Journal of Medicinal Plants Research Vol. 5(21), pp. 5284-5290, 9 October, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Antioxidant, antibacterial and cytotoxic activities of essential oils and ethanol extracts of selected South East Asian herbs

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Accepted 29 August, 2011

Antioxidant, antibacterial and cytotoxic activities of essential oils and ethanol extracts of four South East Asian traditional herbs were investigated. Their essential oils were analyzed by Gas chromatography mass spectrometry (GC-MS) and a total of 57 types of volatile organics were identified. *Artemisia argyi, Centella asiatica, Cosmos caudatus,* and *Polygonum hydropiper* contained 30, 20, 19 and 21 types of volatiles, respectively. The major volatile hydrocarbon for *A. argyi, C. asiatica* and *C. caudatus* was α-cadinene while dodecanal was the major component for *P. hydropiper*. Total phenolic content of ethanol extracts analyzed by Folin-Ciocalteu method were in the range of 31.58±3.08 to 84.03±8.15 mg GAE/100 g. Free radical-scavenging activity of the four ethanol extracts were in the range of 654.43±17.22 to 5857.54±164.13 mg AA/100 g; while ferric-reducing antioxidant power were in the range of 26.58±1.10 to 50.08±0.71 mg AAE/g. Essential oils of all four species were inactive for both these assays. Ethanol extracts of these herbs were active against 60 to 80% of ten strains of human pathogenic bacteria tested, compared to 20 to 50% inhibition for essential oils. Only ethanol extracts of *A. argyi* and *C. caudatus* showed activity against P388 murine leukemia cell line, while all the essential oils were inactive.

Key words: Antimicrobial, antioxidant, cytotoxicity, ethanol extracts, essential oils, total phenolic content.

INTRODUCTION

Aromatic herbs have long been part of the South East Asian cultures as they are consumed regularly as part of the diet. These herbs are eaten raw as salad, or used in cooking to flavor the dishes. Some of these plants have been used as folk remedies for the treatment of ailments such as diabetes, high blood pressure, arthritis and fever, as well as health tonic (Ong and Norzalina, 1999). It is now known that these plants contain a wide variety of biologically active phytochemicals constituents.

Based on available reports, ethanol extracts of *Anacardium occidentale, Garcinia atroviridis, Averrhoa bilimbi, Polygonum minus, Diplazium esculentum* and *Etlingera elatior* are known to inhibit pathogenic microorganisms. In addition, *A. occidentale, G. atroviridis, P. minus*, *E. elatior, Sesbania grandiflora, Barringtonia racemosa* and *Kaempferia galanga* are also known to display cytotoxic activity against human cervical carcinoma (HeLa) cell line (Mackeen et al., 1997).

Antioxidant properties of traditional vegetables reported by Faridah et al. (2006) described the methanolic extract of *A. bilimbi, Poortulaca oleracea, S. nigrum, Persicaria tenella* (= *Polygonum hydropiper*), *Cosmos caudatus, Curcuma mangga, Ocimum basilicum, A. occidentale and Melicope ptelefolia* to exhibit potent antioxidant activity. In addition, *M. ptelefolia, P. oleraceae* and *P. tenella* were also shown to inhibit nitric oxide production in murine peritoneal macrophages *in vitro*. In another study, Wong et al. (2006) evaluated the antioxidant activities of aqueous extracts of tropical plants which include *Centella asiatica, Piper betel, Sauropus androgyus, Coriandrum sativum, Eugenia polyantha, Polygonum hydropiper, O. basilicum and C. caudatus* that are widely consumed in the Southeast Asian region. Antioxidant activity of ethanol and water extracts of *C. asiatica* had also been evaluated

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in a separate study by Abdul et al. (2002).

However, there is still limited information pertaining to the chemical constituents of essential oil and its associated properties from the herbs investigated in this report. Therefore, we studied the biological activities of the essential oils as well as the ethanol extracts of four species o f traditional herbs *A. argyi*, *C. asiatica*, *C:. caudatus* and *P. hydropiper*.

