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Evaluation of phytochemical constituents and biological activities of leaves and stems of *Marsdenia glabra* Cost.

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Abstract

The leaf and stem-based methanol extracts of *Marsdenia glabra* Cost., which has been used as food in northern Thailand, were evaluated in term of their phytochemical profile and biological activities. The GC-MS results showed that various saturated and unsaturated fatty acid compounds were dominant in extracts acquired from both parts of the *M. glabra* plant. The highest level of total flavonoid and total phenolic contents were observed in the leaf extracts, and this extract also possessed the greatest antioxidant activity (DPPH; IC_{50} 513.38 µg/mL, FRAP; 15.17 mg gallic acid equivalent/ g extract). Both the leaf and stem extracts could show slightly good antidiabetic activity when compared with acarbose, while showing high level of antihypertensive activity (approx. 87.00%), and also exhibiting antibacterial activity against methicillin-resistant *Staphylococcus aureus*. Both extracts did not display any adverse effects on human normal cells, while showing low inhibitory effects on human lung cancer cells. *Marsdenia glabra* extracts may be applied as active ingredient in food, pharmaceutical, cosmetic and other industries.

Introduction

Many Thai plants have been used in a wide variety of different fields, such as medicinal, pharmaceutical, cosmetic, and the food and beverage industries (Pandey and Rizvi, 2009; Adeyemi, 2011). *Marsdenia glabra* Cost., an edible plant that belong to the Asclepiadaceae family, is widely distributed in Thailand and adjacent regions. This plant is a climbing plant that has slender stems, ovate to elliptic leaves with an acuminated apex, and typically has length from 2.5-9.1 cm and 1.0-4.0 cm (Konta, 1989). This plant has traditionally been used as a digestive tonic, but has also displayed restorative and also antipyretic properties (Tachakittirungrod *et al*., 2007). Chanwitheesuk *et al*. (2005) reported about the *M. glabra* leaves's antioxidant activity and some of its antioxidant compound (vitamin C, vitamin E, carotenoids, xanthophylls, tannins and phenolic compounds). However, there has been very little complied research data on *M. glabra* with regard to its biological activities. In addition, many previous works reported that the phenolic acid and other phenolic components, such as flavonoids, are often extract in higher amounts in more polar solvents, especially methanol (Harborne, 1998; Perveen *et al.*, 2013; Belyagoubi *et al.*, 2016; Butsat *et al*., 2016). Therefore, the aim of this study was to investigate the chemical compositions of the leaves and stems of the methanol extract of *M. glabra* by GC-MS, and to also evaluate the potential antidiabetic, antihypertensive and anticancer activities, and the plant's possible toxicity to human normal cells.

Materials and Methods

Chemicals

Gallic acid was purchased from Merck (Germany) and Folin-Ciocalteu reagent was bought from BDH Chemicals Ltd. (Poole, England). The 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH) and 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) was purchased from Fluka (Steinheim, Germany). Intestinal acetone powder, angiotensin-I-converting enzyme (ACE) from rabbit lung (A6778), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide and hippuryl-histidyl-leucine (HHL; 859052) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA).

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Dulbecco's modified Eagle medium and fetal bovine serum were purchased from Invitrogen Corp. (NY, USA). All the solvents and other chemicals were an analytical grade.

Collection of plant material

The plant material, *M. glabra*, was collected from a local area in Lampang, Thailand. The plants were dried at 45°C for 72 hours, ground into small pieces and stored at room temperature for further extraction.

Preparation of the extracts

The *M. glabra* extracts were prepared by the method of Chanwitheesuk's work (2005) with some modification. Twenty five grams of dried leaves or stems of *M. glabra* were individually extracted with 250 mL of methanol, and left overnight at room temperature. After that, the extracts were put into ultrasonicator (Crest, USA) for 30 min, filtered through filter paper (Whatman no. 1) and evaporated at 40°C under a vacuum using a rotary evaporator until dry. Dry extracts were kept at room temperature in the dark for further.

