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Full Length Research Paper

High phenolics and antioxidants of some tropical vegetables related to antibacterial and anticancer activities

Jureerut Daduang[1], Sukanda Vichitphan[2], Sakda Daduang[3], Pranithi Hongsprabhas[4] and Patcharee Boonsiri[5]*

[1]Centre for Research and Development of Medical Diagnostic Laboratories, Faculty of Associated Medical Sciences, Khon Kaen University, Khon Kaen 40002, Thailand.
[2]Department of Biotechnology, Faculty of Technology, Khon Kaen University, Khon Kaen 40002, Thailand.
[3]Department of Biochemistry, Faculty of Sciences, Khon Kaen University, Khon Kaen 40002, Thailand.
[4]Department of Medicine, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand.
[5]Department of Biochemistry, Faculty of Medicine, Khon Kaen University, Khon Kaen 40002, Thailand.

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Phytochemicals play an important role in the decrease risk of free radicals associated diseases. In the present study, total phenolic contents, antioxidant, antibacterial and anticancer activities of thirty Thai vegetables have been evaluated. The results showed that phenolic contents of Thai vegetables were highly correlated with total antioxidant activities. Four vegetables in strains of *Spondias pinnata Kurz.*, *Careya sphaerica Roxb., Caesalpinia mimosoides Lamk.* and *Cratoxylum Subsp. Pruniflorum (Kurz.) Gogel.* with high phenolic contents and total antioxidant activities, have strong antibacterial activities against *Staphylococcus aureus*, *Salmonella typhimurium* and *Eschericia coli*. Although, the extracted samples were non-cytotoxic as confirmed on the normal Vero cells, only *C. mimosoides Lamk*. extract had inhibitory effect on oral cavity cell line (IC_{50} 27.17 ± 5.44 µg/ml). Thus, this study suggested that some vegetables can be considered as possible therapeutic agents.

Key words: Antibacterial, antioxidant, anticancer, phenolic contents, vegetables.

INTRODUCTION

Currently, much attention on medicinal plant research has been focused on those vegetables that are health tonic or used for the prevention of various diseases, several epidemiological studies have shown that consumption of vegetables are closely associated with the decrease risks of diseases that resulted from oxidative stress, including cancer, cardiovascular disease, diabetes, neurodegenerative disorders and various infectious diseases (Doll, 1990; Temple, 2000). Phenolic phytochemicals in vegetables are the major bioactive substances which are involve in the prevention of oxidative stress by eliminating free radicals, stimulation of the immune system, regulation of gene expression and antibacterial effects (Liu, 2004). The relationship between the total phenolic contents and the antioxidant activities had been reported (Wang et al., 1996) and it has been demonstrated that antioxidant activity of the plant extracts was stronger than the synthetic ones (Meyers et al., 2003; Parejo et al., 2002). Moreover, antimicrobial activity of the plant extracts was closely related with their phenolic contents (Bendini et al., 2006; Rodriguez et al., 2007). The antibacterial action of the plant phenolic compounds is mediated through their reaction with the cell membrane, inactivation of essential enzymes and destruction or functional inactivation of genetic materials

*Corresponding author. E-mail: patcha_b@kku.ac.th. Tel: +66-43-348386.

(Puupponen-Pimia et al., 2001; Vattem et al., 2004). The variety of vegetables have been investigated in an attempt to find natural antioxidants and to discover new chemical classes of antibacterial and anticancer agents (Essawi and Srour, 2000; Gnanamani et al., 2003; Habsah et al., 2000; Kudi et al., 1999; Nanasombat and Teckchuen, 2009).

Thai traditional plant medicine claims to have health benefits that prevent pathological changes. However, only a few scientific evaluation of the effectiveness of these vegetables have been reported (Chanwitheesuk et al., 2005; Laupattarakasem et al., 2003; Maisuthisakul et al., 2007; Nanasombat and Teckchuen, 2009). The aim of this study, therefore, is to evaluate the total phenolic contents, total antioxidant activities, as well as biological properties, including cytotoxicity, anticancer and anti-bacterial activities of some edible vegetables in Thailand as a new potential source of therapeutic agents.

