Sains Malaysiana 45(2)(2016): 229–235

Evaluation of Antioxidant Properties, Cytotoxicity and Acute Oral Toxicity of *Gynura procumbens* (Compositae)

(Penilaian Sifat Antioksidan, Kesitotoksikan dan Ketoksikan Oral Akut *Gynura procumbens* (Compositae))

WUEN YEW TEOH, NORHANOM ABDUL WAHAB,
JAIME STELLA MOSES RICHARDSON & KAE SHIN SIM*

ABSTRACT

Gynura procumbens *which is locally known as 'Sambung nyawa' in Malay and 'Feng Wei Jian' in Chinese, belongs to the botanical family of Compositae. In this study, antioxidant property of* G. procumbens *extracts was evaluated using DPPH radical scavenging, metal chelating and β-carotene bleaching assays. To the best of our knowledge, this is the first report that evaluated the cytotoxicity of* G. procumbens *extracts on human colon cancer cells (HT-29, HCT 116, HCT-15, SW480, Caco-2) and human normal colon cells (CCD-18Co). The results showed that ethyl acetate extract contained the highest total phenolic content (172.68 mg of GAEs/g of extract) compared to methanol, hexane and water extracts. Methanol extract possessed better overall antioxidant activities while ethyl acetate extract demonstrated better cytotoxic activity. At 24 h treatment, ethyl acetate extract demonstrated selective cytotoxicity against HT-29 and HCT 116 cells with* IC_{50} *values of 35.7 and 42.6 µg/mL, respectively. In addition, methanol extract showed negligible level of toxicity when administered orally. All the results indicated that* G. procumbens *may provide benefits in prevention and treatment of cancer.*

Keywords: Acute oral toxicity; antioxidant; cytotoxic; Gynura procumbens

ABSTRAK

Gynura procumbens *yang dikenali sebagai 'Sambung nyawa' dalam kalangan populasi Melayu dan 'Feng Wei Jian' dalam kalangan populasi Cina, tergolong dalam keluarga botani Compositae. Dalam kajian ini, aktiviti antioksidan ekstrak* G. procumbens *dinilai dengan asai DPPH penyah radikal, pengkelatan logam dan pelunturan β-karotena. Sepanjang pengetahuan kami, ini adalah laporan pertama yang menilai sitotoksiti ekstrak* G. procumbens *terhadap sel kanser kolon manusia (HT-29, HCT 116, HCT-15, SW480, Caco-2) dan sel biasa kolon manusia (CCD-18Co). Hasil kajian menunjukkan bahawa ekstrak etil asetat mengandungi jumlah kandungan fenolik yang paling tinggi (172.68 mg GAEs/g ekstrak) berbanding dengan ekstrak metanol, heksana dan air. Ekstrak metanol mempunyai aktiviti antioksidan yang lebih baik secara keseluruhan manakala ekstrak etil asetat menunjukkan aktiviti sitotoksik yang lebih baik. Dalam perlakuan 24 jam, ekstrak etil asetat menunjukkan kesitotoksikan memilih terhadap HT-29 dan HCT 116 sel dengan nilai* IC_{50} *masing-masing 35.7 dan 42.6 µg/mL. Tambahan pula, ekstrak metanol menunjukkan tahap ketoksikan akut yang boleh diabaikan apabila dimakan. Semua keputusan tersebut menunjukkan* G. procumbens *boleh memberi manfaat dalam pencegahan dan rawatan kanser.*

Kata kunci: Antioksidan; Gynura procumbens*; ketoksikan oral akut; kesitotoksikan*

INTRODUCTION

Cancer is a common disease with high mortality rate in developing and developed countries. It is widely accepted that oxidative stress is associated with cancer as oxidative stress could cause oxidative damage to lipid, protein and DNA. Most of the lipid and DNA oxidation products such as 4-hydroxynonenal and 8-oxo-7,8-dihydro-2'-deoxyguanosine are mutagenic and can lead to cancer (Esterbauer et al. 1991; Valavanidis et al. 2009). Therefore, the use of antioxidants that could reduce oxidative stress may be considered as a potential strategy to prevent the development of cancer. In Asian countries, medicinal plants are still commonly used as complementary or alternative medicine to treat cancer.