Phytochemical analysis

Phytochemical analysis of the methanol extracts of *M. glabra* was determined according to the previous research of Tanruean *et al.* (2013) with some modifications. The extracts were analyzed for their phytochemicals using a gas chromatography (GC) 6890 Agilent Technologies/MSD 5973 Hewlett Packard, equipped with a MS detector and an HP-5MS capillary column (bonded and cross-linked 5% phenyl-methylpolysiloxane 30 m *x* 0.25 µm, film thickness 0.25 µm). The injector and detector temperatures were set at 270 and 280°C, respectively. The oven temperature was set at 80°C and held for 2 min, and then increased at a rate 10°C/min to 120°C and held for 4 min. The oven temperature was then increased at a rate 10°C/min to 155°C and held for 4 min, and then increased at a rate 5°C/min to 280°C and held for 12.50 min. The total running time was 55 min. Helium was used as a carrier gas at a flow rate of 1 mL/min. The sample (1 µL) was injected in the splitless mode. GC-MS detection of an electron ionization system with an ionization energy measurement of 70 eV was used. Injector and MS transfer line temperatures were set at 270 and 290°C, respectively. The components were identified based on a comparison of their relative retention times and the mass spectra with W8N08 and Wiley7n libraries data of the GC-MS system.

Determination of total flavonoid contents

The total flavonoid contents were determined by the method of Kaewnarin's work (2014) with slightly modification. The extract (0.5 mL) was mixed with 2 mL of methanol, followed by the addition 0.15 mL of 50 g/L $NaNO_2$. After 5 min, 0.15 mL of 100 g/L $AlCl_3$ was added. The reaction was mixed and incubated at room temperature for 15 min, and the absorbance was measured at 415 nm. Quercetin solution was used as a standard for the determination and the results were expressed as mg quercetin equivalent (QE)/g dry extract. The data were presented as the average of the triplicate analyses.

Determination of total phenolic contents

Total phenolic contents were estimated using the protocol of Thitilertdecha *et al.* (2008) with slight modifications. The procedure involved of combining 0.25 mL of sample (1 mg/mL) with 2.5 mL of deionized water and 0.5 mL of Folin-Ciocalteu reagent. After 5 min, 0.5 mL of 20% (w/v) Na_2CO_3 was added, and the solution was incubated for 1 hr at room temperature. Absorbance was then measured at 760 nm. Gallic acid solution was used as a standard for the determination and the results were expressed as mg gallic acid equivalent (GAE)/g dry extract. The data were presented as the average of the triplicate analyses.

Determination of antidiabetic activity

α-Glucosidase (AGH) solution was prepared from rat intestinal acetone powder by partial modification of the procedure reported by Oki *et al.* (1999). 100 mg of intestinal acetone powder was added to 3 mL of 0.9% NaCl solution, homogenized with the sonication and kept in an ice bath. After centrifugation at 6,000 rpm for 30 min at 4°C, the resulting supernatant was kept cold and directly subjected to inhibitory assay. The method of Adisakwattana *et al.* (2009) was used to determine AGH inhibitory assay. The assay was defined as the percent inhibition under the assay conditions, which was calculated according to the formula:

$$\text{Percent inhibition} = (A_o - A_s / A_o) \times 100$$

Where A_o is the absorbance of the control, and A_s is the absorbance of the mixture containing the test compound. The data were presented as the average of the triplicate analyses.

