Chemical Composition and Antioxidant activity of methanolic extract of *Spilanthes acmella* Murr.

Wanthani Paengsri, Thanyarat Chuesaard, Napapha Promsawan

***Abstract*—** *Spilanthes acmella* Murr. was extracted with methanol, yielding methanol crude extract 5.86 %w/w. This study aimed to examine the chemical composition and antioxidant activity of methanolic crude extract. The chemical composition of methanolic crude extract was analyzed by gas chromatography-mass spectrometry (GC-MS). The predominant components were found to be palmitic acid (40.08%), 2-hexadecanoyl glycerol (6.96%) and octadecanoic acid (4.06%). Antioxidant activity was determined using 2,2-diphenyl-1-picryl hydrazyl (DPPH) free radical, for evaluating free radicle scavenging activity. The methanolic extract at 150 µg/mL showed an antioxidant activity with high of radical scavenging activity (75.23%).

***Keywords*—** antioxidant activity, GC-MS analysis, *Spilanthes*, *Phak-Kratt Hauwaen*

I. INTRODUCTION

Spilanthes acmella Murr is one of a plants in genus *Spilanthes*, comprising of over 60 species. They are widely distributed in tropical and subtropical regions of the world, such as Africa, America, India, Sri Lanka and Asia. *Spilanthes acmella* Murr., native to Brazil, is a therapeutically important medicinal herb with yellow flowers. Its Thai name is "*Phak-Kratt Hauwaen*" because the yellow flower head look remarkably likes a ring head style [1-2]. It is a half-meter tall plant which takes nearly a year for full life cycle. This plant is typically grown in humid environment with a low rate of seed growth [3]. It is widely utilized as food ingredient and herbal medicine for toothaches, stomatitis, some biological activities such as antibacterials, antimalarials, antifungals, anti-inflammatory, analgesic and antipyretic [4-5]. *Spilanthes acmella* for insecticidal activity it finding indicated the potential of *S.acmella* and *spilanthol* for controlling *plutella xylostella* and other insects of agricultural importance [6]. A great number of bioactive compounds were found in *Spilanthes acmella*, such as alkylamides, phenolic compound, coumarin and triterpenoids [7].

II. PROCEDURE

1.*Plant Material*

The whole plant of *Spilanthes acmella* Murr. was collected from Rong Kwang city, Phrae province, Thailand in January 2014. The plant material was identified by Asst. Prof. Dr. Angkhana Inta, Department of Biology, Faculty of Science, Chiang Mai University.

2. *Extraction*

The whole plant of *Spilanthes acmella* Murr. was cleaned, shade-dried and ground well. The plant (1.3 kg) was extracted with methanol (4 L) by maceration for 7 days, followed by filtration. The methanolic extract was concentrated to dryness under reduced pressure, yielding methanol crude extract 76.13 g (5.86 %w/w) as a brown gum. The extract was stored at a temperature of -5^{o}C and used for further analysis.

3. *Analysis of Methanolic Extract*

GC-MS is used for analysis for chemical composition of the methanolic crude extract from *Spilanthes acmella* Murr. A gas chromatograph (7890A Agilent) was coupled to a mass spectrometer (5975C (EI) Agilent). HP-5MS capillary fused silica column (30 m, 0.25 mm, 1D, 0.25 µm film thickness) has been used in the GC-MS system. Injector and detector temperature were 280 ˚C. An oven temperature program was starting at 100˚C and increasing at 4˚C/min to 270˚C. Helium was used as carrier gas at 1 ml/min. The injection volume of diluted essential oil was 1 µl. Electron ionization mass spectra in the range from *m/z* 30-500 amu were recorded at 70 eV ionization energy. The chemical constituents were identified by comparison and matching their mass spectra with the library data (NIST98 and WILEY7n). The relative percentage amounts of the isolated compounds were calculated by a computerized integrator.