MATERIALS AND METHODS

Sample preparation and extraction

A total of 30 fresh vegetables were purchased from three different local markets in Khon Kaen municipality area, Northeast Thailand during May 2007. The pooled edible parts from each vegetable were mixed and homogenized by an electrical blender. Then, 2.5 g of each homogenized sample was mixed twice with 12.5 ml of 70% (v/v) ethanol using a vortex mixer for 5 min. The extracts were then centrifuged at 2,000 g for 10 min at room temperature. Two extracted supernatants were pooled and the final volume was adjusted to 25 ml with 70% (v/v) ethanol. The supernatant was then divided into two parts; the first part was used to determine the total phenolic contents and total antioxidant activities, while the other part was dried under nitrogen gas and reconstituted in 20% (v/v) dimethylsulfoxide (DMSO) for assaying cytotoxicity, anticancer and antibacterial activities.

Determination of total phenolic contents

The phenolic contents in vegetables were determined by modification of the Folin-Ciocalteu spectrometric method (Singleton and Rossi, 1965). Briefly, 200 µl of the vegetable extracts at appropriated dilutions were mixed with 1 ml of 0.2 M Folin-Ciocalteu reagent. After leaving the solution in dark at room temperature for 30 min, 800 µl of 7% sodium carbonate was added to it. The absorbance of the resulting blue color was measured at 750 nm (Shimadzu, UV mini 1240, Japan). Phenolic contents were expressed as mg of gallic acid equivalent (GAE)/g dry wt of vegetables.

Determination of antioxidant activities

Ferric reducing antioxidant power (FRAP) assay

FRAP total antioxidant activity was carried out according to the modified method of Benzie and Strain (1996). The FRAP reagent was freshly prepared from 300 mM acetate buffer pH 3.6, 10 mM of 2,4,6-tris(2-pyridyl)-1,3,5-triazine (TPTZ) solution in 40 mM HCl and 20 mM iron (III) chloride ($FeCl_3.6H_2O$) solution at a ratio of 10:1:1 (v/v), respectively. One milliliter of vegetable extracts was added to 1 ml of the FRAP reagent, then after 5 min the absorbance of the reaction mixtures were then recorded at 593 nm (Shimadzu, UV mini 1240, Japan). The standard curve was constructed using iron (II) sulfate ($FeSO_4$) solution. The results were expressed as µmol Fe(II)/g dry wt of vegetables.

1,1-diphenyl-2-picrylhydrazyl (DPPH) method

Antioxidant activity of vegetable extracts was evaluated by DPPH spectrophotometric assay modified from Mensor et al. (2001). Briefly, the extracts were diluted with 70% (v/v) ethanol, and 20 µl of the dilution with 1 ml of 0.25 mM DPPH solution were added to it. After mixing, the reaction mixtures were kept in dark for 10 min and the changes in absorbance at 540 nm were then measured (Shimadzu, UV mini 1240, Japan). Radical scavenging activity was shown as mM Trolox equivalent antioxidant capacity (TEAC)/g dry wt of vegetables.

Determination of antibacterial activity and minimum inhibitory concentration

Antibacterial activities of the vegetable extracts were determined by the agar well diffusion method modified from Rodriguez et al. (2007). Their extracts were also determined against the growing bacteria in different strains of bacteria, including *Staphylococcus aureus* (TISTR 746, TISTR 038 and TISTR 029), *Salmonella typhimurium* (TISTR 292, MSU and ATCC 14028) and *Eschericia coli* (W 3310, ATCC 25922 and TISTR 073). Chloramphenical drug (500 µg/ml) and DMSO were used as positive and negative controls, respectively. The test organisms, 1 x 10^8 colony forming units per milliliter (CFU/ml) grown in nutrient broth media for 24 h, were spread over the surface of nutrient agar medium in 9-cm-diameter Petri dishes by sterile swab sticks. The agar medium was punched with a 5-mm-diameter cork-borer. The extracted residue was reconstituted with 400 µl of 20% (v/v) DMSO and then 40 µl of the reconstituted sample was applied on each well. The Petri dishes were incubated at 37°C for 24 h and obtained in diameters of their inhibition zones. An inhibition zone > 15 mm was considered as a high antibacterial activity.