Determination of antihypertensive activity

The angiotensin-I-converting enzyme (ACE) inhibitory activity was evaluated by the modified

method of Park *et al.* (2003). The sample (50 µL) was mixed with 25 mU/mL ACE (50 µL) and pre-incubated at 37°C for 10 min. Then, 6 mM hippuryl-histidyl-leucine (HHL) in 50 mM Tris with 300 mM NaCl 100 µL was added and further incubated for 30 min. The reaction was stopped by adding 200 µL of 1.0 M HCl. Hippuric acid was extracted by ethyl acetate (600 µL), followed by centrifugation at 4,880 rpm for 15 min. The supernatant (200 µL) was transferred to a test tube and evaporated at 95°C to remove the ethyl acetate. Hot distilled water (1.0 mL) was added to dissolve the hippuric acid and the absorbance was determined at 228 nm. The ACE inhibition was calculated from this equation:

$$\text{Percent inhibition} = [1 - (A_s / A_o)] \times 100$$

Where A_o is the absorbance of the control (containing all reagents except the test compound), and As is the absorbance of the mixture containing the test compound. The results of all experiments were expressed as mean ± standard deviation.

DPPH free radical scavenging assay

The free radical scavenging ability was determined according to the method of Gülçin *et al.* (2003) with slight modifications. The 2,2'-diphenyl-1-picrylhydrazyl radical (DPPH•) solution in ethanol (0.1 mM, 1.5 mL) was mixed with 0.5 mL of different concentrations of each extract, and methanol was used as the control. The mixtures were well shaken and kept at room temperature for 30 min in the dark. The absorbance was measured at 517 nm and gallic acid was used as the comparative standard. The percent of DPPH• discoloration of the samples was calculated according to the formula:

$$\text{Percent inhibition} = (A_o - A_s / A_o) \times 100$$

Where A_o is the absorbance of the control (containing all reagents except the test compound), and A_s is the absorbance of the mixture containing the test compound. The test sample concentrations providing 50% inhibition (IC_{50}) were calculated from the plot of inhibition percentage against extract concentration values. The radical scavenging ability was presented IC_{50} values. The data were presented as the average of the triplicate analyses.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was determined according to the protocol of Li *et al.* (2006) with some modifications. The FRAP reagent containing 10 mM of 2,4,6-tris(2-pyridyl)-s-triazine (TPTZ) solution in 40 mM hydrochloric acid (20 mL), 20 mM ferric (III) chloride (20 mL) and acetate buffer (5 mL, 300 mM, pH 3.6) was prepared freshly prior to being used. Different concentrations of each extract (0.1 mL) was mixed with the FRAP reagent (1.5 mL) and 1.4 mL of acetate buffer (300 mM, pH 3.6) and were then incubated at an ambient temperature for 30 min. The absorbance was measured at 593 nm. Gallic acid was used as a standard and FRAP value was calculated as the gallic acid equivalent (mg GAE/ g dry extract). The data were presented as the average of the triplicate analyses.

Antibacterial activity

Antibacterial test, methicillin-resistant *S. aureus*, was carried out by disc diffusion method using 100 µL suspensions containing 10^6 CFU/mL of methicillin-resistant *S. aureus* spread on Mueller-Hinton agar (MHA) medium. The disc (6 mm in diameter) was impregnated with 100 mg/mL of the extracts and then placed on the inoculated agar. Negative control was prepared using the methanol. The inoculated plates were incubated at 37°C for 24 hr. Antibacterial activity was evaluated by measuring the zone of inhibition against the test bacteria.

Antitumor activity and cell toxicity assay

Antitumor activity and cell toxicity assay of the methanol extracts of the leaves and stems of *M. glabra* were determine according to the protocol of Wang *et al.* (2010) with some modifications. Tumor cells, A549 (human lung cancer cell) and SW1353 (Chondrosarcoma), were cultured in Dulbecco's modified Eagle medium (DMEM) until reaching 80% confluence. Trypan blue exclusion method was applied to determine the cell viability. In this experiment, optimum cell viability was above 98% and concentration level was adjusted for further experimentation. Human lymphocyte cells, peripheral blood mononuclear cells (PBMCs), were obtained from healthy volunteers' by venipuncture and heparin was used as an anticoagulant. The blood solution was diluted with one-fold sterile phosphate buffer saline (PBS) and was centrifuged with Ficoll-Hypaque gradient centrifugation to separate PBMCs from the other specimens. Briefly, the diluted blood solution was overlaid in Ficoll-Hypaque solution and centrifuged at 1,300 rpm, 25°C for 30 min. The PBMC layer was collected, washed two times with sterile PBS and the re-dissolved PBMC pellets were treated with RPMI-1640 cell medium with 10% fetal bovine serum (FBS). Cell viability was determined and the concentration level was adjusted for further experiment.

