper nigrum* L.> *Albizia myriophylla* Benth.>*Conioselinum univitatum* Turcz.>*Zingiber officinale* Roscoe.> *Derris scandens* Benth.>*Alpinia galangal* Swartz.>*Cymbopogon citratus* Stapf.>*Pterocarpus santalinus* L.>*Curcuma longa* L.> *Globba laeta* K. Larsen extracts, which were 86.35, 85.94, 85.14, 84.33, 83.94, 82.93, 82.73, 82.53, 82.33, 81.93, 81.93, 80.19, 79.92, 78.92, 78.51, 75.90, 63.05 and 29.72%, respectively, at the concentration of 2.00 mg mL^{-1} (Table 2). Among the 18 plants tested, 16 plants showed more than 75% decolorization. They had good DPPH radical scavenging activity. These extracts exhibited a remarkable antioxidant effect at low concentrations. In particular, the methanolic extracts of *K. parviflora* Wall., *R. officinale* Baill. and *P. chaba* Hunt. exhibited at 0.01 mg mL^{-1} an extraordinary antioxidant effect (5.71, 5.52 and 5.48% successively) whereas the ascorbic acid showed at this concentration an effect of 5.46%. The activity of *Curcuma longa* L. (63.05%) showed moderate radical scavenging capacity, while the extract of *Globba laeta* K. Larsen (29.72%) showed negligible activity. However, free radical scavenging activity also increased with increasing concentration.

Table 2: Free radical scavenging activities of the methanolic extracts of Thai medicinal plants[a]

	Radical scavenging activity (%)						
	0.01	0.10	0.30	0.50	1.00	1.50	2.00
Scientific name	(mg mL^{-1})						
Allium sativum L.	0.73	4.29	30.52	50.20	73.49	77.51	82.53
Saussurea lappa Clarke.	1.74	14.86	51.00	65.26	81.53	83.13	83.94
Cymbopogon citratus Stapf.	2.01	17.49	53.61	70.68	72.09	77.91	78.51
Cinnamomum iners Blume.	3.26	19.65	37.95	54.62	76.70	81.35	84.33
Derris scandens Benth.	3.36	16.06	39.16	46.39	65.86	78.71	79.92
Albizia myriophylla Benth.	1.13	7.94	16.06	23.49	42.17	75.10	81.93
Caesalpinia sappan L.	1.50	7.30	25.70	45.98	75.10	81.33	82.93
Pterocarpus santalinus L.	5.44	21.59	53.21	61.85	66.67	71.29	75.90
Piper chaba Hunt.	5.48	10.22	35.54	52.21	78.11	82.30	85.14
Piper nigrum L.	1.46	11.33	31.53	61.65	76.51	79.12	82.33
Rheum officinale Baill.	5.52	10.67	20.68	28.92	41.97	73.90	85.94
Mansonia gagei Drumm.	3.69	22.67	59.03	71.69	80.32	81.93	82.73
Conioselinum univitatum Turcz.	1.20	15.52	35.54	61.24	79.52	80.19	81.33
Alpinia galangal Swartz.	1.77	15.13	39.16	55.82	71.69	75.90	78.92
Curcuma longa L.	0.45	4.97	17.27	27.11	39.76	52.00	63.05
Globba laeta K. Larsen	0.09	1.79	20.48	21.69	23.70	27.11	29.72
Kaempferia parviflora Wall.	5.71	24.56	42.77	60.64	82.53	85.34	86.35
Zingiber officinale Roscoe.	2.12	19.63	52.61	70.88	77.11	78.71	80.19
Ascorbic acid	5.46	22.89	57.42	71.46	85.52	91.94	96.38

[a]Values are the means (n = 3)

Table 3: Antimicrobial activity of the methanolic extracts of Thai medicinal plants