In addition, the residues were two-fold serially diluted with 20% DMSO (4.00 to 6.25 µg/ml), after which the extracts 40 µl were applied in each well of an agar plate to obtain the minimum inhibitory concentration (MIC). The lowest concentration of the extract showing a clear inhibition zone was reported as MIC.

Scanning electron microscopy

Scanning electron microscopy (SEM) was performed by the method of Uawonggul et al. (2007) with slight modification. After the bacteria were grown in agar plates for 24 h, the cells at inhibition zone were harvested. The bacterial cells were then washed twice with 20 mM phosphate buffer saline (PBS) pH 7.0 and fixed with 2.5% w/v glutaraldehyde overnight. The fixed cells were carefully pipetted and settled onto a 0.2 µM polycarbonate membrane filter (Whatman, Germany) for 5 min. The materials were then dehydrated for 15 min in each graded ethanol concentrations (30, 50, 70 and 90%). The dehydrated materials in the absolute ethanol were dried in a critical point drier (Thermo VG Scientific, CPD7510, England) with carbon dioxide (CO_2) as the drying agent. Dry materials were coated by sputter coater (Polaron, SC7620, England) with gold palladium and examined by a scanning electron microscope (LEO Electron Microscopy Ltd, LEO1450VP, England) operation at 12 to 20 kV. For negative control, all were performed in

a similar manner except that the bacterial cells were incubated with PBS buffer instead of the vegetable extracts.

Determination of cytotoxicity and anticancer activity

Cytotoxicity of the vegetable extracts against normal Vero cells (African green monkey kidney fibroblast) was determined by colorimetric method adapted from Skehan et al. (1990). Briefly, in a 96-well-microplate, 190 µl of Vero cell suspension containing 1 x 10^5 cells/ml and 10 µl of extracted samples were added into each well and incubated in 5% CO_2 at 37°C for 72 h. The different concentrations of the extracts were exposed to Vero cells for the determination of IC_{50}. Positive and negative controls were 0.76 µg/ml ellipticine and 0.5% DMSO, respectively. Cell growth >50% was considered as non-cytotoxic, while the cytotoxic to Vero cell line was defined at ≤ 50%.

Anticancer activity of extracted samples in the human epidermal carcinoma oral cavity cell line ATCC #CCL-17 (KB) was evaluated by using the method of Skehan et al. (1990). However, the positive control was 0.31 µg/ml ellipticine and 0.18 µg/ml doxorubicine, while DMSO was used as negative control.

RESULTS AND DISCUSSION

Thirty fresh vegetables were illustrated with their common and scientific names, their edible parts and pharmaceutical uses (Table 1). According to traditional medicine, they have been used for several pharmaceutical purposes, such as: (1) carminative and appetizer, (2) antipyretic, anti-inflammation and astringent, and (3) anti-helmintic, anti-diarrhea and laxative.

Total phenolic content and antioxidant activity

Total phenolic contents of all extracts were presented in a wide range at 5.41 to 284.28 mg GAE/g dry wt (Table 1). *Spondias pinnata Kurz.*, *Careya sphaerica Roxb., Barringtonia acutangula Gaertn., Caesalpinia mimosoides Lamk.* and *Cratoxylum Subsp. Pruniflorum (Kurz.) Gogel.* had high total antioxidant activities in the standard methods. High antioxidant activities in the different methods were reported in previous studies (Chanwitheesuk et al., 2005; Maisuthisakul et al., 2007). Antioxidant activities were highly correlated with phenolic contents in vegetable samples at $r^2 = 0.733$, $p<0.0001$ and at $r^2 = 0.495$ and $p<0.0001$ (Figures 1A and B). This finding agreed with a previous report by Zheng and Wang (2001).

Antibacterial activity

Four vegetables, including *S. pinnata Kurz.*, *C. mimosoides Lamk., Cratoxylum Subsp. Pruniflorum (Kurz.) Gogel.* and *C. sphaerica Roxb.,* had high total phenolic contents and total antioxidant activities. As such, they were selected for antibacterial test by agar well diffusion assay (Table 2). The inhibition zone around the tested bacteria tended to increase with the increasing concentration of phenolic contents. This finding correlated with previous reports by Bendini et al. (2006) and Rodriguez et al. (2007). Interestingly, *S. pinnata Kurz.*, with the highest total phenolic contents, was the most effective against *S. aureus*. *S. aureus* was the most susceptible to all extracts (≤6.25 µg/ml MIC), whereas *S. typhimurium* was quite resistant against *C. sphaerica Roxb.* (50 to 100 µg/ml MIC) and *S. pinnata Kurz.* (200 µg/ml MIC) (Table 3).