The3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to investigate the cytotoxicity of *M. glabra* extracts on A549, SW1353 and human lymphocyte cells. Tumor cells (A549 and SW1353) and human lymphocyte cells were cultured in a 96-well tissue culture plate, which contained 5,000 and 10,000 cells in each well, respectively. Different concentrations of the sample solution were added to each well and they were then incubated at 37°C in a 5% CO_2 incubator for 24 hr. After that, 20 µL of MTT solution (5 mg/mL of MTT in PBS, pH 7.4) was added and the specimens were further incubated at 37°C for another 4 hr. Cell medium was drained out and the formazan dye sediment was dissolved with 100 µL of dimethyl sulfoxide (DMSO). Absorbance was measured at 540 nm and the cell viability ratio was calculated by comparing the absorbance of the wells that did not contain any sample solution.

Statistical analysis

All experimental results were presented as means ± SD in triplicate. The correlation (r) between the two variants was analyzed using the Pearson test. The statistical analyses were performed using SPSS software (SPSS 17.0 for windows; SPSS Inc., Chicago).

Results and Discussion

The extraction yields (g dry extract/ 100 g dry sample) of the leaves and stems extracts were 28.80 and 11.06, respectively. Phytochemicals presented in the both extracts of *M. glabra* were analyzed by GC-MS. Each compound was identified based on mass spectral matching (≥ 90%) from W8N08 and Wiley7n libraries, which is summarized in Table 1. Gas chromatography chromatograms of leaves and stems of *M. glabra* are displayed in Figure 1(A) and Figure 1(B), respectively. The GC-MS analysis results revealed that extracts acquired from both parts of the *M. glabra* plant consisted of 19 components that could be categorized into 7 groups, which were phenolic compounds, terpenes, saturated and unsaturated fatty acids and methyl ester of fatty acids, hydrocarbons, vitamin E, and sterols. Saturated and unsaturated fatty acids and the methyl ester of fatty acids were found as the major components in both of the extracts. Furthermore, linolenic acid methyl ester (26.66%), palmitic acid methyl ester (16.53%) and linoleic acid methyl ester (11.72%) were found to be present as the major component in the leaf extracts, while oleic acid amide (28.37%), linolenic acid methyl ester (15.55%) and palmitic acid methyl ester (14.15%) were identified as the major constituents in the stem extracts. The leaf extracts also contained high contents of terpene (11.74%) and sterols (9.42%), while high contents of sterols (11.61%) and linoleic acid methyl ester (9.71%) were found in the stem extracts. Moreover, the vitamin E content in the leaf extracts was found to be 6 times higher than in the stem extracts. Based on these results, unsaturated fatty acids and their methyl ester forms were the dominant compounds identified in both extracts, and they both contained good amounts of linoleic acid, linolenic acid and oleic acid, the essential fatty acids. These essential fatty acids cannot be synthesized in human body and must only be obtained from the diet. Previous studies have suggested that linolenic acid has been associated with certain health benefits and has been identified for its ability to treat several diseases such as coronary artery disease and rheumatoid arthritis (Freeman, 2000; Rennie *et al*., 2003). Therefore, it can be concluded that *M. glabra* may be a good source of essential fatty acids and may also be identified for its potential applications in the pharmaceutical industry.

Table 1. Chemical compositions of the methanol extracts acquired from the leaves and stems of *Marsdenia glabra*.