Gynura procumbens which belongs to the botanical family of Compositae, is locally known as 'Sambung nyawa' in Malay and 'Feng Wei Jian' in Chinese. The native geographic distribution of *G. procumbens* is tropical West Africa, India, China, Myanmar, Thailand, Malaysia, Philippines, Indonesia and Papua New Guinea (Vanijajiva & Kadereit 2011). Traditionally, *G. procumbens* is used to treat fever, kidney disease, hypertension, diabetes and cancer (Perry 1980). Previous studies showed that *G. procumbens* display a wide range of bioactivities such as anti-herpes simplex virus (Nawawi et al. 1999), anti-hyperglycaemic (Akowuah et al. 2002; Algariri et al. 2013), anti-inflammatory (Iskander et al. 2002), anti-hyperlipidaemic (Zhang & Tan 2000), antioxidant (Rosidah

et al. 2008), anti-hypertensive (Hoe et al. 2007; Kim et al. 2006) effects as well as cytotoxicity (Hew et al. 2013; Nurulita et al. 2012).

The present study aimed to evaluate the total phenolic content, antioxidant activities, cytotoxic effect and acute oral toxicity of leaves from locally grown *G. procumbens*. The data collected from this study could be useful in evaluating the potential benefits of *G. procumbens* in prevention and treatment of cancer.

Materials and Methods

Chemicals and Reagents

Gallic acid, BHA (butylated hydroxyanisole), DPPH (1,1-diphenyl-2-picrylhydrazyl), potassium ferricyanide, Folin-Ciocalteu's phenol reagent, Ferrozine, ferrous chloride tetrahydrate, β-carotene, linoleic acid, MTT (Methylthiazolyldiphenyl-tetrazolium bromide), RPMI 1640 medium, McCoy's 5A medium, EMEM (Eagle's Minimum Essential Medium), sodium bicarbonate, *cis*-platin, carboxymethyl cellulose, EDTA (ethylene diamine tetra acetic acid) and DMSO (dimethyl sulfoxide) were purchased from Sigma-Aldrich company. Foetal bovine serum, penicillin/streptomycin (100X), amphotericin B (250 μg/mL) and sodium pyruvate (100 mM) were from PAA Laboratories. Methanol, hexane, ethyl acetate and chloroform were purchased from Fisher Scientific Company.

Plant Sample Collection and Identification

Fresh leaves of *G. procumbens* were collected from Seremban, Negeri Sembilan, Malaysia in February 2011. Dr Yong Kien Thai of Institute of Biological Sciences, Faculty of Science, University of Malaya, carried out identification of the plant. A voucher specimen (herbarium no: KLU47743) was deposited at the herbarium of the Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia.

Preparation of Extracts

Fresh leaves of *G. procumbens* (8469.70 g) were washed, dried and ground to fine powder (670.80 g, 7.92%). The powder was extracted with methanol at room temperature to yield methanol extract (58.90 g, 8.78%). The methanol extract (41.23 g) was further extracted with hexane to obtain hexane-soluble extract (14.00 g, 34.00%) and hexane-insoluble residue. Hexane-insoluble residue was further partitioned with ethyl acetate-water (1:1) to yield ethyl acetate (2.5 g, 6.10%) and water (7.00 g, 17.00%) extracts. All the extracts were kept at 4ºC before further testing.

Determination of Total Phenolic Content

The phenolic content of *G. procumbens* extracts was determined by Folin-Ciocalteu method as described previously by Sulaiman and Ooi (2012). Folin-Ciocalteu reagent was used to determine total phenolic content in extracts. Absorbance was read at 760 nm using a microplate reader (Thermo Scientific Multiskan GO). A standard curve was plotted using gallic acid (0 - 1000 mg/L). All experiments were carried out in triplicate and the results were expressed as milligram of gallic acid equivalents per gram of extract (mg of GAEs/g of extract).

DPPH Radical Scavenging Activity

DPPH radical scavenging activity of *G. procumbens* extracts was measured according to the method described by Sulaiman and Ooi (2012). BHA was used as a positive reference standard and the results expressed as IC_{50} value, which is the inhibition concentration at which 50 % of DPPH radicals were scavenged.

Metal Chelating Assay

Metal chelating assay was performed based on the protocol described by Sulaiman and Ooi (2012). EDTA was used as a positive reference standard for the experiment. The experiment was carried out in triplicate and results expressed as IC_{50}, which is the concentration at which 50% of metal ions were chelated.

