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Full Length Research Paper

In vitro antibacterial and radical scavenging activities of Malaysian table salad

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Six types of table salad (*ulam*) namely *Mentha arvensis* (mint), *Lactuca sativa* var *capitata* (cabbage lettuce), *Psophocarpus tetragonolobus* (winged bean), *Pluchea indica* Less. (beluntas), *Premna cordifolia* (bebuas) and *Limnocharis flava* L. buchenau *(*yellow velvetleaf) were investigated for their antimicrobial and radical scavenging activities. In addition, total phenolic content (TPC) test was also carried out for the six plants. All the plants were extracted successively with dichloromethane (DCM) and ethanol (EtOH). The results showed that the DCM and EtOH extracts of *L. flava* displayed good antimicrobial activity against *Pseudomonas aeruginosa, Staphylococcus aureus* and *Streptococcus pyogenes*. The EtOH extract of this plant was the only one that showed inhibition against *Candida albicans*. *P. tetragonolobus* had the highest scavenging activity in both extracts, while the ethanolic extract of *P. indica* showed the highest phenolic contents (56.287 µg/mg). The total phenolic contents of the extracts ranged from 56.287 to 46.461 µg/mg gallic acid equivalents (GAE/g dry extract). The results showed that some table salads (*ulam)* possess antimicrobial and antioxidant activities and therefore, could be used as a natural preservative ingredient in food and/or pharmaceutical industries.

Key words: Table salad, antimicrobial, antioxidant, disc diffusion, total phenolic content.

INTRODUCTION

Ulam is a Malaysian term for table salad which refers to any vegetable that is eaten raw. Malaysians usually eat *ulam* because of their therapeutic effect. Some Malaysian *ulam* are used to cure cough, fever, flu and headache. Numerous studies have been reported on the antioxidant and antimicrobial activities of plant extracts against different types of microbes, including food-borne pathogens (Beuchat, 1994; Lis-Balchin and Deans, 1997; Smith-Palmer et al., 1998; Hara-Kudo et al., 2004).

The exploration of naturally-occurring antimicrobial food preservation receive increasing attention due to consumer awareness of natural food products and a growing concern of microbial resistance towards conventional preservatives (Gould, 1995). Nowadays, consumers are also concerned about the safety of foods that contain synthetic preservatives. Therefore, there has been increasing interest in the development of new types of effective and nontoxic natural antioxidant and antimicrobial compounds (Smid and Gorris, 1999).

Traditionally, *Mentha arvensis* is used to expel gas from the digestive system, induce sweating, assist the flow of menstrual fluid, promote milk secretion and relieve fever (Wan, 2007), while aqueous extract of the leaves and roots of *Premna cordifolia* can reduce fever and asthma and the young leaves can stimulate milk production in lactating mothers (Mat, 2008). In Malaysia, young leaves of *Pluchea indica* are taken as salad to increase appetite, ease digestion and reduce stomach ache (Wan, 2007). A decoction of *Lactuca sativa* leaves is taken as a diuretic, stomachic to treat bladder problems, constipation, coughs, kidney ailments, urinary problems and used as lotion to treat eye inflammation (Ong, 2008). *Phosphocarpus tetragonolobus* pods are considered roborant (Stopp, 1962); the leaves and seeds are eaten to cure skin sores such as boils and ulcers (Perry, 1980). Continuing our investigation for the search of bioactive plants of Malaysia (Ahmat et al., 2010) we report, another study that evaluated the *in-vitro* antimicrobial and antioxidant activities of six Malaysian *ulam*

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which are *Mentha arvensis* (mint), *L. sativa* var *capitata* (lettuce), *P. tetragonolobus (*winged bean), *P. indica* (beluntas), *P. cordifolia* (bebuas) and *Limnocharis flava* (yellow velvetleaf).