Scanning electron microscopy

To observe the effects of the four potential extracts, *S. pinnata Kurz.*, *C. sphaerica Roxb., C. mimosoides Lamk.* and *Cratoxylum Subsp. Pruniflorum (Kurz.) Gogel.* on the disruption of bacterial cell membrane, SEM was performed. In the absence of the vegetable extracts, *S. aureus* TISTR 746, *E. coli* W 3310 and *S. typhimurium* TISTR 292 exhibited a regular and smooth surface membrane (Figures 2A, G and M). Morphological changes of the membrane clearly appeared when the bacteria were treated with chloramphenical (Figures 2B, H and N). After treatment separately with 400 µg/ml of vegetable extracts, *S. aureus* TISTR 746 was enlarged in size with rough surface and asymmetrical shape (Figures 2C to F), whereas the surface deformations of bacterial cells, such as grooves and cavities, to the overall cell wall wrinkling were illustrated in *E. coli* W 3310 (Figures 2I to L) and *S. typhimurium* TISTR 292 (Figures 2O to R). Several studies reported that the plant phenolic compounds can disrupt bacterial cell membrane because they acted as strong oxidizers (Puupponen-Pimia et al., 2001; Vattem et al., 2004). The variation of bacterial size and shape observed in the present study may be due to the formation of chemical bonds between polysaccharide components at the surface of the cell wall as a result of the disrupted equilibrium of surface macromolecules upon increasing phenolic contents.

Cytotoxicity against cancer cell line and Vero cells

As a result of strong antibacterial and antioxidant activities, the extracts of *S. pinnata Kurz.*, *C. mimosoides Lamk., Cratoxylum Subsp Pruniflorum (Kurz.) Gogel*. and *C. sphaerica Roxb.* were obtained in anticancer activities. Although all extracts showed non-cytotoxicity, particularly confirmed on Vero cells, only the extract from *C. mimosoides Lamk.* had inhibitory effect on the oral cavity cell line (KB) proliferation (IC_{50} of 27.17 ± 5.44 µg/ml) (Table 4). The phenolic phytochemicals of *C. mimosoides Lamk.* may effectively be related to the anticancer activity, in that the kaempferol and quercetin, which are phenolic components in *Ginkgo biloba* extract, induced caspase-3-

Table 1. Vegetables and their therapeutic uses, total phenolic contents and antioxidant activities of ethanolic extracts.