β-Carotene Bleaching Assay

β-Carotene bleaching activities of extracts were measured as described by Murugan and Iyer (2012). BHA was used as positive reference standard in the present study. The rate of β-carotene bleaching (R) was calculated according to the equation below: $R = (\ln (A_0/A_t))/t$, where ln is natural logarithm; A_0 is absorbance reading at time 0 min; A_t is absorbance reading at time 120 min. The antioxidant activity (%) was calculated in terms of percentage inhibition relative to control using equation below:

$$[(R_{control} - R_{sample})/ R_{control}] \times 100\%.$$

MTT Cytotoxic Assay

MTT cytotoxic assay was carried out as described previously by Teoh et al. (2013). Briefly, all the extracts were firstly dissolved in DMSO (except water extract which was dissolved in distilled water) to form stock solutions. Cells were seeded into 96-well plates for 24 h before treatment with various concentrations of the extract. The final concentration of DMSO in each well was 0.5%. Untreated cells were used as negative controls. After 24, 48 and 72 h of incubation, 20 μL of MTT (5 mg/mL) was added into all wells and incubated for another 4 h. The medium was then removed and replaced with DMSO. The absorbance was measured at 570 nm with 650 nm as background. *Cis*-platin was used as a positive reference standard. IC_{50} value is the concentration of extract or positive reference standard that inhibits 50% of cell growth.

ACUTE ORAL TOXICITY

Acute oral toxicity was tested in Sprague-Dawley rats (male, aged 8-12 weeks) as described previously by Teoh et al. (2013). Current animal research protocol was approved by Institutional Animal Care and Use Committee, University of Malaya (UM IACUC) before commencement of the study (Ethics approval number: ISB/29/06/2012/SKS (R)). Briefly, methanol extract was firstly suspended in 0.3% carboxymethyl cellulose suspension (vehicle). The rats were randomly assigned to three treatment groups and one control group (n=3). The treatment groups were dosed at 300, 2000 and 5000 mg of crude methanol extract per kg of body weight while the control group was administered with vehicle only. After 12 h of fasting, the rats were force-fed with the extract and the volume of administration is 1 mL/100 g of body weight. After dosing, food was withheld for 4 h before providing food. All rats were observed for mortality, signs of toxicity and behavioral changes at 4 h after dosing and daily for 14 days. Individual weights were recorded from day 1 to 14. The experiment was performed twice for each dose.

STATISTICAL ANALYSIS

The antioxidant data were subjected to one-way analysis of variance (ANOVA) and the significance of the difference between the means was determined by the Duncan's multiple range tests at $p<0.05$. The IC_{50} values for cytotoxic activity were obtained by non-linear regression using GraphPad Prism statistical software.

RESULTS AND DISCUSSION

TOTAL PHENOLIC CONTENT OF EXTRACTS

Ethyl acetate extract of *G. procumbens* showed the highest phenolic content (172.68 mg of GAE/g of extract) among all extracts tested (Table 1). The current findings were in agreement with that obtained in a previous study by Rosidah et al. (2008) which reported ethyl acetate extract of *G. procumbens* had the highest phenolic content although the extraction method was different from that used in the current study. Similar phenolic content ranging from 67.18 to 67.59 mg of GAE/g of extract was observed in methanol and water extracts. The non-polar hexane extract which lacked polar and semi-polar phenolic constituents showed significantly the lowest phenolic content (22.03 mg of GAE/g of extract) among all the extracts tested.

ANTIOXIDANT ACTIVITIES OF EXTRACTS

Methanol extract showed the highest DPPH radical scavenging activity (IC_{50} value of 1.13 mg/mL), followed by water (IC_{50} 1.51 mg/mL), hexane (IC_{50} 2.44 mg/mL) and ethyl acetate (IC_{50} 4.16 mg/mL) extracts, as shown in Table 2. To our surprise, ethyl acetate extract which had highest phenolic content showed the lowest scavenging activity against DPPH radical. The hydroxyl group of phenolic constituents from ethyl acetate extract were expected to donate electron or hydrogen to convert DPPH radical to non-radical products (Bendary et al. 2013). Thus, semi-polar phenolic constituents of *G. procumbens* may not play a major role in scavenging DPPH-related radicals. The combination of semi-polar phenolics, polar phenolics and other constituents were found to be more effective in scavenging DPPH radical as obtained for methanol extract in the current study.