MATERIALS AND METHODS

Plant materials and extraction

Mentha arvensis (mint), *L. sativa* var *capitata* (cabbage lettuce) and *P. tetragonolobus* (winged bean) were collected from Shah Alam, Selangor, Malaysia, while *P. indica L.* (beluntas), *P. cordifolia* (bebuas) and *L. flava L. buchenau* (paku rawan) were obtained from Taiping, Perak, Malaysia. Successive extractions were carried out in dichloromethane (DCM) and ethanol (EtOH). The dry powdered samples (10 g) were macerated with dichloromethane (500ml) for two days. The solvent was then evaporated using the rotary evaporator. This process was repeated for five times. The samples were re-extracted with ethanol (500 ml) for two days and dried in the same manner.

Microorganisms and culture

The extracts were tested against four bacteria namely *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa*, *Streptococcus pyogenes* and a yeast *Candida albicans*. 20 g of nutrient agar powder was dissolved in 1 L of distilled water while 39 g of potatodextrose agar was dissolved in 1 L distilled water. The mixtures were sterilized by autoclaving for 15 min at 121°C and pressure 15 Psi. After sterilization, the agar was poured in sterile Petri dishes. To ensure nothing was grown on the agar, the Petri dishes were left for 3 days before used. The strains were cultured on nutrient agar (NA) for bacteria and on potato dextrose agar (PDA) for yeast. Bacteria strains were incubated at 37°C for 24 h and yeast for 48 h.

Antimicrobial activity by disc diffusion assay

The method of disc diffusion was employed for the determination of antimicrobial activity of the six Malaysian *ulam* extracts. All extracts were dissolved in their solvents (DCM or EtOH) to a final concentration of 1, 5, 10, 20 and 30 mg/ml. For example, to prepare 30 mg/ml concentration, 300 mg of extract was dissolved in 10 ml of the solvent (either dichloromethane or ethanol). Each dissolved extracts were sterilized by filtration with 0.22 µm Millipore filters. Antimicrobial tests were then carried out by disc diffusion method (Murray et al., 1995) using the suspension containing 10^8 CFU ml^{-1} bacteria, 10^6 CFU ml^{-1} and yeast obtained from a 0.5 McFarland turbidity standard spread on nutrient agar (NA) or potato dextrose agar (PDA), respectively. The suspension was standardised by adjusting the optical density to 0.1 at 600 nm (SHIMADZU UV-120-01 spectrophotometer). The discs (6 mm in diameter) were impregnated with 50 µl of five different concentrations of extracts and placed on the inoculated agar. Ethanol acted as the negative control for ethanol extracts while dichloromethane acted as the negative control for dichloromethane extracts. Streptomycin (0.02 g/ml) was used as the positive control to determine the sensitivity of each bacterial species tested. Cycloheximide (100 mg/ml) was used as the positive control for yeast. The inoculated plates were incubated at 37°C for 24 h for bacteria and 48 h for yeast. Antimicrobial activity was evaluated by measuring the zones of inhibition against the test organisms.

Total phenolic contents

The amount of total phenolic contents (TPC) in the extracts was determined according to the Folin-Ciocalteu method. About 0.01 g of gallic acid was prepared in 100 ml volumetric flask and marked up with distilled water (stock solution). Gallic acid standard solution with concentrations of 0.0, 5.0, 10.0, 20.0, 30.0 and 50.0 ppm was prepared.

Sample preparation

Each prepared standard solution was poured into a 10 ml volumetric flask, 1.5 ml of Folin-Ciocalteu reagent was added and allowed to stand at room temperature for 3 to 8 min. 1.5 ml of Na_2CO_3 solution was added, shaken and allowed to stand for 2 h at room temperature. Absorbance at 760 nm was measured after the reading was auto zeroed with the blank solution.