MATERIALS AND METHODS

Plant materials

Samples of *P. hydropiper*, *C. caudatus*, *C. asiatica* and *A. argyi* were obtained fresh from local farmer at Kota Belut, Kota Kinabalu, Sabah, Malaysia. Plants were identified by comparison with herbariums available at BORNEENSIS at Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah. Upon arrival at the laboratory, the plants were rinsed, air-dried and kept at 4°C prior to extraction.

Extraction and analysis of essential oil

Whole plants were cut into small pieces and hydrodistillated for 8 h, using a Clevenger-type apparatus to obtain the essential oils. The oils were dried over anhydrous sodium sulphate and stored under N_2 at -20°C until analyzed. The analysis of essential oils was carried out on a Shimadzu GC-2010 gas chromatographer equipped with a SGE BPX-5 capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm), coupled to a GCMS-QP2010 PLUS gas chromatography mass spectrometer. Electron ionization energy was 70 eV.

Helium gas was used as carrier gas at a flow rate of 27.7 ml/min. Injector and MS transfer line temperatures were set at 200 and 280°C, respectively. Oven temperature was increased gradually from 50 for to 300°C at 3°C/min and held at this temperature for 5 min. Diluted samples (2% w/v, in *n*-hexane) of 1 µl were injected by autosampler AOC 5000. The main constituents of essential oils were identified by comparison of their retention indices (RI) to those stored in FFNSC 1.2, NIST08 and NIST08s Libraries. The retention indices were determined in relation to a homologous series of n-alkanes (C_8 to C_{40}) run under the same operating conditions. Component relative concentrations were calculated based on GC peak area with correction factors.

Extraction of ethanol crude and determination of total phenolic content

Whole plants were cut into small pieces, weighed and extracted with ethanol in a Soxhlet apparatus for 24 h over duration of 3 days. The ethanol extracts were evaporated in vacuo and stored under N_2 at -20 °C until tested.

Total phenolic content (TPC) of the extracts was determined using the Folin-Ciocalteu assay as described by Chan et al. (2007) with slight modifications. A total of 100 µl of Folin-Ciocalteu reagent (10% v/v) was added to 20 µl of extracts (1 mg/ml methanol) in a 96 wells microplate, followed by 80 µl sodium bicarbonate (7.5% w/v) after 5 min.

Absorbance was measured on a Tecan Infinite M200 microplate reader at 765 nm after 30 min. All analyses were carried out in triplicate. Total phenolic concentration was expressed as gallic acid equivalents (GAE) in mg/100 g. The calibration equation for gallic acid was $y = 6.2511x + 0.015$ ($r^2 = 0.9998$).

Antioxidant assays

Free radical-scavenging activity (DPPH assay)

The free radical-scavenging activity of the extracts was determined by the DPPH assay as described by Chan et al. (2007) with modifications. A total of 50 µl of different dilutions (0.2 to 2.0 mg/ml) of extract were added to 100 µl of DPPH (5.9 mg/100 ml methanol) in a 96 wells microplate. The mixtures were vortex vigorously and left at room temperature in the dark. Absorbance was measured at 517 nm after 30 min with a Tecan Infinite M200 microplate reader. All analyses were carried out in triplicate. Free radical-scavenging activity was calculated as IC_{50} and expressed as ascorbic acid equivalent antioxidant capacity (AEAC) in mg AA/100 g as given by the equation as (The IC_{50} of ascorbic acid used for the calculation of AEAC was 0.0107 mg/ml):

AEAC (mg AA/100 g) = IC_{50} (ascorbate) / IC_{50} (extract) × 100,000

Ferric-reducing antioxidant power (FRAP assay)

The ferric-reducing antioxidant power (FRAP) assay as described by Chan et al. (2007) was adopted with modifications. 200 µl of different dilutions of extract (2 to 20 mg/ml) were added to 500 µl phosphate buffer (0.2 M, pH 6.6) and 500 µl potassium ferricyanide (1% w/v). The mixtures were incubated at 50°C for 20 min. 500 µl trichloroacetic acid (10% w/v) was added to stop the reaction. Each mixture was then separated into aliquots of 500 µl and diluted with 500 µl of distilled water. To each diluted aliquot, 100 µl ferric chloride (0.1% w/v) was added. After 30 min, 100 µl of each mixture was loaded on a 96 well microplate and absorbance was measured at 700 nm a Tecan Infinite M200 microplate reader. FRAP of extracts was expressed as mg AAE/g. The calibration equation for ascorbic acid was $y = 1.6175x + 0.0151$ ($r^2 = 0.9991$).