The metal chelating activity of plant chemical constituents is widely considered as part of the mechanism that contributes to the antioxidant property besides the direct free radical scavenging mechanism. Free transition iron ions are well known as pro-oxidants which could generate reactive oxygen species (ROS) *via* Fenton reaction (Srivastava et al. 2006). Metal chelating activity of hexane extract of *G. procumbens* was significantly higher than the other extracts tested (Table 2). The metal chelating activity of *G. procumbens* extracts in descending order was hexane > methanol > ethyl acetate > water. This indicated that non-polar constituents of *G. procumbens* appeared to be the main contributor in chelating metal ions and preventing oxidative stress. However, all the extracts demonstrated low metal chelating activity compared to EDTA, the positive standard reference.

Generally, antioxidant activity of *G. procumbens* extracts in β-carotene bleaching assay increased in a concentration-dependent manner (Table 3). Overall, methanol and ethyl acetate extracts demonstrated significant higher antioxidant activity compared to hexane and water extracts. At 4 mg/mL, antioxidant activity of methanol extract was significantly higher than the other extracts (43.49%). At 20 mg/mL, antioxidant activity of ethyl acetate extract was significantly higher than the other extracts (90.41%). Methanol and ethyl acetate extracts may contain constituents that inhibited the co-oxidation of linoleic acid and β-carotene.

CYTOTOXIC ACTIVITIES OF EXTRACTS

To the best of our knowledge, this is the first report on the cytotoxicity of *G. procumbens* extracts toward human colon cancer cells (HT-29, HCT 116, HCT-15, SW480, Caco-2) and human normal colon cells (CCD-18Co). According to specifications from the National Cancer Institute plant screening program (Lee & Houghton 2005), all *G. procumbens* extracts are thus considered not active in terms of cytotoxicity (IC_{50} > 20 μg/mL). However, ethyl acetate extract showed moderate cytotoxicity against the six tested cancer cell lines at 72 h treatment (Table 4). At 24 h treatment, ethyl acetate extract was more selective towards HT-29 and HCT 116 cells than other cell lines with IC_{50} values of 35.7 and 42.6 μg/mL, respectively. At this point, it is difficult to hypothesize the sensitivity of both cell lines towards ethyl acetate extract because both cell lines have very different genetic makeup according to COSMIC database. Ethyl acetate extract showed low cytotoxicity against CCD-18Co normal colon cells (IC_{50}

TABLE 1. Total phenolic content of *G. procumbens* extracts

Extracts	Concentration of total phenolics (mg of GAEs/g of extract)
Methanol	67.59±3.62^{b}
Hexane	22.03±1.19^{a}
Ethyl acetate	172.68±2.73^{c}
Water	67.18±0.77^{b}

GAEs, gallic acid equivalents; Values are expressed as mean±standard deviation (n=3), means with different letters (a-c) in the same column were significantly different (p<0.05, ANOVA)

TABLE 2. The IC_{50} values of *G. procumbens* extracts in DPPH radical scavenging activity and metal chelating assay

Extract	IC_{50} value (mg/mL)	
	DPPH radical scavenging activity	Metal chelating assay
Methanol	1.13±0.01^{b}	4.59±0.09^{c}
Hexane	2.44±0.07^{d}	1.99±0.08^{b}
Ethyl acetate	4.16±0.04^{e}	11.37±0.31^{d}
Water	1.51±0.02^{c}	16.50±0.36^{e}
BHA*	0.03±0^{a}	-
EDTA*	-	0.04±0^{a}

*Positive reference standard. Values are expressed as mean±standard deviation (n=3), means with different letters (a-e) in the same column were significantly different (p<0.05, ANOVA)

TABLE 3. The antioxidant activity (%) of *G. procumbens* extracts measured by β-carotene bleaching method

Extracts	Concentration of extracts (mg/mL)				
	4.0	8.0	12.0	16.0	20.0
Methanol	43.49±0.46^{dv}	50.27±0.94^{cw}	52.70±1.11^{cx}	61.05±0.54^{cy}	80.10±1.13^{cz}
Hexane	33.44±0.39^{cv}	37.72±0.93^{bw}	34.02±0.86^{bv}	43.12±1.35^{bx}	61.26±0.66^{by}
Ethyl acetate	22.87±1.45^{bv}	52.95±1.47^{dw}	89.52±0.90^{dy}	85.17±1.31^{dx}	90.41±1.80^{dy}
Water	18.04±2.52^{aw}	16.85±1.27^{aw}	15.56±1.82^{aw}	7.39±1.21^{av}	29.98±0.00^{ax}
BHA*	69.51±1.46^{ev}	78.84±1.53^{ew}	83.52±1.10^{ex}	88.06±1.44^{ey}	89.71±1.54^{dy}