About 0.01 g each of plant extracts was dissolved in 2 ml solvent. The plant extract (0.01 ml) was mixed with 2.5 ml Folin-Ciocalteu reagent (diluted 10 times with water) and incubated at room temperature for 3 min followed by the addition of 2 ml of Na_2CO_3 (75 g/l) to the mixture. The sample was incubated for 2 h before total polyphenols was determined. For the control sample, 0.5 ml of the distilled water was used. The absorbance was measured at 760 nm. The results were expressed in milligram of gallic acid per 100 g dry matter (mg GA / 100 g Dwt) or in milligram of gallic acid per 100 g of fresh weight (mg GA / 100 g Fwt).

The content of phenolic compounds in the sample extract was determined from the regression equation of calibration curve of a standard gallic acid:

$$Y = 0.013x + 0.058, \quad R^2 = 0.999$$

Free radical scavenging activity

The DPPH radical-scavenging activity was determined by using the method proposed by Yamaguchi et al. (1998) with some modification. The positive control was prepared by adding 4 ml of quercetin (0.05 mg/ml) to 1 ml of DPPH (0.4 mg/ml), whereas the negative control was prepared by each solvents used and 1 ml of DPPH solution. The radicals' stock solution was prepared fresh daily. The DPPH solution (1 ml) was added to 3 ml of polyphenol extracts with different concentrations (200, 400, 600, 800, 1000 and 1200 ppm). The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 10 min. The decrease in absorbance of the resulting solution was monitored at 517 nm for 10 min. Lower absorbance of the reaction mixture indicates higher free radical-scavenging activity. Radical scavenging activity is expressed as the inhibition percentage (IP) and was calculated as follows:

$$IP = (A_{control} - A_{sample}) \times \frac{100\%}{A_{control}}$$

The decolouration was plotted against the sample extract concentration and a logarithmic regression curve was established in order to calculated the IC_{50} (inhibitory concentration 50), which is the amount of sample extract concentration necessary to decrease by 50% the absorbance of DPPH.

RESULTS

Antimicrobial activity

Six types of *ulam* typically eaten by Malaysians have

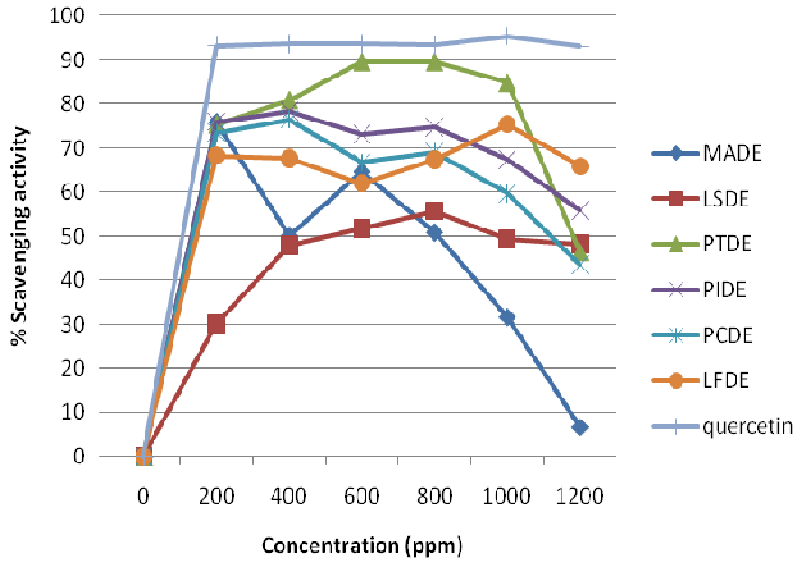

Figure 1. Radical scavenging activity of dichloromethane extract of the six Malaysian's *'Ulam'*.

been investigated for the presence of antimicrobial activity. Each plant was extracted with dichloromethane (DCM) and ethanol (EtOH) successively (Figures 1 and 2).