Common Thai name	Scientific name	Uses	Edible part used	Total phenolic contents (mg GAE/g dry wt)*	Antioxidant activity with FRAP assay (µmol Fe (II) /g dry wt)*	Antioxidant activity with DPPH assay (mM TEAC/g dry wt)*
Krajiewdang	*Curcuma aeruginosa Roxb.*	Carminative	Flower	20.18±0.04	301.20±0.13	0.77±0.00
Paew	*Polygonum odoratum Lour.*	Carminative, appetizer	Leaf	36.20±0.03	297.25±0.09	0.80±0.09
Cheelao	*Anethum graveolens Linn.*	Carminative, appetizer	Leaf	17.29±0.01	144.29±0.15	0.47±0.00
Cha-om	*Acacia pennata Wild*	Carminative	Leaf	64.33±0.01	120.22±0.11	0.36±0.00
Chaplu	*Piper sarmentosum Roxb.*	Carminative, anti-hyperglycemia	Leaf	18.42±0.02	87.16±0.20	0.37±0.00
Kaya	*Caesalpinia mimosoides Lamk.*	Appetizer, improve quality of blood, anti-vertigo	Shoot tip	246.61±0.06	841.47±0.20	1.07±0.01
Pham	*Wolffia globosa Hartog* and *Plas*	Appetizer	Flower	27.19±0.07	441.18±0.15	0.72±0.04
Tuapoo	*Psophocarpus tetragonlobus (L.) DC.*	Appetizer	Pod	5.80±0.08	194.28±0.11	0.31±0.02
Kanjong	*Limnocharis flava Buch.*	Appetizer	Stem	8.13±0.01	79.46±0.15	0.36±0.05
Kajorn	*Telosma minor Craib.*	Appetizer, antidote	Flower	6.31±0.02	78.69±0.13	0.28±0.00
Keelekban	*Cassia siamea Lamk.*	Antipyretic	Leaf	116.23±0.02	516.73±0.16	0.54±0.00
Krached	*Neptunia oleracea Lour.*	Antipyretic, antidote	Leaf	42.98±0.02	236.62±0.11	0.96±0.00
Sadao	*Azadirachta indica A. Juss.*	Anti-malaria, appetizer	Flower	46.28±0.03	206.09±0.11	0.81±0.01
Kaeban	*Sesbania grandiflora Desv.*	Antipyretic, antidote	Flower	9.14±0.04	166.70±0.11	0.29±0.09
Cheefarang	*Eryngium foetidum Linn.*	Antipyretic, anti-flatulent	Leaf	6.99±0.03	78.89±0.12	0.14±0.01
Makok	*Spondias pinnata Kurz.*	Anti-inflammation	Leaf	284.28±0.02	1390.10±0.31	1.66±0.00
Paetumpueng	*Gynura divaricata DC.*	Anti-inflammation, anti-hyperglycemia	Leaf	5.41±0.04	223.79±0.15	0.16±0.01
Tamlueng	*Coccinia grandis (Linn.) Voigt.*	Anti-inflammation, antidote, anti-hyperglycemia	Shoot tip	22.51±0.02	118.26±0.13	0.30±0.00
Kradone	*Careya sphaerica Roxb.*	Astringent	Leaf	121.27±0.05	1139.05±0.11	0.99±0.02
Hom	*Amaranthus lividus Linn.*	Astringent, antidote	Leaf	11.00±0.01	195.67±0.25	0.27±0.02
Paknok	*Hydrocotyle javanica Thunb.*	Astringent, antidote, diuretic	Leaf	6.72±0.07	72.49±0.33	0.48±0.04
Jikna	*Barringtonia acutangula Gaertn.*	Anti-diarrhea	Leaf	105.25±0.02	1022.27±0.13	1.13±0.00
Kratin	*Leucaena leucocephala (Lamk.) de Wit.*	Anti-diarrhea, astringent	Leaf	133.62±0.08	352.91±0.25	0.35±0.02
Krajeabkiew	*Abelmoschus esculentus (L) Moench.*	Anti-helmintic, diuretic	Pod	10.02±0.01	189.01±0.18	0.57±0.01
Katokrok	*Passiflora foetida Linn.*	Anti-helmintic	Leaf	24.54±0.06	95.72±0.18	0.25±0.02
Kaepa	*Dolichandrone serrulata (DC.) Seem.*	Anti-helmintic, appetizer	Flower	12.98±0.02	86.02±0.09	0.30±0.01
Teawdang	*Cratoxylum Subsp. Pruniflorum (Kurz.) Gogel.*	Laxative	Leaf	155.31±0.01	672.23±0.21	0.43±0.01
Kayang	*Limnophila aromatica (Lamk.) Merr.*	Laxative, relieve stomachache, astringent	Shoot tip	37.64±0.01	259.70±0.20	0.53±0.04
Plung	*Bassella rubra Linn.*	Laxative, diuretic, anti-inflammation	Leaf	14.62±0.06	149.58±0.19	0.50±0.02
Chamuang	*Garcinia cowa Roxb.*	Laxative, anti-expectorant, anti-dispepsia	Leaf	18.98±0.02	134.84±0.10	0.45±0.01

* = Mean ± SD; n = 3.