*Positive reference standard; Values are expressed as mean±standard deviation (n=3), means with different letters (a-e) in the same column were significantly different (p<0.05, ANOVA), means with different letters (v-z) in the same row were significantly different (p<0.05, ANOVA)

values > 200 μg/mL), thus suggesting its medicinal efficacy which may selectively kill cancer cells. Previous phytochemical analysis showed that *G. procumbens* leaves contained kaempferol-3-*O*-glucoside, kaempferol-3-*O*-rutinoside, rutin and quercetin-3-*O*-rhamnosyl(1-6) galactoside (Akowuah et al. 2002; Rosidah et al. 2008). Cytotoxic effects of quercetin and kaempferol on HT-29 and HCT 116 cancer cells have been previously reported (Kim et al. 2010; Li et al. 2009). These flavonol aglycones may be present in ethyl acetate extract and therefore contribute to the cytotoxicity observed.

ACUTE ORAL TOXICITY OF METHANOL EXTRACT

Acute oral toxicity test was carried out in the current study to evaluate the safety of *G. procumbens* for human consumption by using an animal model. Throughout the 14 days of observation, no deaths or changes in the outer appearance (skin, fur, eyes and mucous membranes) were observed in treated rats were dosed with 300, 2000 and 5000 mg of crude methanol extract per kilogram of body weight. The treated rats also did not show any signs of toxicity (loss of appetite, vomiting, constipation, diarrhoea, dysphagia, hematemesis, hematochezia, tremors, convulsions, salivation and coma) and behavioral changes (hyperactivity and hypoactivity). The overall condition of treated rats was similar to control rats. As shown in Table 5, the mean body weight of all treatment groups gradually increased over the observation period. The present findings on the acute oral toxicity are in agreement with that reported in a previous study by Rosidah et al. (2009) in which the methanol extract of the plant prepared by Soxhlet extraction displayed no toxicity.

CONCLUSION

In the present study, *G. procumbens* extracts showed antioxidant activities. Overall, methanol extract of the plant demonstrated relatively better DPPH radical scavenging, metal chelating and antioxidant activity against β-carotene bleaching. It appears that crude extracts prepared from *G. procumbens* worked better as an antioxidant compared

TABLE 4. Cytotoxic activity (IC50 values) of G. procumbens extracts against selected human cell lines

Extract	Treatment duration (h)	Cytotoxicity (IC50) in µg/mL						
		HT-29	HCT 116	HCT-15	SW480	Caco-2	MCF7	CCD-18Co
Methanol	24	>100	>100	>100	>100	>100	>100	>200
	48	>100	>100	>100	>100	>100	>100	>200
	72	>100	>100	>100	>100	>100	>100	>200
Hexane	24	>100	>100	>100	>100	>100	>100	>200
	48	>100	>100	>100	>100	>100	>100	>200
	72	>100	>100	>100	>100	>100	>100	>200
Ethyl acetate	24	35.7±1.2	42.6±1.7	60.0±6.6	55.7±4.7	>100	81.5±3.4	>200
	48	57.9±3.9	47.9±3.6	62.0±2.0	57.3±4.0	56.7±8.6	61.6±0.0	>200
	72	62.4±8.4	66.1±1.4	78.7±1.2	65.2±1.8	66.7±5.5	58.3±4.8	>200
Water	24	>100	>100	>100	>100	>100	>100	>200
	48	>100	>100	>100	>100	>100	>100	>200
	72	>100	>100	>100	>100	>100	>100	>200
Cis-platin*	24	>12.5	12.0±0.7	6.2±0.4	>12.5	>12.5	11.2±0.5	>12.5
	48	10.1±0.2	4.0±0.3	3.9±0.2	6.8±0.5	4.3±0.4	4.2±0.4	>12.5
	72	6.4±0.6	2.9±0.1	1.7±0.4	3.2±0.6	1.9±0.2	2.7±0.3	>12.5