The DCM extracts of the six plants were found to show selective inhibition against the bacteria as shown in Table 1. *Limnocharis flava* L. buchenau dichloromethane extract (LFDE) was found to inhibit skin pathogen microbes such as *S. aureus, P. aeruginosa* and *S. pyogenes*. The zones of inhibition were not dependent on the concentration of extract. Inhibition of LFDE at 5 mg/ml against *S. pyogenes* was comparable to streptomycin (positive control) and showed moderate inhibition against *S. pyogenes* even at low concentration of 1 mg/ml. *Mentha arvensis* dichloromethane extract (MADE) and *Lactuca sativa* var *capitata* dichloromethane extract (LSDE) displayed weak inhibition against *P. aeruginosa* at all concentrations. It is probably because maximum inhibition was achieved that led to non-increase of the inhibition even though higher concentration was applied. All DCM plant extracts did not display any inhibition against *E. coli* and yeast *C. albicans*.

The inhibition of EtOH extracts of the six *ulam* are displayed in Table 2. *Limnocharis flava* L. buchenau ethanol extract (LFEE) was able to weakly inhibit the three types of bacteria namely *S. aureus*, *P. aeruginosa, S. pyogenes* and a yeast *C. albicans* at higher concentrations of 20 and 30 mg/ml. *Premna cordifolia* ethanol extract (PCEE) displayed weak inhibition against three microbes; *S. aureus, P. aeruginosa* and *S. pyogenes. Mentha arvensis* ethanol extract (MAEE) selectively inhibited *P. aeruginosa* at all concentrations, while *Psophocarpus tetragonolobus* ethanol extract (PTEE) selectively inhibited *S. aureus* weakly at 20 and 30 mg/ml.

Total phenolic contents

The antioxidant activity of vegetable extracts has been correlated to their content of phenolic components (Velioglu et al.,1998) due to their property of scavenging free radicals. Therefore, it is important to consider the effect of the total phenolic quantity in the antioxidant activity of some selected Malaysian *'ulam'* extracts. The TPC was expressed in equivalent of gallic acid (µg/mg of extract) and the results for the plant extracts are presented in Table 3.

Based on the data collected, plants extracted in EtOH gave higher and persistant results compared to those extracted with DCM. This behavior is probably due to the EtOH capacity to solubilize flavonoid components from the samples; substances detected by the Folin-Ciocalteu method. *P. indica* gave a higher value among the other samples (56.287 µg/mg), followed by *P. tetragonolobus* (42.760 µg/mg), *L. sativa* (40.717 µg/mg), *M. arvensis* (39.648 µg/mg), and *P. cordifolia* (16.370 µg/mg). *L. flava* showed the lowest content of phenolic compounds (15.210 µg/mg).

Furthermore, in DCM extract, the higher value of TPC was obtained by *L. flava* (51.307 µg/mg), followed by *L. sativa* (23.923 µg/mg), *M. arvensis* (17.307 µg/mg), *P. tetragonolobus* (9.123 µg/mg) and *P. indica* (7.0769 µg/mg). *P. cordifolia* gave the lowest value which was 4.6461 µg/mg.

DPPH radical scavenging activity

The results showed that *P. tetragonolobus* exhibited the highest radical scavenging activity compared to the other

Table 1. Zone of inhibition of the dichloromethane extracts of Malaysian Ulams against bacteria and yeast.