Antimicrobial assay

Antibacterial assay was performed via disc diffusion method against 10 strains of food pathogenic bacteria: *Pseudomonas aeruginosa* (HP0108), *Salmonella* sp. (HP0208), *Proteus mirabilis* (HP0308), *Escherichia coli* (HP0408), *Salmonella typhi* (HP0508), *Salmonella enteritidis* (HP0608), *Salmonella thyphimunium* (HP0708), *Staphylococcus aureus* (HP0808), *Listeria monocytogenes* (HP0908) and *Vibrio cholerae* (HP1008). These bacteria were obtained from Department of Pathology, Queen Elizabeth Hospital, Kota Kinabalu, Sabah, Malaysia.

One loopful of bacteria was pre-cultured in Muller Hinton broth for 24 h and turbidity of culture was adjusted to optical density of McFarland 0.5. Then, 0.1 ml of pre-cultured bacterial suspension was seeded on nutrient agar plates. Paper discs impregnated with 500 µg/disc extracts were placed on the seeded agar plates, and the diameters of inhibition zone were measured after 24 h incubation at 30°C. Commercial antibiotics, streptomycin (Merck, Germany) were used as a positive control.

Cytotoxicity assay against P388 murine leukemia cells

Murine leukemia cells (P388) were cultured in RPMI-1640 medium containing 10% fetal bovine serum, 100 µg/ml kanamycin, and 10 µg/ml 2-hydroxyethyl disulfide at 37°C at 5% CO_2. A total of 100 µl test solution dissolved in RPMI-1640 medium was added to each well of the 96-well microplate containing 100 µl tumor cell suspension (1×10^4 cells/ml) and incubated at 37°C for 96 h. Then, 50 µl of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium

bromide (MTT) saline solution (1 mg/ml) was added to each well and incubated for 3 h under the same condition in order to stain live cells. Upon incubation, the plate was centrifuged and the supernatant removed and cells mixed with 150 µl of DMSO to determine the IC_{50} values.

RESULTS AND DISCUSSION

Essential oil composition analysis

Yields of essential oils based on fresh weight were 0.08, 0.04, 0.08 and 0.07% for *A. argyi*, *C. asiatica*, *C. caudatus* and *P. hydropiper*, respectively. A total of 57 types of volatile organics were extracted and identified *via* GCMS. Distribution of the volatiles varied with species of herb analyzed. Relative abundance of the volatiles identified in the herbs were in the following order; *A. argyi* (30)> *P. hydropiper* (21)>*C. asiatica* (20)>*C. caudatus* (19). The main constituents of the essential oils are shown in Table 1. Major compounds for *A. argyi* were γ-cadinene (12.51%), caryophyllene (10.13%), δ-elemene (7.41%), phytol (6.85%) and β-elemene (5.52%); *C. asiatica*: γ-cadinene (26.44%), β-farnesene (15.19%), caryophyllene (10.02%), α-humulene (9.20%) and neophytadiene (5.15%); *C. caudatus*: γ-cadinene (33.29%), caryophyllene (9.73%), α-farnesene (6.06%) and (*E*)-ocimene (5.64%); *P. hydropiper*: dodecanal (29.90%), drimenol (11.33%), caryophyllene (7.39%), neoisoligifolene (7.18%) and decanal (5.99%). γ-Cadinene was the most abundant compound for *A. argyi*, *C. asiatica* and *C. caudatus*. All four species were found to have caryophyllene and α-humulene in common. Zheng et al. (2004) identified 36 compounds from China's *A. argyi.* Germacrene D, α-phellandrene and α-mycrene were the major compounds. As for *C. asiatica,* Ali (2008) reported 23 compounds in this plant from Peninsular Malaysia, with α-cubebene, α-caryophyllene, α-humulene, and δ-muurolene as the predominant constituents. In another study, the similar plant growing in South Africa was reported to contain α-humulene, β-caryophyllene, bicyclogermacrene, germacrene B and myrcene as the major constituents (Oyedeji and Afolayan, 2005). Recently, Wongfun et al. (2010) reported the main component of the plant from Thailand to be humulene, β-caryophyllene, α-copaene, β-farnesene, β-elemene and alloaromadendrene. To our best knowledge, there has been no report on the essential oil from *C. caudatus* and *P. hydropiper* and the findings here are the first for these two species.