Peak no.	RT[a]	Components[b]	Total peak (%)	
			Leaves	Stems
1	9.95	4-vinylguaiacol	0.14	1.81
2	11.42	*p*-carbomethoxybenzaldehyde	0.2	ND
3	13.22	*trans*-isoeugenol	ND	2.35
4	22.76	neophytadiene	4.87	1.75
5	24.78	palmitoleic acid methyl ester	0.52	ND
6	24.91	palmitic acid methyl ester	16.53	14.15
7	25.12	methyl di-tert-butyl hydroxyhydrocinnamate	0.38	1.62
8	25.86	palmitic acid	1.26	ND
9	28.35	linoleic acid methyl ester	11.72	9.71
10	28.51	linolenic acid methyl ester	26.66	15.55
11	28.68	phytol	6.87	1.64
12	28.97	steric acid methyl ester	3.72	3.9
13	30.11	palmitic acid amide	0.9	2.9
14	32.51	arachidic acid methyl ester	0.9	0.72
15	33.20	oleic acid amide	6.77	28.37
16	33.62	stearic acid amide	0.56	1.87
17	38.19	*β*-monolinolein	1.31	ND
18	38.32	pelargonic acid	1.88	ND
19	38.62	lignoceric acid methyl ester	0.59	0.87
20	40.85	nanocosane	0.58	0.46
21	42.98	γ- tocopherol	0.42	0.22
22	44.10	*α*-tocopherol	3.72	0.44
23	45.79	campesterol	0.84	1.87
24	46.28	stigmasterol	2.25	3.39
25	47.47	*β*-sitosterol	6.33	6.35
Phenolic compounds			0.72	5.78
Hydrocarbons			0.58	0.46
Saturated fatty acids and methyl ester of fatty acids			26.34	24.41
Unsaturated fatty acids and methyl ester of fatty acids			45.67	53.63
Unsaturated glyceride			1.31	ND
Vitamin E			4.14	0.66
Terpenes			11.74	3.39
Sterols			9.42	11.61
Total			99.92	99.94

[a]Retention time (as minutes); [b]Compounds listed in order of elution from a HP-5 MS column; "ND"= not detected

Table 2. Total flavonoid content, total phenolic content and biological activities of the *Marsdenia glabra* methanol extracts

Assays	*M. glabra* methanol extracts	
	Leaves	Stems
Total flavonoid content (mg QE/g extract)	48.42±2.63	17.08±0.88
Total phenolic content (mg GAE/g extract)	38.43±1.41	22.58±1.90
DPPH free radical scavenging activity (IC_{50}µg/mL)	513.38±7.08	853.13±18.37
Ferric reducing antioxidant power (mg GAE/ g extract)	15.17±0.41	13.76±0.14
Antidiabetic activity (%)	46.33±1.49	40.05±1.20
Antihypertensive activity (%)	87.30±0.03	87.16±0.04

*Average ± standard deviation from three replicates

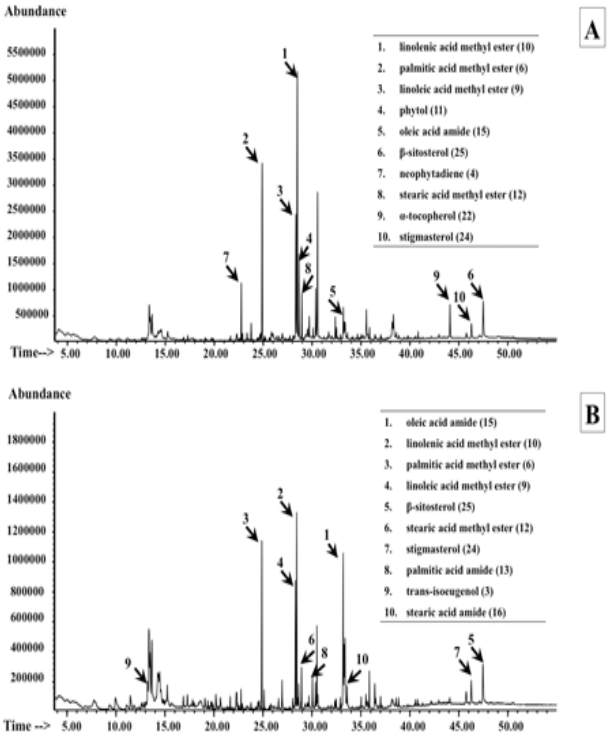

Figure 1. GC chromatogram of the leaves (A) and stems (B) extracts of *Marsdenia glabra*; the number represented the highest top ten compounds in each extract.