Wanthani Paengsri is with the Basic Science Program, Maejo University Phrae Campus, 54140, Thailand (corresponding author, phone: (+66) 81 8830960**;** e-mail: wanthanip@gmail.com)
Thanyarat Chuesaard is with the Basic Science Program, Maejo University Phrae Campus, 54140, Thailand (e-mail: thanyaratc@hotmail.com)

Napapha Promsawan is with Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai, 50200, Thailand (e-mail: np.promsawan @gmail.com)

4. *Antioxidant Activity*

The antioxidative activity of the extract was elucidated by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. DPPH is a highly stable radical compound with a purple color. It can be reduced to form a light-yellow color of diphenylpicrylhydrazine. When a solution of DPPH is mixed with a substrate (RH), hydrogen atom donor, then it traps a hydrogen radical ($H^{\bullet}$) form an antioxidant to become a reduced form with loss of this violet color. Formation of DPPH upon absorption of hydrogen from antioxidant was shown in **Figure I**.

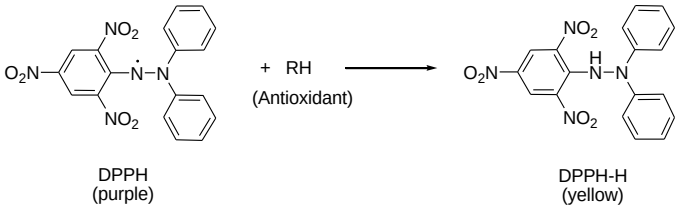

Fig. I Formation of DPPH upon absorption of hydrogen from antioxidant

Color changes from purple to yellow which can be monitored by spectrophotometer. In this study, an experimental system was initiated by preparing 300 µM solution of DPPH in methanol. 1 mL of the DPPH solution was added to sample solution (0.3 mL, dissolved in methanol 1.70 mL). After 30 min, absorbance of the sample solution was measured at 517 nm and the percentage of radical scavenging activity was calculated from the following equation:

$$\% \text{ Radical scavenging} = \frac{(1-\text{Abs.sample}/\text{Abs.cont})}{} \times 100$$

Where;

Abs.cont is an absorbance of the control reaction.

Abs.sample is the absorbance of the presence of sample.

III. Results and Discussion

The methanolic extract of the whole plant of *Spilanthes acmella* Murr. was obtained as a dark brown amorphous with percentage yield of 5.86 % (w/w). The chemical composition of the extract was investigated using gas chromatography-mass spectrometry (GC-MS) technique equipped with the HP-5MS column. The chemical constituents were mainly identified by comparisons of their mass data with those of reference compounds from NIST98 (National Institute of Standards and Technology, Gaithersburg, MD, USA) and WILEY7n (Wiley, New York, USA) libraries.

The identified major compounds and their quantitative amounts in the methanolic extract are given in **Table I** in order of their retention times with the relative peak area, expressed as a percentage of chemical composition. Nine major constituents in the alcoholic extract were identified and their chemical structures were shown in **Fig. II**

Palmitic acid (**4**) was found at the highest composition from the extract as of 40.08%, followed by 2-hexadecanoyl glycel (**7**, 6.96%) and octadecanoic acid (**5**, 4.06%).

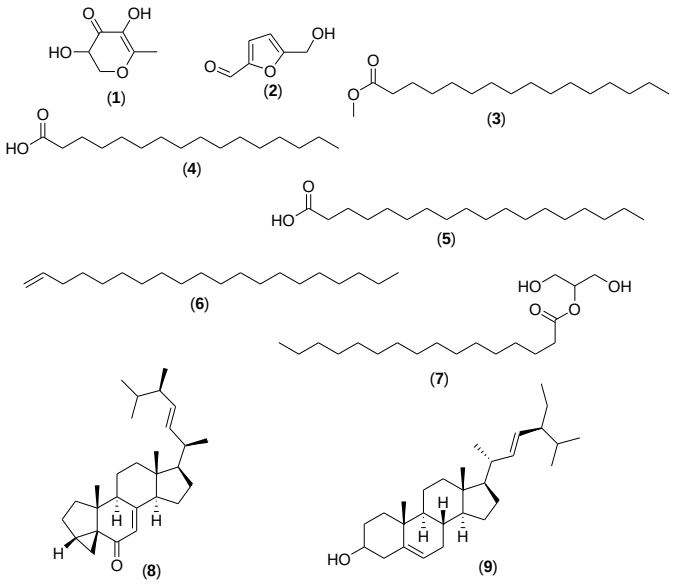

Fig. II Chemical structures of major compounds (**1**)-(**9**)