Yield of ethanol extracts and total phenolic content

Yields of ethanol extracts based on fresh weight were 2.07, 2.48, 2.00 and 1.47% for *A. argyi*, *C. asiatica*, *C. caudatus* and *P. hydropiper*, respectively. Total phenolic content (TPC) of the extracts were determined by Folin-Ciocalteu method and expressed in mg GAE/100 g. Total phenolic content is higher in *C. caudatus* (84.03 ± 8.15 mg GAE/100 g) and *P. hydropiper* (74.84 ± 7.04 mg GAE/100 g) as compared to *A. argyi* (39.13 ± 2.48 mg GAE/100 g) and *C. asiatica* (31.58 ± 3.08 mg GAE/100 g). Huda-Faujan et al. (2007) reported total phenolic content based on fresh weight of *C. caudatus*, *Polygonum minus* and *C. asiatica* to be 21.41 mg TAE/100 g, 44.35 mg TAE/100 g and 3.72 mg/100 g, respectively.

Antioxidant activity

Antioxidant activities of the essential oils and ethanol extracts were evaluated by DPPH and FRAP assays. However, only the ethanol extracts exhibited activity and the results are shown in Table 2. In DPPH assay, *P. hydropiper* showed highest activity (5867.54 ± 164.13 mg AA/100 g) followed by *C. caudatus* (3200.37 ± 54.11 mg AA/100 g), *C. asiatica* (717.68 ± 46.46 mg AA/100 g) and *A. argyi* (654.43 ± 17.22 mg AA/100 g). Whereas in FRAP assay, *C. caudatus* exhibited highest activity (50.08 ± 0.71 mg AAE/g), followed by *P. hydropiper* (39.31 ± 0.51 mg AAE/g), *A. argyi* (36.99 ± 1.20 mg AAE/g) and *C. asiatica* (26.58 ± 1.10 mg AAE/g). Similar trend of antioxidant activity for extracts of *C. caudatus* and *P. hydropiper* had been observed by researchers who had used other assays (Faridah et al., 2006; Wong et al., 2006). The potent antioxidant activities in few of these herbs could be associated with the presence of flavonoids and phenolics in these plants, as suggested by various researchers. (Faridah et al., 2003; Peng et al., 2003; Yagi et al., 1994). Correlation analysis showed that TPC correlated well with FRAP (r = 0.96) and free radical scavenging activity (r = 0.90). Our findings agree with the widely accepted idea that antioxidant activities of botanical extracts are contributed by polyphenols (Wong et al., 2006).

Antibacterial and cytotoxic activities

Result of disc diffusion antimicrobial assay is shown in Table 3. Ethanol extracts of these herbs exhibited broad activity, with inhibition against 60 to 80% of the ten strains of tested bacteria as compared to 20 to 50% by its essential oils. Essential oils of all four species exhibited moderate inhibition against *Salmonella* sp. and *V. cholerae.* Essential oil of *P. hydropiper* was also active against *P. aeruginosa, E. coli* and *S. enteritidis*. It was reported that oxygenated monoterpenes such as camphor, terpinen-4-ol, linalool, α-terpineol and borneol, which are representative components in some of the oils investigated as being responsible for antimicrobial activities (Lopes-Lutz et al., 2008). On the other hand, all four ethanol extracts showed activity against

Table 1. Constituents of essential oils of *Artemisia argyi*, *Centella asiatica*, *Cosmos caudatus* and *Polygonum hydropiper*.