	Inhibition zones (mm) against														
Species	BC	BS	SA	ST	SF	SCr	EC	SD	STy	PV	VC	CK	CT	SC	KM
Allium sativum L.	12	10	9	-	12	7	9	10	10	-	-	-	6	6	7
Saussurea lappa Clarke.	8	8	7	-	9	6	6	7	7	-	-	-	-	-	-
Cymbopogon citratus Stapf.	7	-	-	-	8	-	7	8	8	-	-	-	-	-	-
Cinnamomum iners Blume.	-	-	-	-	10	10	8	9	9	8	-	-	-	-	-
Derris scandens Benth.	10	-	9	-	11	-	-	9	7	7	7	7	9	7	7
Albizia myriophylla Benth.	7	9	11	7	10	10	9	8	7	12	7	10	9	9	7
Caesalpinia sappan L.	12	8	-	7	11	7	13	10	10	-	7	12	9	8	7
Pterocarpus santalinus L.	10	10	7	-	10	7	6	7	10	6	-	-	-	-	-
Piper chaba Hunt.	6	6	-	-	11	-	9	8	7	-	-	-	-	8	-
Piper nigrum L.	10	-	7	-	12	7	11	10	7	-	11	8	-	7	7
Rheum officinale Baill.	10	10	-	-	10	10	10	9	8	-	-	7	-	6	7
Mansonia gagei Drumm.	14	-	13	-	9	-	7	8	12	14	11	13	-	7	7
Conioselinum univitatum Turcz.	7	-	-	-	8	-	6	7	6	-	-	-	-	-	-
Alpinia galangal Swartz.	6	-	6	7	10	10	8	7	12	-	7	-	-	6	6
Curcuma longa L.	6	8	-	-	9	13	7	6	7	-	-	-	6	-	-
Globba laeta K. Larsen	11	8	6	-	10	-	7	7	7	6	7	-	9	9	7
Kaempferia parviflora Wall.	10	-	10	-	12	-	-	10	13	7	7	-	7	7	-
Zingiber officinale Roscoe.	6	-	6	-	10	8	6	7	10	-	-	-	8	7	6
Antibiotic susceptibility values															
Ampicillin (10 μg disc^{-1})	16	26	25	12	16	14	11	15	-	12	14	NT	NT	NT	NT
Ciprofloxacin (10 μg disc^{-1})	20	18	20	30	20	23	30	25	21	25	22	NT	NT	NT	NT
Ketoconazole (10 μg disc^{-1})	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	25	8	18	20

(-/): No inhibition at the concentration tested., NT: Not tested. Microbial strains: BC: *Bacillus cereus* ATCC 11778; BS: *Bacillus subtilis* ATCC 6633; SA*: Staphylococcus aureus* ATCC 13150; ST: *Streptococcus thermophilus* ATCC 19258; SF: *Streptococcus faecalis* TISTR 459; SCr: *Streptococcus cremoris* TISTR 058; EC: *Escherichia coli* ATCC 29214; SD: *Shigella dysenteriae* ATCC 13313; STy, *Salmonella typhi* ATCC 43579; PV: *Proteus vulgaris* TISTR 100; VC: *Vibrio chlolerea* ATCC 14033; CK: *Candida krusei* TISTR 5256; CT: *Candida tropicalis* ATCC 9968; SC: *Saccharomyces cerevisiae* ATCC 18824; KM: *Kluyveromyces marxianus* ATCC 8554