Microorganisms	Dosage (mg/ml)	*Mentha arvensis* (MADE)	*Lactuca sativa* var *capitata* (LSDE)	*Psophocarpus tetragonolobus* (PTDE)	*Pluchea indica* (PIDE)	*Premna cordifolia* (PCDE)	*Limnocharis flava* L. buchenau (LFDE)
		Inhibition (mm)					
E. coli	30	-	-	-	-	-	-
	20	-	-	-	-	-	-
	10	-	-	-	-	-	-
	5	-	-	-	-	-	-
	1	-	-	-	-	-	-
	Positive control	18	18	18	18	18	18
S. aureus	30	-	8±0.62	-	-	-	8±0.58
	20	-	11±1.00	-	-	-	11±0.58
	10	-	11±1.53	-	-	-	10±0.00
	5	-	-	-	-	-	10±1.54
	1	-	11±0.58	-	-	-	9±0.58
	Positive control	18	18	18	18	18	18
P. aeruginosa	30	6±0.00		-	-	-	8±1.00
	20	6±0.00		-	-	-	8±0.58
	10	6±0.00		-	-	-	7±1.00
	5	6±0.00		-	-	9	6±0.00
	1	6±0.00		-	-	-	6±0.00
	Positive control	18	18	18	18	18	18
S. pyogenes	30	-	-	-	-	-	9±0.58
	20	-	-	-	-	-	9±2.20
	10	-	-	-	-	-	-
	5	-	-	-	-	-	14±2.02
	1	-	-	-	-	-	12±2.00
	Positive control	14	14	14	14	14	14
C. albicans	30	-	-	-	-	-	-
	20	-	-	-	-	-	-
	10	-	-	-	-	-	-
	5	-	-	-	-	-	-
	1	-	-	-	-	-	-
	Positive control	18	18	18	18	18	18

Zone of inhibition: 15 – 20 mm: strong inhibition , 10 – 14 mm: moderate inhibition, 0 – 9 mm : weak inhibition
- : No inhibition
Data are: zone of inhibition ± standard deviation of triplicate readings.

plants. It can be seen in Figure 2 that PTDE and PTEE possessed higher percentage of radical scavenging activity compared to the other extracts. PTDE showed high scavenging activity at 600 and 800 ppm, while PTEE showed high scavenging activity at 1000 ppm. At these points, the scavenging activity of these extracts was just slightly less than the positive control, quercetin which supported the claim by Mustafa et al. (2010) that *P. tetragonolobus* methanol extract contains a high amount of quercetin.

M. arvensis displayed good scavenging activity in ethanol and DCM extracts at 200 ppm with 79.4 and 75.8% scavenging activity, respectively. *L. sativa* showed moderate scavenging activity where the LSDE and LSEE were just around 40 to 55%. *P. indica* dichloromethane extract (PIDE) showed good scavenging activity at 400

Table 2: Zone of inhibition of the ethanol extracts of Malaysian ulams against bacteria and yeast.

Microorganisms	Dosage (mg/ml)	*Mentha arvensis* (MAEE)	*Lactuca sativa* var *capitata* (LSEE)	*Psophocarpus tetragonolobus* (PTEE)	*Pluchea indica* (PIEE)	*Premna cordifolia* (PCEE)	*Limnocharis flava* L. buchenau (LFEE)
		Inhibition (mm)					
E. coli	30	-	-	-	-	-	-
	20	-	-	-	-	-	-
	10	-	-	-	-	-	-
	5	-	-	-	-	-	-
	1	-	-	-	-	-	-
	Positive control	18	18	18	18	18	18
S. aureus	30	-	-	8±0.58	-	6±0.58	7±0.58
	20	-	-	7±1.00	-	7±0.58	6±0.58
	10	-	-	-	-	6±0.58	-
	5	-	-	-	-	-	-
	1	-	-	-	-	-	-
	Positive control	18	18	18	18	18	18
P. aeruginosa	30	8±0.58	-	-	11±2.02	9±1.00	9±0.58
	20	9±1.00	-	-	8±1.15	7±0.58	9±0.58
	10	9±0.58	-	-	-	6±1.00	-
	5	9±0.58	-	-	-	-	-
	1	7±0.58	-	-	-	-	-
	Positive control	18±0.58	18	18	18	18	18
S. pyogenes	30	-	6±0.00	-	6±0.00	6±0.00	6±0.00
	20	-	-	-	6±0.00	6±0.00	6±0.00
	10	-	-	-	6±0.00	-	-
	5	-	-	-	-	-	-
	1	-	-	-	-	-	-
	Positive control	14	14	14	14	14	14
C. albicans	30	-	-	-	-	-	6±0.58
	20	-	-	-	-	-	6±0.58
	10	-	-	-	-	-	-
	5	-	-	-	-	-	-
	1	-	-	-	-	-	-
	Positive control	18	18	18	18m	18	18