Total flavonoid and total phenolic contents were also evaluated from both of the extracts and the results are shown in Table 2. The total flavonoid contents in the leaf and stem extracts were 48.42 and 17.08 mg QE/ g extract, respectively. The amounts of total phenolic contents were found in the leaf extracts of 38.43 mg GAE/ g extract, while the stem extracts contained the phenolic content of 22.58 mg GAE/ g extract. Chanwitheesuk *et al.* (2005), the leaf extract of *M. glabra* contained total phenolic contents of 51.50 mg/100 g dried weight, which was much more lower than the extracts of leaves of other tested plants within the same family Asclepiadaceae such as, *Gymnema inodorum* (188.00 mg/100 g dried weight) and *Dregea volubilis* (100.00 mg/100 g dried weight). However, the extract possessed higher total phenolic contents than the leaves of other tested plants, such as *Polycia fruticosa, Ocimum americanum, Coriandrum sativum* and *Apium graveolens,* which contained total phenolic contents of 46.30 and 43.60, 33.00 and 31.10 mg/100 g dried weight, respectively.

The results of the study on the bioactivities of the *M. glabra* extracts are presented in Table 2. Based on a comparison of antidiabetic activity between the both extracts, it was found that α-glucosidase inhibition of the leaf extracts (46.33%) was higher than the stem extracts (40.05%). However, this plant extract from both leaf and stem could exhibit 72.30 and 62.50% of antidiabetic activity, which were slightly good when compared the standard antidiabetic agent (acarbose). The results correspond with Lim and Loh's work (Lim and Loh, 2016) which reported that the inhibitory effect in α-glucosidase inhibition assay could be found in the free- and bound phenolic extracts of white Tambun pomelo peels, kaffir lime peels, lime peels and calamansi peels ranging from 15.63 to 43.99%. Previous studies have suggested that the phenolic and flavonoid compounds in the plant extracts are responsible for the antidiabetic activity (Ardestani and Yazdanparast, 2007; Wang *et al*., 2011). On the other hand, with regard to the antihypertensive activity, both the leaf and stem extracts exhibited high levels of inhibition. Both extracts could inhibit angiotensin-I converting enzyme (ACE) activity by about 87%. The present scientific research has revealed that the medicinal plants or herbs are capable of inhibiting hypertension with significant potential (Tabassum and Ahmad, 2011). In this study, correlations between the antidiabetic and antihypertensive activities with the total flavonoid and total phenolic contents of both the leaf and stem extracts of *M. glabra* were investigated (Table 3). There were strong correlations between

Table 3. The Pearson correlation coefficient of total flavonoid and total phenolic contents with antidiabetic, antihypertensive and antioxidant activities of the *Marsdenia glabra* extracts

	Antidiabetic activity	Antihypertensive activity	Antioxidant activity	
			DPPH assay	FRAP assay
Total flavonoid contents	0.917	0.937	0.990	0.731
Total phenolic contents	0.877	0.886	0.978	0.689

Table 4. Effects of the *Marsdenia glabra* extracts against human lung cancer cells (A549) and chondrosarcoma (SW1353)

Sample	Concentration (µg/mL)	Percent inhibition (%)	
		A549	SW1353
Leaves	100	NA	NA
	1000	25.00	NA
Stem	100	NA	NA
	1000	15.00	NA

*Average ± standard deviation from three replicates ; "NA"= not active

their antidiabetic and antihypertensive activities with their total flavonoid contents (rantidiabetic = 0.917 and rantihypertension = 0.937, $p<0.05$) and their total phenolic contents (rantidiabetic = 0.877 and rantihypertension = 0.886, $p<0.05$) of both the leaf and stem extracts of *M. glabra*. Therefore, *M. glabra*, has been of significant interest for the purpose of isolating the effective components for antidiabetic and antihypertensive drug development.