The results of the antimicrobial screening of a total of 18 extracts against fifteen microbial species are shown Table 3, among the 18 plant extracts, 8 plants showed the most pronounced activity with inhibition zones of more than 12 mm shown by the methanolic extracts of *A. sativum* L. (bulb), *A. myriophylla* Benth. (root), *C. sappan* L. (heartwood), *P. nigrum* L. (seed), *M. gagei* Drumm. (heartwood), *A. galangal* Swartz. (rhizomes), *C. longa* L. (rhizomes) and *K. parviflora* Wall. (rhizomes). The inhibitory effect of the studied medicinal plant extracts was exhibited mainly against the Gram-positive bacteria namely, *Bacillus cereus* ATCC 11778, *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 13150, *Streptococcus faecalis* TISTR 459 and *Streptococcus cremoris* TISTR 058 and Gram-negative bacteria namely, *Escherichia coli* ATCC 29214, *Shigella dysenteriae* ATCC 13313 and *Salmonella typhi* ATCC 43579. Some of the extracts include *Derris scandens* Benth., *Albizia myriophylla* Benth. and *Caesalpinia sappan* L. showed any activity against fungi. It was interesting to note that *Bacillus* strains, *Streptococcus* strains, *E. coli* ATCC 29214, *Sh. dysenteriae* ATCC 13313 and *Salm. typhi* ATCC 43579 showed more sensitivity to the investigated extracts than the other antibiotic susceptible bacteria or fungi.

The results obtained from the screening of the extracts of 18 Thai medicinal plants were very promising, especially, the activity of the methanolic extract of *A. sativum* L. (Alliaceae), as it was

Table 4: Minimal inhibitory concentrations (MIC) of the methanolic extracts of Thai medicinal plants

	MIC (μg mL^{-1})														
Species	BC	BS	SA	ST	SF	SCr	EC	SD	STy	PV	VC	CK	CT	SC	KM
Allium sativum L.	4	32	32	NT	16	64	32	32	32	NT	NT	NT	128	128	64
Saussurea lappa Clarke.	64	64	64	NT	32	128	128	64	64	NT	NT	NT	NT	NT	NT
Cymbopogon citratus Stapf.	64	NT	NT	NT	64	NT	64	64	64	NT	NT	NT	NT	NT	NT
Cinnamomum iners Blume.	NT	NT	NT	NT	32	32	64	32	32	64	NT	NT	NT	NT	NT
Derris scandens Benth.	32	NT	32	NT	16	NT	NT	32	64	128	64	64	32	64	64
Albizia myriophylla Benth.	64	32	16	64	32	32	32	64	64	16	64	32	32	32	64
Caesalpinia sappan L.	16	64	NT	64	16	64	4	32	32	NT	64	16	32	64	64
Pterocarpus santalinus L.	32	32	64	NT	32	64	128	64	32	128	NT	NT	NT	NT	NT
Piper chaba Hunt.	128	128	NT	NT	16	NT	32	64	64	NT	NT	NT	NT	64	NT
Piper nigrum L.	32	NT	64	NT	16	64	8	32	64	NT	16	64	NT	64	64
Rheum officinale Baill.	32	32	NT	NT	32	32	32	32	64	NT	NT	64	NT	256	64
Mansonia gagei Drumm.	4	NT	4	NT	32	NT	64	64	16	4	16	4	NT	64	64
Conioselinum univitatum Turcz.	64	NT	NT	NT	64	NT	128	64	256	NT	NT	NT	NT	NT	NT
Alpinia galangal Swartz.	128	NT	128	64	32	32	64	64	4	NT	NT	NT	NT	128	256
Curcuma longa L.	128	64	NT	NT	32	4	64	128	64	NT	NT	NT	128	NT	NT
Globba laeta K. Larsen	16	64	128	NT	32	NT	64	64	64	128	64	NT	32	32	64
Kaempferia parviflora Wall.	32	NT	32	NT	16	NT	NT	32	4	128	NT	NT	64	64	NT
Zingiber officinale Roscoe.	128	NT	128	NT	32	64	128	64	32	NT	NT	NT	64	64	256