Zone of inhibition: 15 – 20 mm: strong inhibition , 10 – 14 mm: moderate inhibition, 0 – 9 mm : weak inhibition
- : No inhibition
Data are: zone of inhibition ± standard deviation of triplicate readings.

ppm (78.4%), but PIEE was a poor radical scavenger with activity below 30% at all concentrations. Premna cordifolia (PCDE) also exhibited good scavenging activity at 400 ppm, while PCEE showed moderate activity at all concentrations, in the range of 30 to 60%. L. flava showed different patterns of activity compared to other plant extracts in which it exhibited better scavenging activity with increasing concentration.

DISCUSSION

Antimicrobial activity

Based on the results, DCM extracts and EtOH extracts gave different pattern of activity. In the DCM extract, *P. indica* did not inhibit the bacteria compared to EtOH extract; *P. indica* can inhibit *S. pyogenes* and *P.*

Table 3. Total phenolic content of six selected table salad in DCM and EtOH.

Sample	TPC in DCM (µg/mg)	TPC in EtOH (µg/mg)
M. arvensis	17.31 ± 0.01	39.65 ± 0.10
L. sativa	23.92 ± 0.03	40.72 ± 0.12
P. tetragonolobus	9.12 ± 0.04	42.76 ± 0.11
P. cordifolia	4.65 ± 0.07	16.37 ± 0.15
P. indica	7.08 ± 0.02	56.29 ± 0.08
L. flava	51.31 ± 0.03	15.21± 0.08

Data are ± standard deviation of triplicate readings.

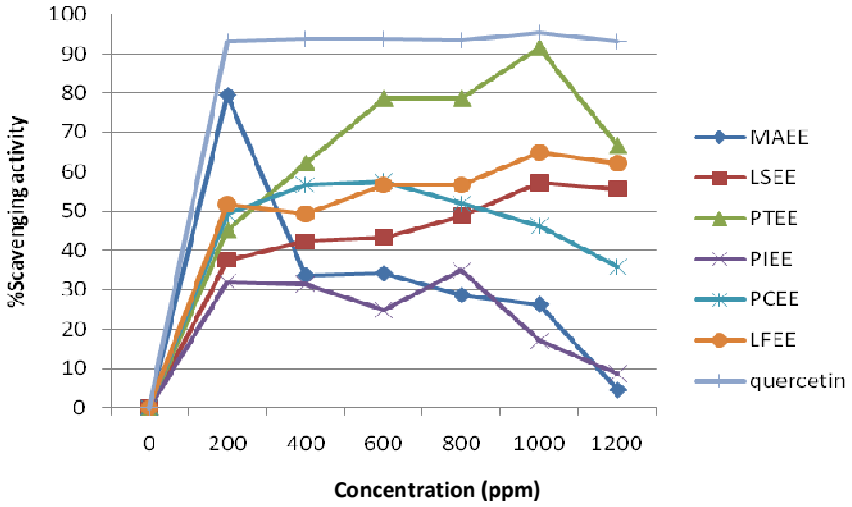

Figure 2. Radical scavenging activity of ethanolic extracts of the six Malaysians '*Ulam*'

aeruginosa. Sittiwet (2009) reported that *P. indica* aqueous extract possess antimicrobial activity against *E. coli* at 25 to 100 mg/ml and *K. pneumoniae* at 50 to 100 mg/ml. However, our ethanol extract of this plant did not inhibit *E. coli*, but was able to inhibit skin pathogen micro organism namely P. aeruginosa (moderate inhibition) and S. pyogenes (weak inhibition). The difference in activity between the two studies may be due to the different solvent used in the extraction as well as soil and weather conditions of the plant origin. It is also well established that solvents with different polarity will extract compounds similar to its polarity. This is supported by Freiburghans (1996) that different solvent extracts of the same plant may exhibit different pharmacological properties.