RT (min)	RI	Component	Relative concentration (%)			
			Aa	***Ca***	***Cc***	***Ph***
13.21	1046[a]	(*E*)-Ocimene			5.64	
17.21	966[b]	2,6-Dimethyl-1,3,5,7-octatetraene			4.69	
18.26	1149[a]	Camphor	1.44			
19.36	1088[b]	Borneol	2.46			
19.69	1137[b]	(-)-Terpen-4-ol	0.40			
20.43	1145[b]	α-Terpineol	0.62			
20.83	1204[c]	Decanal				5.99
22.59	1200[a]	γ-Terpineol	2.70			
23.79	1278[a]	Decanol				0.73
24.36	1285[a]	Borneol acetate	0.44			
25.46	1309[a]	Hendecanal				0.44
25.56	1128[c]	7-Oxabicyclo[4.1.0]heptane	0.96			
26.46	1335[a]	δ-Elemene	7.41			
28.32	1375[a]	α-Copaene	0.57	1.37	0.84	
28.92	1390[a]	β-Elemene	5.52	1.29	3.30	
29.96	1410[a]	Dodecanal				29.90
30.29	1494[b]	Caryophyllene	10.13	10.02	9.73	7.39
30.73	1432[a]	α-Bergamotene				0.79
31.53	1452[a]	β-Farnesene	1.05	15.19		0.84
31.83	1454[a]	α-Humulene	4.61	9.20	2.28	3.00
32.02	1492[a]	Valencene		4.29		
32.51	1502[b]	2-Isopropenyl-4a,8-dimethyl 1,2,3,4,41,5,6,7octahydronaphthalene	1.53			
32.59	1476[a]	Dodecanol				2.59
32.64	1435[c]	γ-Muurolene		0.97	0.75	
32.94	1512[a]	γ-Cadinene	12.51	26.44	33.29	
33.10	1449[a]	α-Himachalene	0.80			
33.14	1432[a]	Bergamotene			2.92	
33.28	1487[a]	β-Selinene	0.38		0.57	0.51
33.52	1497[a]	Bicyclogermacrene	2.82	2.85	3.17	
33.70	1504[a]	α-Farnesene	0.58		6.06	
34.02	1570[b]	8-Isopropenyl-1,5-dimethyl-cyclodeca-1,5-diene	1.74	0.44		
34.38	1518[a]	δ-Cadinene	1.08	1.21	2.52	
34.54	1507b	Eudesma-3,7(11)-diene				0.47

Table 1. Contd.

34.59	1523[a]	β-Sesquiphillandrene	5.11			
35.42	1754[b]	Nerolidyl acetate				1.01
36.02	1562[a]	Nerolidol	0.56	0.61		0.58
36.74	1456[b]	Butanedioic acid, methyl- bis(1-methylpropyl) ester		0.27	0.49	0.50
36.83	1660[b]	Germacrene D-4-ol	0.71			
36.93	1536[b]	(-)-Spathulenol			0.61	
37.11	1573[a]	Tridec-2(E)-enal		0.48		
37.14	1587[a]	Caryophyllene oxide			3.22	0.83
38.01	1530[c]	Ledol		0.28		
38.16	1601[b]	Tetradecanal				0.48
38.22	1688[a]	α-Bisabolol	0.47			
38.80	1594[a]	Viridiflorol	0.52			
38.90	1576[a]	Spathulenol	0.86			
39.42	1641[a]	α-Muurolol		0.23	0.54	
39.45	1673[b]	Neoisolongifolene				7.18
39.97	1669[a]	α-Cadinol	1.04	0.74	2.21	
40.14	1647[b]	Eudesm-7(11)-en-4-ol	0.65			
44.36	1769[a]	Drimenol				11.33
45.96	1836[a]	Neophytadiene		5.15		
46.82	2045[b]	3,7,11,15-tetramethyl-2-hexadecen-1-ol		0.40		
47.44	1763[b]	E-10-pentadecenol		0.20		
50.70	1807[b]	Drimenin				0.69
52.45	1763[b]	Acetic acid				3.22
54.94	2106[a]	Phytol	6.85		2.02	1.57
		Total	76.52	81.63	84.85	80.04