All of the identified major components could be classified into eight groups; fatty acids, long chain alcohol, heterocyclic aromatic compound, triterpene, oxygenated triterpene, ester, alkene and flavonoid fraction. The percentage composition for groups of major compounds are also listed in **Table I**. The methanol extract of this plant was found to be rich in fatty acids and hydrocarbons. The major components, identified in the extract, were fatty acids (**4**,**5**), quantified for 44.14%. Long chain alcohol (6.96%) was classified as the second largest group of major compounds in the extract. Among the terpenoids, triterpene and oxygenated triterpene were found at 1.14%, 2.52%, respectively. However, the other classes of terpenoids were not observed. In addition, heterocyclic aromatic compound was also detected for 3.29%.

TABLE I
CHEMICAL COMPOSITION OF IDENTIFIED MAJOR COMPOUNDS FROM METHANOLIC EXTRACT OF *SPILANTHES ACMELLA* MURR.

No.	Retention time (min)	Major Compounds	RA(%)[a]	Match(%)[b]
1	3.93	2,3-dihydro-3,5-dihydroxy-6-methyl-4*H*-pyran-4-one	2.01	93
2	5.21	5-(hydroxymethyl)-2-furan carboxaldehyde	3.29	95
3	21.89	hexadecanoic acid methyl ester	2.41	99
4	23.12	palmitic acid	40.08	99
5	27.44	octadecanoic acid	4.06	99
6	31.23	1-eicosane	1.17	92

7	34.38	2-hexadecanoyl glycerol	6.96	90
8	44.08	(3β,5α,22*E*)-3,5-cycloergosta-7,22-dien-6-one	2.52	92
9	48.19	(22*E*)-stigmasta-5,22-dien-3-ol	1.14	99
		Fatty acids (4,5)	44.14%	
		Long chain alcohol (7)	6.96%	
		Heterocyclic aromatic compound (2)	3.29%	
		Oxygenated triterpene (8)	2.52%	
		Ester (3)	2.41%	
		Flavonoid fraction (1)	2.01%	
		Alkene (6)	1.16%	
		Triterpene (9)	1.14%	

[a]RA, relative area (peak area relative to total peak area)
[b]MS, from a composition of the mass spectrum with MS libraries

Antioxidant activity of methanol extract of *Spilanthes acmella* Murr. has been investigated by DPPH assay. The percentage of radical scavenging of the methanolic extract at many concentrations were shown in **Fig. III** and **Table II**.

At higher concentration, the antioxidant activity of the extract is also higher. It was found that the methanolic extract at 150 µg/mL showed an antioxidative activity with high of radical scavenging activity (75.23%). However, its antioxidant activity is lower than that of ascorbic acid and α-Tocopherol, used as positive controls.