E. coli, S. aureus, P. aeruginosa, S. pyogenes and *C. albicans* strains exhibited varying sensitivities to the 12 extracts studied. Among the five microorganisms tested, *P. aeruginosa* was the most sensitive, while *E. coli* was the most resistant. *E. coli* was also reported resistant against *Orthosiphon stamineus* Benth. and *C. macropodium* extracts (Ho et al., 2010; Ebrahimadi et al., 2010), but sensitive to the essential oil of *Chaerophyllum macropodum* and *Zataria multiflora* (Ebrahimadi et al., 2010; Sharififar et al., 2007).

Bushra and Ganga (2003) stated that when solvents like ethanol, hexane and methanol are used to extract plants, most of them are found able to exhibit inhibitory effect on Gram-positive and Gram-negative bacteria. Nearly all of the identified compounds from plants that are active against microorganisms are aromatic or saturated organic compounds, which are mostly obtained through ethanolic or methanolic extraction (Nostro et al., 2000). It was clearly demonstrated in this study, that a plant can give different antimicrobial profiles when extracted by different solvents. This may possibly be due to the different active compounds extracted in different solvents. Antibacterial effects of PCEE, LFDE and LFEE on *S. aureus*, *S. pyogenes* and *P. aeruginosa* showed that the plants can be used in the treatment of gastrointestinal infection in man and skin diseases as suggested by Roggers et al. (1990). These extracts can also be used in the treatment of boils, sores and wounds, since *S. aureus* and *P. aeruginosa* have been implicated as causative agents of these diseases (Braude, 1982).

Latha et al. (2007) stated that *P. tetragonolobus* ethanolic extract can inhibit *S. aureus, E. coli, P. aeruginosa* and *C. albicans* but at a higher concentration than the concentration applied in this study. This suggests that inhibition might occur at higher concentrations. However, only LFEE was found to be able to inhibit *C. albicans* growth and none of the plant extracts was able to inhibit the growth of *E. coli*.

Correlation between total phenolic content and free radical scavenging activity

The correlation between the total content of phenolics and the antioxidant capacity were studied by many authors. Several studies established a linear correlation between the total content of phenolics and the antioxidant capacity (Cai et al., 2004) whereas, some studies reported that there was no correlation (Yu et al., 2002). Based on this study, we found that there was no correlation between phenolic content of the *ulam* extracts with their antioxidant activity. *P. indica* ethanolic extract had the highest phenolic contents but poor radical scavenging activity, while the dichloromethane extract of *P. tetragonolobus* on the other hand, had low phenolic content but exhibited good antioxidant activity. A lot of reports declared that organic solvents either used in single or mixed forms especially polar ones are most preferable for extraction of biologically active plant ingredients (De Pasquale et al., 1995: Ferrero et al., 2007).

Methanol and ethanol are reported as efficient extracting solvents for saponins and sterols (De Pasquale et al., 1995: Hui et al., 2007), alkaloids (Ivanovska et al., 1996), polyphenols (Ferrero et al., 2007) and terpenoids (Taylor et al., 1996), while DCM is efficient in terpenoids extraction (Mendoza et al., 1997: Cowan, 1999).

Conclusion

In conclusion, a plant displays different antimicrobial and antioxidant profiles when extracted in different solvents. Of all the six plants tested, *L. flava* L. *buchenau* was able to inhibit moderately and weakly three bacteria; *S. aureus*, *P. aeruginosa* and *S. pyogenes.* Furthermore, only this plant could inhibit the growth of *C. albicans.* As for the radical scavenging activity, *P. tetragonolobus* showed the best activity among all the extracts. This study showed that the six investigated *ulam* normally eaten by the Malaysians contain antimicrobial and antioxidant properties. These *ulam* can also be uses as antioxidant and antimicrobial agents in the development of drugs of therapy for infection diseases especially skin diseases since most of the extracts could inhibit skin pathogens.

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