Aa: *Artemisia argyi*, *Ca*: *Centella asiatica*, *Cc*: *Cosmos caudatus*, *Ph*: *Polygonum hydropiper*. [a] FFCNS 1.2, [b] NIST08, [c] NIST08s.

P. mirabilis, *S. thyphimunium*, *S. aureus* and *L. monocytogenes*. In particular, ethanol extract of *C. caudatus* showed strong inhibition against *S. thyphimunium*. The bacterium was also moderately inhibited by the extracts of *A. argyi*. However, essential oils and ethanol extracts of all four species were inactive against *S. typhi*. Based on the available information, Mackeen et al. (1997) reported ethanol extracts of *P. minus* and five other herbs showed antimicrobial activity with minimum inhibitory concentration values in the range of 100 to 800 µg/ml. Further research on some of these herbs also reported cytotoxic activity of *P. minus* and six other herbal extracts to inhibit HeLa cell line with CD50 values of 10 to 30 µg/ml. In the present study, ethanol extracts of *A. argyi* and *C. caudatus* showed weak cytotoxic activity against P388 murine leukemia cells with IC50 values of 50 and 25 µg/ml, respectively. While, the extracts of *P. hydropiper* and *C. asiatica*, as well as the essential oils of all four

Table 2. Total phenolic content (TPC), ascorbic acid equivalent antioxidant capacity (AEAC), and ferric-reducing antioxidant power (FRAP) of four species of herbs.

Species	TPC (mg GAE/100 g)	Antioxidant activity	
		AEAC (mg AA/100 g)	FRAP (mg AAE/g)
Artemisia argyi	39.13 ± 2.48	654.43 ± 17.22	36.99 ± 1.20
Centella asiatica	31.58 ± 3.08	717.68 ± 46.46	26.58 ± 1.10
Cosmos caudatus	84.03 ± 8.15	3200.37 ± 54.11	50.08 ± 0.71
Polygonum hydropiper	74.84 ± 7.04	5867.54 ± 164.13	39.31 ± 0.51

Table 3. Antibacterial activity of essential oils and ethanolic extracts of *Artemisia argyi*, *Centella asiatica*, *Cosmos caudatus* and *Polygonum hydropiper* against 10 human pathogenic bacteria. (Inhibition zone diameter in mm).

Test bacteria	Essential oil (500 µg disc^{-1})				Ethanolic extract (500 µg disc^{-1})				Control (500 µg disc^{-1})
	Aa	Ca	Cc	Ph	Aa	Ca	Cc	Ph	Streptomycin
Pseudomonas aurelis	-	7	-	9	7	8	-	7	31
Salmonella sp.	11	9	10	10	7	7	7	-	20
Proteus mirabilis	-	-	-	-	7	7	8	7	20
Escherichia coli	-	-	-	7	-	-	-	-	22
Salmonella typhi	-	-	-	-	-	-	-	-	25
Salmonella enteritidis	-	-	-	9	-	7	-	-	22
Salmonella thyphimunium	-	-	-	-	10	7	16	8	18
Staphylococcus aureus	-	-	-	-	7	7	7	7	20
Listeria monocytogenes	-	-	-	-	8	7	8	7	23
Vibrio cholerae	7	7	7	9	-	8	8	7	12

Aa: *Artemisia argyi*, *Ca*: *Centella asiatica*, *Cc*: *Cosmos caudatus*, *Ph*: *Polygonum hydropiper* - : no inhibition.

species were inactive.