*Positive reference standard. Values are expressed as mean±standard deviation (n=3)

TABLE 5. The effect of methanol extract of *G. procumbens* on rat body weight

Treatment dose (mg/kg)	Body weight (g)													
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Control	183 ± 6	183 ± 10	187 ± 12	188 ± 10	192 ± 16	197 ± 16	195 ± 13	197 ± 12	198 ± 14	200 ± 17	205 ± 23	210 ± 20	212 ± 18	215 ± 18
300	224 ± 24	225 ± 25	226 ± 26	230 ± 27	230 ± 27	233 ± 24	235 ± 25	239 ± 22	240 ± 24	243 ± 20	246 ± 22	251 ± 20	256 ± 22	259 ± 20
2000	195 ± 13	193 ± 16	193 ± 13	228 ± 45	200 ± 15	200 ± 15	203 ± 13	202 ± 10	207 ± 10	210 ± 10	213 ± 13	215 ± 10	220 ± 10	222 ± 13
5000	223 ± 21	226 ± 20	229 ± 20	231 ± 19	235 ± 20	238 ± 18	239 ± 20	243 ± 20	244 ± 20	247 ± 19	249 ± 20	253 ± 21	253 ± 21	257 ± 21

Values are expressed as mean ± standard deviation (n=6)

to fractionated mixtures. Ethyl acetate extract exhibited moderate cytotoxicity against cancer cells while low cytotoxicity was observed against normal cells. The acute oral toxicity test indicated that *G. procumbens* has negligible level of toxicity when administered orally. Taken together, the results suggested the potential use of *G. procumbens* in cancer treatment. Lastly, investigations on the synergistic effect of ethyl acetate extract with oxaliplatin against colon cancer cells are under way to provide evidence that support the use of *G. procumbens* as complementary medicine.

ACKNOWLEDGEMENTS

The authors wish to acknowledge the Ministry of Education, Malaysia (MOE) and University of Malaya for the financial assistance through FRGS FP013/2012A grant and PPP PV042/2012A grant.

REFERENCES

Akowuah, G.A., Sadikun, A. & Mariam, A. 2002. Flavonoid identification and hypoglycaemic studies of the butanol fraction from *Gynura procumbens*. *Pharmaceutical Biology* 40: 405-410.

Algariri, K., Meng, K.Y., Atangwho, I.J., Asmawi, M.Z., Sadikun, A., Murugaiyah, V. & Ismail, N. 2013. Hypoglycemic and anti-hyperglycemic study of *Gynura procumbens* leaf extracts. *Asian Pacific Journal of Tropical Biomedicine* 3: 358-366.

Bendary, E., Francis, R.R., Ali, H.M.G., Sarwat, M.I. & El Hady, S. 2013. Antioxidant and structure-activity relationships (SARs) of some phenolic and anilines compounds. *Annals of Agricultural Science* 58: 173-181.

Catalogue of Somatic Mutations in Cancer (COSMIC). http://cancer.sanger.ac.uk/cancergenome/projects/cell_lines/.

Esterbauer, H., Schaur, R.J. & Zollner, H. 1991. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radical Biology and Medicine* 11: 81-128.

Hew, C.S., Khoo, B.Y. & Gam, L.H. 2013. The anti-cancer property of proteins extracted from *Gynura procumbens* (Lour.) Merr. *PLoS ONE* 8: e68524.

Hoe, S.Z., Kamaruddin, M.Y. & Lam, S.K. 2007. Inhibition of angiotensin-converting enzyme activity by a partially-purified fraction of *Gynura procumbens* in spontaneously hypertensive rats. *Medical Principles and Practice* 16: 203-208.

Iskander, M.N., Song, Y., Coupar, I.M. & Jiratchariyakul, W. 2002. Anti-inflammatory screening of the medicinal plant *Gynura procumbens*. *Plant Foods for Human Nutrition* 57: 233-244.

Kim, H.J., Kim, S.K., Kim, B.S., Lee, S.H., Park, Y.S., Park, B.K., Kim, S.J., Kim, J., Choi, C., Kim, J.S., Cho, S.D., Jung, J.W., Roh, K.H., Kang, K.S. & Jung, J.Y. 2010. Apoptotic effect of quercetin on HT-29 colon cancer cells via the AMPK signalling pathway. *Journal of Agricultural and Food Chemistry* 58: 8643-8650.