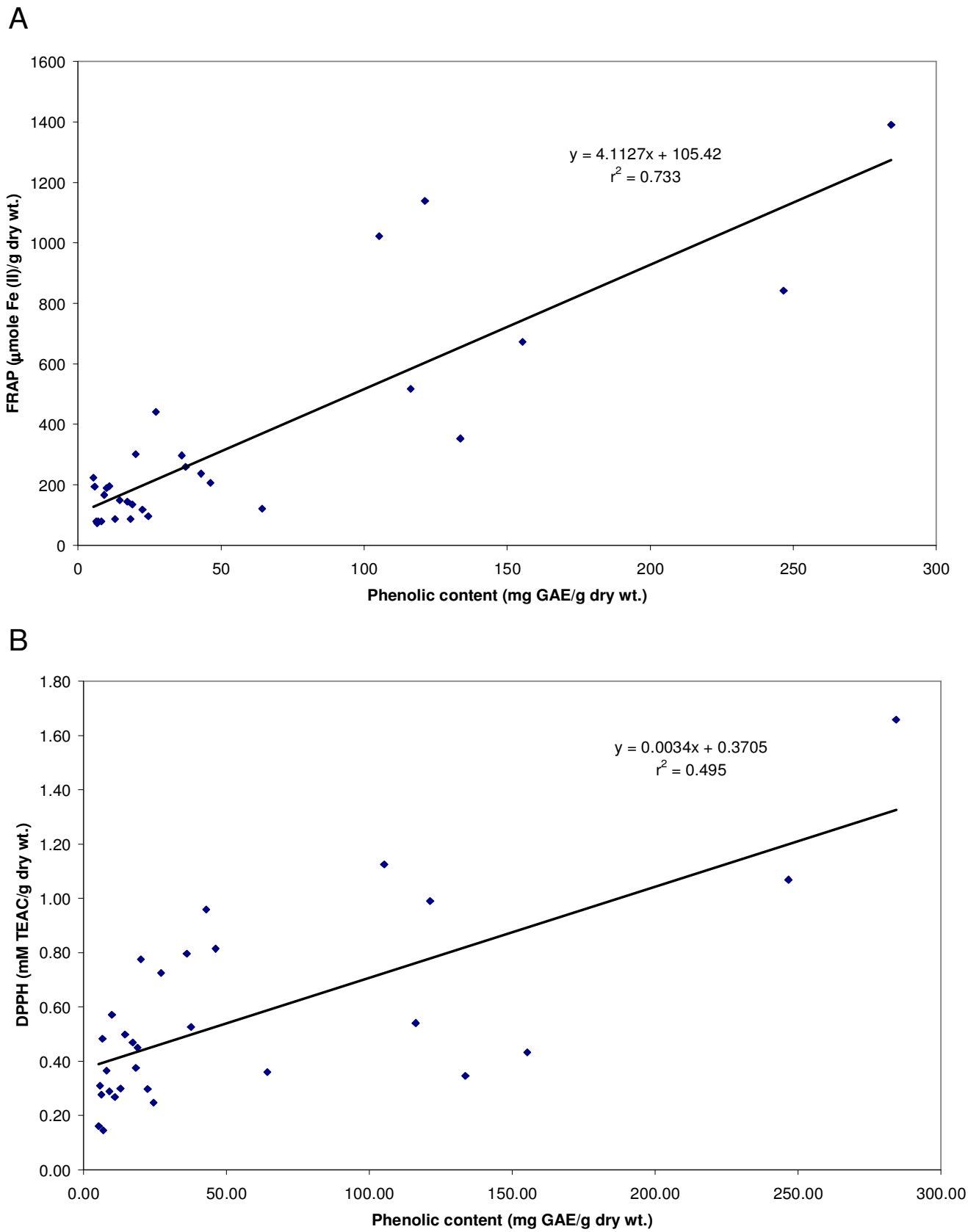

Figure 1. Correlation between (A) FRAP total antioxidant activity and phenolic content and (B) DPPH antioxidant activity and phenolic content, in the ethanolic extracts of Thai vegetables.

Table 2. Inhibition zone of vegetable extracts.