Conclusion

Ethanol extracts of *P. hydropiper* and *C. caudatus* exhibited stronger antioxidant activities in comparison to *C. asiatica* and *A. argyi.* It is suggestive that the consumption of these vegetables may play an important role in preventing the formation of free radical and reduce the damages caused by these radicals. Both the essential oils and ethanol extracts showed various levels of antibacterial activities against the tested food pathogens. Thus, the essential oils and extracts of these plants may also be useful for the development of safe food products and natural additives in the formulation of total organic products.

ACKNOWLEDGEMENT

The authors would like to acknowledge Professor Dr. Shigeki Matsunaga and Ms. Reiko Ueoka of The University of Tokyo for assistance in cytotoxicity assay. Technical advice from Ms Ang May Yen of Thermo Fisher Scientific Company Limited in processing the Retention Index using Hydrocarbon Standard is acknowledged.

REFERENCES

Abdul HA, Shah ZM, Muse R, Mohamed S (2002). Characterization of antioxidative activities of various extracts of *Centella asiatica* (L.) Urban. Food Chem., 77: 465-469.

Ali MSM (2008). Analysis of phenolics and other phytochemicals in selected Malaysian traditional vegetables and their activities *in vitro*. PhD Thesis, University of Glasgow, UK.

Chan EWC, Lim YY, Omar M (2007). Antioxidant and antibacterial activity of leaves of *Etlingera* species (Zingiberaceae) in Peninsular Malaysia. Food Chem., 104: 1586-1593.

Faridah A, Shaari K, Lajis NH, Israf DH, Umi KY (2003). Antioxidative and radical scavenging properties of the constituents isolated from *Cosmos caudatus* Kunth. Nat. Prod. Sci., 9: 245-248.

Faridah A, Lajis NH, Israf DA, Shaari K, Umi KY (2006). Antioxidant and nitric oxide inhibition activities of selected Malay traditional vegetables. Food Chem., 95: 566-573.

Huda-Faujan N, Noriham A, Norrakiah AS, Babji AS (2007). Antioxidant activities of water extracts of some Malaysian herbs. ASEAN Food J., 14: 61-68.

Mackeen MM, Ali AM, El-Sharkaw SH, Manap MY, Salleh KM, Lajis NH, Kawazu K (1997). Antimicrobial and cytotoxic properties of some Malaysian traditional vegetables (Ulam). Pharm. Biol., 35(3): 174-178.

Lopes-Lutz D, Alviano DS, Alviano CS, Kolodziejczyk PP (2008). Screening of chemical composition, antimicrobial and antioxidant

activities of *Artemisia* essential oils. Phytochemisrty, 69: 1732-1738.
Ong HC, Norzalina J (1999). Malay herbal medicine in Gemencheh, Negeri Sembilan, Malaysia. Fitoterapia, 70: 10-14.
Oyedeji OA, Afolayan AJ (2005). Chemical composition and antibacterial activity of the essential oil of *Centella asiatica* growing in South Africa. Pharm. Biol., 43: 249-252.
Peng ZF, Strack D, Baumert A, Subramaniam R, Goh NK, Chia TF, Chia LS (2003). Antioxidant flavonoids from leaves of *Polygonum hydropiper* L. Phytochemistry, 62: 219-228.
Wong SP, Leong LP, Koh JHW (2006). Antioxidant activities of aqueous extracts of selected plants. Food Chem., 99: 775-783.
Wongfhun P, Gordon MH, Apichartsarangoon A (2010). Flavour characterization of fresh and processed pennywort (*Centella asiatica* L.) juices. Food Chem., 119: 69-74.
Yagi A, Uemura T, Okamura N (1994). Antioxidative sulphated flavodnoids in leaves of *Polygonum hydropiper*. Phytochemistry, 34: 885-887.
Zheng X, Deng C, Song G, Hu Y (2004). Comparison of essential oil composition of *Artemisia argyi* leaves at different collection times by headspace solid-phase microextraction and gas chromatography-mass spectrometry. Chromatographia, 59: 729-732.