NT: Not tested. Microbial strains: BC: *Bacillus cereus* ATCC 11778; BS: *Bacillus subtilis* ATCC 6633; SA: *Staphylococcus aureus* ATCC 13150; ST: *Streptococcus thermophilus* ATCC 19258; SF: *Streptococcus faecalis* TISTR 459; SCr: *Streptococcus cremoris* TISTR 058; EC: *Escherichia coli* ATCC 29214; SD: *Shigella dysenteriae* ATCC 13313; STy: *Salmonella typhi* ATCC 43579; PV: *Proteus vulgaris* TISTR 100; VC: *Vibrio chlolerea* ATCC 14033; CK: *Candida krusei* TISTR 5256; CT: *Candida tropicalis* ATCC 9968; SC: *Saccharomyces cerevisiae* ATCC 18824; KM: *Kluyveromyces marxianus* ATCC 8554.

effective against *B. cereus* ATCC 11778 (MIC = 4 μg mL^{-1}) (Table 4) while *C. sappan* L. (Papilionaceae) showed outstanding antimicrobial properties against *E. coli* ATCC 29214 with a MIC value of 4 μg mL^{-1}. It was interesting to note that the extract of *M. gagei* Drumm (Sterculiaceae) was very effective against *B. cereus* ATCC 11778, *Staph. aureus* ATCC 13150, *Pr. vulgaris* TISTR 100 and *C. krusei* TISTR 5256 (MIC = 4 μg mL^{-1}) than the other strains tested. In addition, the *A. galangal* Swartz., *C. longa* L. and *K. parviflora* Wall. (Zingiberaceae) extracts also showed good antimicrobial effects against *Strep. cremoris* TISTR 058 and *Salm. typhi* ATCC 43579 (MIC = 4 μg mL^{-1}).

DISCUSSION

Free radicals have been implicated in many disease conditions, the important ones being superoxide radicals, hydroxy radicals, peroxyl radicals and single oxygen, from both endogenous and exogenous sources, may be involved in the etiologies of such diverse human diseases as arteriosclerosis, ischemic injury, cancer and neurodegenerative diseases, as well as in processes like inflammation and ageing (Alho and Leinonen, 1999). There is evidence that indigenous antioxidants may be useful in preventing the deleterious consequences of oxidative stress and there is increasing interest in the protective biochemical functions of natural antioxidants contained in spices, herbs and medicinal plants (Farrukh *et al*., 2006; Al-Fatimi *et al*., 2007). Our attention has been focused, in particular, on the parts of 18 commonly used Thai medicinal plants. The antioxidant activities of the plant extracts were measured by DPPH radical assay. The

characteristics of the used medicinal plants and the inhibitory effects of their methanolic extracts on DPPH radical oxidation, expressed as IC_{50}. Considering the large variation of IC_{50} values, ranging from 0.02 mg mL^{-1} in *Mansonia gagei* Drumm., *Pterocarpus santalinus* L., *Cinnamomum iners*Blume. and *Kaempferia parviflora* Wall. to 2.06 mg mL^{-1} in *Globba laeta* K. Larsen and *Zingiber officinale* Roscoe., the potential of antioxidant activity of plant materials of this study was divided into 3 groups: high (IC_{50}<0.10 mg mL^{-1}), moderate (0.10 mg mL^{-1} <IC_{50}<1.00 mg mL^{-1}) and low (IC_{50}>1.00 mg mL^{-1}). Six plant materials out of 18 samples showed a high effective antioxidant capacity (IC_{50}<0.06 mg mL^{-1}) that order: *Mansonia gagei* Drumm., *Pterocarpus santalinus* L., *Cinnamomum iners* Blume., *Kaempferia parviflora* Wall., *Caesalpinia sappan* L., *Rheum officinale* Baill. and *Albizia myriophylla* Benth., respectively. These plants can be considered as good sources of natural antioxidants since their extracts were found to possess high antioxidant activity. Chanwitheesuk *et al.* (2005) suggest that the antioxidant activities of medicinal plants may be attributed to the antioxidant components present, especially vitamin E, xanthophylls and phenolics. Antioxidants are secondary metabolites produced by plants to protect against oxidative damage by free radicals (Larson, 1988). In the family Zingiberaceae, i.e., *Alpinia galangal* Swartz., *Curcuma longa* L., *Globba laeta* K. Larsen, *Kaempferia parviflora* Wall. and *Zingiber officinale* Roscoe., the antioxidants produced by the plant are transported to the rhizomes where they are accumulated. This implies that rhizomes would have higher antioxidant activity than other plant parts (Chan *et al.*, 2008). On the other hand nine medicinal plant species, namely *Cymbopogon citratus* Stapf., *Piper nigrum* L., *Saussurea lappa* Clarke., *Conioselinum univitatum* Turcz., *Piper chaba* Hunt., *Allium sativum* L., *Alpinia galangal* Swartz. and *Derris scandens* Benth. were in moderate range whereas the *Curcuma longa* L., *Globba laeta* K. Larsen and *Zingiber officinale* Roscoe. extracts showed low antioxidant activity. Similar observations have been made by Chan *et al.* (2008), who reported the rhizomes of *Zingiber officinale* Roscoe. had low concentration of total phenolic content and its extracts showed low antioxidant activity (0.96 mg AA g^{-1} fresh weight). The antiradical activity (A_{AR}) defined as $1/IC_{50}$ was seen in extracts from various medicinal plants. As expected, extracts with higher A_{AR} were obtained from the medicinal plant extracts mentioned above.