Bacterial strains/ vegetable extracts	Diameter of inhibition zone (mm)[a]				
	Chloram phenical (500 µg/ml)	*Careya sphaerica Roxb.*	*Cratoxylum Subsp. Pruniflorum (Kurz.) Gogel.*	*Spondias pinnata Kurz.*	*Caesalpinia mimosoides Lamk.*
			Staphylococcus aureus		
TISTR 746	25.75±0.53	18.00±0.87	20.00±0.48	25.00±0.89	23.75±0.25
TISTR 038	24.00±0.97	15.25±0.29	21.75±0.25	22.25±0.87	20.25±0.96
TISTR 029	25.75±0.78	18.50±0.96	21.75±0.49	24.50±0.82	24.00±0.22
			Salmonella typhimurium		
TISTR 292	33.00±0.15	13.00±0.71	23.50±0.85	22.00±0.32	23.50±0.55
MSU	27.25±0.19	10.75±0.75	15.75±0.82	14.50±0.55	19.50±0.75
ATCC 14028	26.50±0.93	12.50±0.91	14.50±0.71	13.00±0.75	18.00±0.31
			Escherichia coli		
W 3310	28.25±0.27	14.00±0.32	21.75±0.19	21.75±0.71	21.50±0.65
ATCC 25922	29.50±0.95	11.00±0.75	20.50±0.65	21.50±0.25	21.50±0.50
TISTR 073	26.00±0.80	10.00±0.50	15.25±0.75	12.50+0.50	25.50±0.29

[a] signifies at 400 µg/ml of vegetable extracts.

Table 3. Minimum inhibition concentration of vegetable extracts.

Bacterial strains/ vegetable extracts	MIC (µg/ml)			
	Careya sphaerica Roxb.	*Cratoxylum Subsp. Pruniflorum (Kurz.) Gogel.*	*Spondias pinnata Kurz.*	*Caesalpinia mimosoides Lamk.*
Staphylococcus aureus				
TISTR 746	12.50	≤ 6.25	≤ 6.25	≤ 6.25
TISTR 029	50	≤ 6.25	≤ 6.25	≤ 6.25
TISTR 038	25	≤ 6.25	≤ 6.25	≤ 6.25
Salmonella typhimurium				
TISTR 292	100	≤ 6.25	200	≤ 6.25
MSU	50	≤ 6.25	200	≤ 6.25
ATCC 14028	100	≤ 6.25	200	≤ 6.25
Escherichia coli				
W 3310	25	≤ 6.25	50	≤ 6.25
ATCC 25922	25	≤ 6.25	≤ 6.25	≤ 6.25
TISTR 073	50	≤ 6.25	≤ 6.25	≤ 6.25

dependent apoptosis in oral cavity cancer cells (Kang et al., 2010). However, the extract should be further sub-fractionated to identify the individual molecular component whose bioactivity towards oral cavity cancer cell can be separately assessed.

Conclusion

This investigation indicates that a number of vegetables have high potential of antibacterial activity. *C. mimosoides Lamk.* is the only one which had inhibitory