Kim, M.J., Lee, H.J., Wiryowidagdo, S. & Kim, H.K. 2006. Antihypertensive effects of *Gynura procumbens* extract in spontaneously hypertensive rats. *Journal of Medicinal Food* 9: 587-590.

Lee, C.C. & Houghton, P. 2005. Cytotoxicity of plants from Malaysia and Thailand used traditionally to treat cancer. *Journal of Ethnopharmacology* 100: 237-243.

Li, W., Du, B., Wang, T., Wang, S. & Zhang, J. 2009. Kaempferol induces apoptosis in human HCT116 colon cancer cells via the Ataxia-Telangiectasia Mutated-p53 pathway with the involvement of p53 upregulated modulator of apoptosis. *Chemico-Biological Interactions* 177: 121-127.

Murugan, K. & Iyer, V.V. 2012. Antioxidant and antiproliferative activities of marine algae, *Gracilaria edulis* and *Enteromorpha lingulata* from Chennai Coast. *International Journal of Cancer Research* 8: 15-26.

Nawawi, A., Nakamura, N., Hattori, M., Kurokawa, M. & Shiraki, K. 1999. Inhibitory effects of Indonesian medicinal plants on the infection of Herpes simplex virus type 1. *Phytotherapy Research* 13: 37-41.

Nurulita, N.A., Meiyanto, E., Sugiyanto, Matsuda, E. & Kawaichi, M. 2012. *Gynura procumbens* modulates the microtubules integrity and enhances distinct mechanism on doxorubicin and 5-flurouracil-induced breast cancer cell death. *Oriental Pharmacy and Experimental Medicine* 12: 205-218.

Perry, L.M. 1980. *Medicinal Plants of East and Southeast Asia*: *Attributed Properties and Uses*. Cambridge: MIT Press.

Rosidah, Yam, M.F., Sadikun, A., Ahmad, M., Akowuah, G.A. & Asmawi, M.Z. 2009. Toxicology evaluation of standardized methanol extract of *Gynura procumbens*. *Journal of Ethnopharmacology* 123: 244-249.

Rosidah, Mun Yam, Amirin Sadikun & Mohd Asmawi. 2008. Antioxidant potential of *Gynura procumbens*. *Pharmaceutical Biology* 46: 616-625.

Srivastava, A., Harish, S.R. & Shivanandappa, T. 2006. Antioxidant activity of the roots of *Decalepis hamiltonii* (Wight & Arn.). *LWT-Food Science and Technology* 39: 1059-1065.

Sulaiman, S.F. & Ooi, K.L. 2012. Polyphenolic and vitamin C contents and antioxidant activities of aqueous extracts from mature-green and ripe fruit fleshes of *Mangifera* sp. *Journal of Agricultural and Food Chemistry* 60: 11832-11838.

Teoh, W.Y., Sim, K.S., Richardson, J.S.M., Wahab, N.A. & Hoe, S.Z. 2013. Antioxidant capacity, cytotoxicity and acute oral toxicity of *Gynura bicolor*. *Evidence Based Complementary and Alternative Medicine* 2013: 958407.

Valavanidis, A., Vlachogianni, T. & Fiotakis, C. 2009. 8-hydroxy-2' -deoxyguanosine (8-OHdG): A critical biomarker of oxidative stress and carcinogenesis. *Environmental Carcinogenesis and Ecotoxicology Reviews* 27: 120-139.

Vanijajiva, O. & Kadereit, J.W. 2011. A revision of Gynura (Asteraceae: Senecioneae). *Journal of Systematics and Evolution* 49: 285-314.

Zhang, X.F. & Tan, B.K.H. 2000. Effects of an ethanollic extract of *Gynura procumbens* on serum glucose, cholesterol and triglyceride levels in normal and streptozotocin-induced diabetic rats. *Singapore Medical Journal* 41: 9-13.

Wuen Yew Teoh, Jaime Stella Moses Richardson
& Kae Shin Sim*
Institute of Biological Sciences, Faculty of Science
University of Malaya, 50603 Kuala Lumpur
Malaysia

Norhanom Abdul Wahab
Biology Division, Centre for Foundation Studies in Science

University of Malaya, 50603 Kuala Lumpur
Malaysia

*Corresponding author; email: simkaeshin@um.edu.my

Received: 10 February 2015
Accepted: 5 August 2015