The radical scavenging activity of plant extracts may be an indicator of its antioxidant activity. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants. Hence, DPPH is often used as a substrate to evaluate antioxidative activity of antioxidants. Different plant extracts reduce DPPH radicals significantly. The methanolic extract of the rhizomes of *K. parviflora* Wall. exhibited the highest level of radical scavenging activity with average value 86.35%. The plant *K. parviflora* Wall. is a medicinal plant naturally distributed throughout Southeast Asia, In Thailand, it is widely grown in the country's northeastern region, especially in Leoi province where there are suitable conditions for growth and production of the active compounds. It is widely used as material for organic red wine and traditional medicine. The rhizomes of *K. parviflora* Wall. have been known to be effective against some diseases, including stomachic, hypertension, diuretic and diabetics. A few studies on pharmacological activities of the *K. parviflora* Wall. extract have been scientifically investigated. It has been reported that the extract of *K. parviflora* Wall. has health-promoting for longevity, treatment for colic disorder, duodenal ulcer, gastric ulcer and allergy (Yenjai *et al.*, 2004; Rujjanawate *et al.*, 2005; Tewtrakul and Subhadhirasakul, 2007). In addition, the alcoholic

infusion of its rhizome has been used as a tonic for rectifying male impotence, body pains and gastrointestinal disorders. The result of this study suggests that the *K. parviflora* Wall. can be used as a potential source of natural antioxidants, with pharmaceutical applications.

After evaluating the effects on the Gram-negative bacteria we found that extracts of sixteen plants inhibited *E. coli* ATCC 29214, all medicinal plant extracts inhibited *Sh. dysenteriae* ATCC 13313 and *Salm. typhi* ATCC 43579, seven extracts inhibited *Pr. vulgaris* TISTR 100 and six extracts inhibited *V. chlolerea* ATCC 14033. Among the Gram-positive bacteria, all medicinal plant extracts inhibited *Strep. faecalis* TISTR 459, seventeen extracts inhibited *B. cereus* ATCC 11778M, nine extracts inhibited *B. subtilis* ATCC 6633, eleven extracts inhibited *Staph. aureus* ATCC 13150 and *Strep. cremoris* TISTR 058. It is remarkable that the *A. myriophylla* Benth., *C. sappan* L. and *A. galangal* Swartz. extracts exhibited only antibacterial activity against *Strep. thermophilus* ATCC 19258. The highest activity was shown against the Gram-positive bacterium, *B. cereus* ATCC 11778, *B. subtilis* ATCC 6633, *Staph. aureus* ATCC 13150, *Strep. faecalis* TISTR 459 and *Strep. cremoris* TISTR 058. It has been reported that Gram-negative bacteria had low susceptibility to plant extracts when compared to Gram-positive bacteria (Kumarasamy*et al*., 2002; Al-Fatimi *et al*., 2007). The resistance of Gram-negative bacteria towards antibacterial substances is related to lipopolysaccharides in their outer membrane.