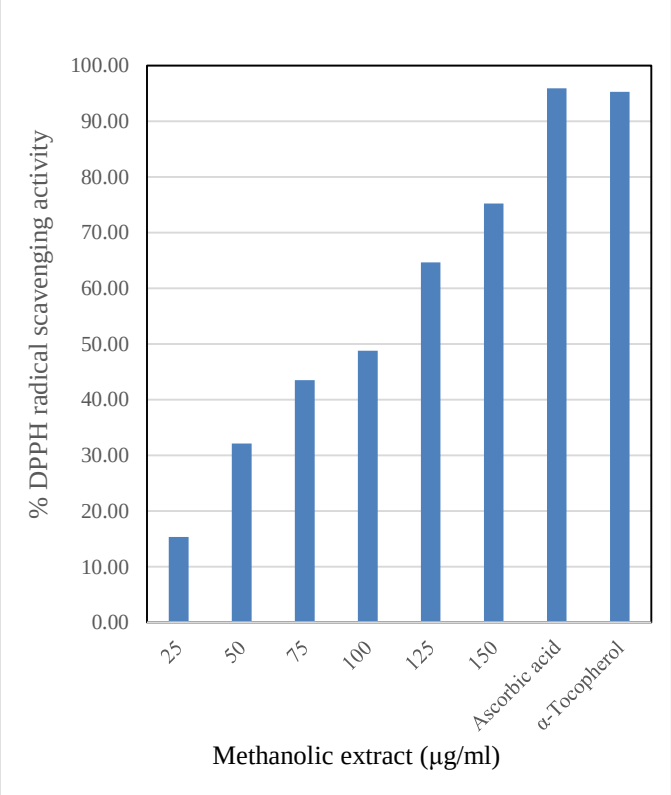

Fig. III DPPH radical scavenging activity at concentrations of methanolic extract at 25, 50, 75, 100, 125 and 150 µg/mL

TABLE II
PERCENTAGE OF RADICAL SCAVENGING OF METHANOLIC EXTRACT AT MANY CONCENTRATIONS

Concentration of methanolic extract (µg/mL)	% radical scavenging
25	15.35
50	32.14
75	43.52
100	48.83
125	64.68
150	75.23
Ascorbic acid (1 mM)	95.93
α-Tocopherol(1 mM)	95.27

IV. CONCLUSION

This study presented the GC-MS analysis of *Spilanthes acmella* Murr. from methanolic crude extract and antioxidant activity. It was found that GC-MS analysis showed nine major chemical constituents rich of fatty acid and hydrocarbons. The antioxidant activity of methanol crude extract by DPPH assay revealed that the sample at 150 µg/mL showed the highest activity with radical scavenging 75.23% compared with positive controls.

ACKNOWLEDGMENT

We are grateful to Maejo University Phrae Campus for financial supporting this research and Department of Chemistry, Faculty of Science, Chiang Mai University for scientific facilities.

REFERENCES

[1]. V. Prachayasittikul, S. Prachayasittikul, S. Ruchirawat, V. Prachayasittikul, "HIGH THERAPEUTIC POTENTIAL OF *SPILANTHES ACMELLA*: A REVIEW", *EXCLI Journal*. Vol. 12, pp. 291-312, 2013.

[2]. O. Wongsawatkul, S. Prachayasittikul, C. Isarankura-Na-Ayudhya, J. Satayavivad, S. Ruchirawat, V. Prachayasittikul, " Vasorelaxant and Antioxidant Activities of *Spilanthes acmella* Murr.", *Int. J. Mol Sci*. Vol. 9, pp. 2724-2744, 2008.

[3]. KL. Tiwari, SK. Jadhav, V. Joshi, "An updated review on medical herb genus *Spilanthes*", *Chin. J. Integr. Med*. Vol. 9, pp. 1170-1178, 2011.

[4]. A. Chakraborty, BR. Devi, R. Sanjeb am, S. Khumbong, IS. Thokchom, "Preliminary studies on local anesthetic and antipyretic activities of *Spilanthes acmella* Murr. in experimental animal models", *Indian J. Pharmacol*. Vol. 42, pp. 277-279, 2010.

[5]. WD. Ratnasooriya, KP. Pieris, U. Samaratunga, JR. Jayakody, "Diuretic activity of *Spilanthes acmella* flowers in rats", *J. Ethnopharmacol*. Vol. 91, pp. 317-320, 2004.

[6]. A. Sharma, V. Kumar, RS. Rattan, N. Kumar, B. Singh, "Insecticidal oxicity of spilanthol from *Spilanthes acmella* Murr. Against *Plutella xylostella* L", *American J. Plant Sci*. Vol. 3, pp. 1568-1572, 2012.

[7]. S. Prachayasittikul, S. Suphapong, A. Worachartcheewan, R. Lawung, S. Ruchirawat, et al. “Bioactive metabolites from *Spilanthes acmella* Murr.” *Molecules*. Vol. 14, pp. 850-867, 2009.