For antioxidant activity assay, higher effective radical scavenging activity was noticed in the leaf extracts (IC_{50} of 513.38 µg/mL) than the stem extracts (853.13 µg/mL). Similar to the result of ferric reducing antioxidant power (FRAP) system, the leaf extracts exhibited a little bit higher activity than the stem extracts with FRAP values of 15.17 and 13.76 mg gallic acid equivalent/g dry extract, respectively. In a previous study, Neakham (2008) reported that 400 µg/mL of the acetone extract conducted by refluxing the leaves of *M. glabra* revealed high DPPH free radical scavenging activity with a level of 95.47 percent. From the study of Neakham, the higher DPPH free radical scavenging activity that was recorded may be due to the use of the refluxing method in the extraction step, and the use of acetone may have extracted higher molecular weight of flavanols in the plant materials (Dai and Mumper, 2010), which may have helped to increase the scavenging activity. Additionally, previous research studies have suggested that the antioxidant activity of various plant extracts have been partly associated with some constituents other than fatty acids, such as phenolic compounds, terpenes and vitamin E. These compounds are already well known to possess high levels of antioxidant activities (Tasioula-Margari and Okogeri, 2001; Lee *et al*., 2002; Sroynak *et al.*, 2013; Santos *et al*., 2013). Based on our results, higher content levels of total flavonoid and total phenolic compounds, vitamin E and phytol found in the leaf extracts of *M. glabra* than the stem extracts may actually be related to the antioxidant activities of the extracts. Additionally, high correlations were observed between antioxidant activities of both the leaf and stem extracts of *M. glabra* with their total flavonoid contents (rDPPH = 0.990 and rFRAP = 0.731, $p<0.05$) and their total phenolic contents (rDPPH = 0.978 and rDPPH = 0.689, $p<0.05$). However, the other antioxidant compounds such as, vitamin C, carotenoids, xanthophylls and tannins that were present in the extracts of *M. glabra* (Chanwitheesuk *et al.*, 2005), may also have played a role in the antioxidant activity.

In the antibacterial activity assay, both the leaf and stem extracts of *M. glabra* could protect against methicillin-resistant *Staphylococcus aureus* with an inhibition zone of 11 and 10 mm, respectively. The bacterial strain is a frequent cause of serious infections, such as endocarditis, pneumonia, osteomyelitis, and bacteremia (Moise-Broder *et al*., 2004). In addition, this study also investigated the effect of the leaf and stem extracts of *M. glabra* against two human tumor cells, human lung cancer cell (A549) and chondrosarcoma (SW1353), as well as the toxicity toward human normal cells (peripheral blood mononuclear cell; PBMCs) (Table 4). Low inhibitory activity was noticed in the A549 inhibition with both *M. glabra* extracts (1,000 µg/mL). No inhibition was observed in the PBMCs and SW1353 cell culture with the same concentration value of the extracts. These results revealed that the leaf and stem extracts of *M. glabra* possessed low activity level of inhibiting human cancer cells, nevertheless, both extracts were observed to have no effect on human normal cells.

Conclusion

The results reported here explore the possibility of further determinations of the efficacy of the biological properties of the plant extracts. Both

quality and quantity of extracts, particularly active biological ingredients, required to be commenced. This study can be concluded that the methanol extracts of *M. glabra*, especially the leaf extracts could be identified as candidates for a natural antidiabetic, antihypertension and antioxidant agents that have no side effects on human normal cells in food, pharmaceutical, cosmetic and other industries.

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