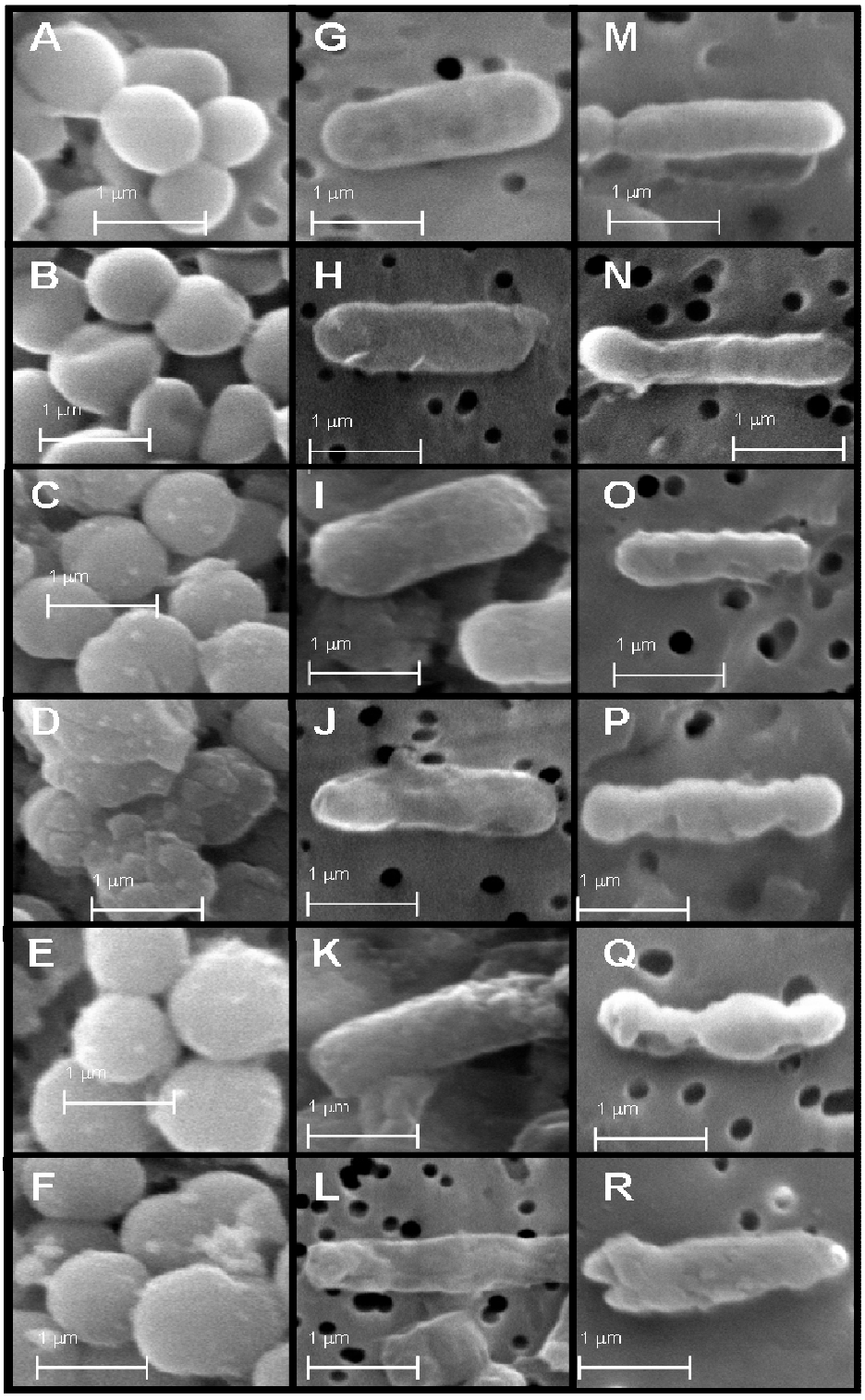

Figure 2. The scanning electron microscopic photographs of *S. aureus* TISTR 746 (2A-F), *Escherichia coli* W 3310 (2G-L) and *S. typhimurium* TISTR 292 (2M-R), after separate treatment with four vegetable extracts (400 µg/ml). Figures 2 A, G and M = Negative control treated with 0.9% sodium chloride; Figures 2 B, H and N = Positive control treated with 500 µg/ml chloramphenical; Figures 2 C, I and O = Treated with *S. pinnata Kurz*; Figures 2 D, J and P = Treated with *C. sphaerica Roxb.*; Figures 2 E, K and Q = Treated with *C. mimosoides Lamk.*; Figures 2 F, L and R = Treated with *Cratoxylum Subsp. Pruniflorum (Kurz.) Gogel.*; Bar = 1 micrometer.

Table 4. Cytotoxicity against Vero cells, anticancer against human epidermal carcinoma of oral cavity cell line (KB) and IC_{50} value of selected Thai vegetable extracts.

Vegetable	Cytotoxicity activity[a] to vero cells	Oral cavity cell line (KB) (IC_{50}, µg/ml)[b]
Careya sphaerica Roxb.	Non-cytotoxic	Inactive
Cratoxylum Subsp. Pruniflorum (Kurz.) Gogel.	Non-cytotoxic	Inactive
Spondias pinnata Kurz.	Non-cytotoxic	Inactive
Caesalpinia mimosoides Lamk.	Non-cytotoxic	27.17 ± 5.44[c]
Ellipticine (µg/ml)	0.76	0.31
Doxorubicine (µg/ml)	ND	0.18

[a] The percentage cell growth >50% was considered non-cytotoxic, but ≤ 50% was cytotoxic; [b] The percentage inhibition < 50% was considered inactive, but ≥ 50% was active (reported as IC_{50} value); [c]Mean of three replications. ND = not determined.

effect on oral cavity cell line proliferation. The results suggest that these vegetables can be used as a potential source of natural antioxidants, with their pharmaceutical applications.

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