The disk diffusion method gives the opportunity to determine an approximate MIC indicating the degree of potential antimicrobial activity compared with that of positive control, ampicillin, ciprofloxacin and ketoconazole. Generally, *A. sativum* L. and *M. gagei* Drumm, are well known for its traditional use as an antiseptic. However, it is interesting to note that many other species, like *C. sappan* L., *A. galangal* Swartz., *C. longa* L. and *K. parviflora* Wall., etc., which have traditionally been used to treat wounds and infections, were found to be effective against the microorganism tested in our study. It is remarkable that the methanolic extracts of *A. galangal* Swartz. and *K. parviflora* Wall. showed interesting antibacterial activity against *Salm. typhi* ATCC 43579 with a MIC = 4 μg mL^{-1}. They are the one of most popular herbal remedies which have traditionally been used in Thai traditional medicine for antidiarrhoea, skin diseases, toothaches, gastric diseases, diuretic, antibacterial, antifungal and antioxidant activities (Chanwitheesuk *et al*., 2005; Oonmetta-Aree *et al*., 2006). The activity of the medicinal plant extracts against both Gram-positive and Gram-negative bacteria may be an indication of the presence of a broad spectrum of antibiotic compounds which are mainly distributed in the seed, bulb, heartwood and rhizomes of plants (Kumarasamy *et al*., 2002; Al-Fatimi *et al*., 2007).

CONCLUSION

In conclusion, the findings in the present study are in agreement to a certain degree with the traditional uses of the plants. The findings support the view that some Thai medicinal plants are promising sources of potential antioxidants and antimicrobial activity, which may be efficient as preventive agents in the pathogenesis of some diseases. The study shows that there are differences in the antioxidant and antimicrobial activity of the medicinal plants commonly consumed in Thailand. Some of the plants can be considered as good sources of natural antioxidants since their extracts were found to possess high antioxidant activity. The highest antioxidant activities were detected in *K. parviflora* Wall., followed by *R. officinale* Baill., *P. chaba* Hunt. and *C. inersBlume*., respectively. Based on the antimicrobial activity, the *A. sativum* L., *C. sappan* L., *M. gagei* Drumm., *A. galangal* Swartz., *C. longa* L. and *K. parviflora* Wall. extracts had the greatest inhibitory effect against *B. cereus* ATCC 11778, *E. coli* ATCC 29214, *Staph. aureus* ATCC 13150, *Pr. vulgaris*

TISTR 100, *Strep. cremoris* TISTR 058, *Salm. typhi* ATCC 43579 and *C. krusei* TISTR 5256 among medicinal plant studied. Some Gram-positive, Gram-negative bacteria and fungi were susceptible to the extract. The obtained results could form a good basis for selection of medicinal plant species for further investigation in the potential discovery of new natural bioactive compounds. However, the characteristics of the phytochemicals and the pharmacological mechanisms of the extracts should be further studied to gain more understanding of their antioxidant and antimicrobial activity in body and food systems, which may be further exploited to synthesize new drugs in order to cure infectious disease.

ACKNOWLEDGMENTS

Authors acknowledge S. Sikhruadong, Department of Plant Production Technology, Mahasarakham University, Thailand, that allowed us to benefit from their knowledge of the plants studied. We would like to gratefully acknowledge the staff of the Microbiology Laboratory, Department of Biotechnology, Technology Faculty, Mahasarakham Universty, Thailand, for their collaboration and providing laboratory facilities. Besides this we thank S. Khumkratok, Walai Rukhavej Botanical Research Institute, Mahasarakham University, Mahasarakham, Thailand, for the assistance by the taxonomical identification of some medicinal plant species.

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