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Chemical Constituents of Water Extract of *Acmella uliginosa* (Sw.) Cass. Flowers, Leaves, Stems and Roots from Malaysia

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ABSTRACT

A few reports on the phytochemical analysis of the organic solvent extract of *Acmella uliginosa* (Sw.) Cass. (*A. uliginosa*) have been published previously. Water extract is preferable depending on the intended final applications of the extract, such as those for the food and pharmaceutical industries. Thus, this current study focused on the phytochemical study of water extract from various parts of *A. uliginosa* which was collected from Kelantan, Malaysia. The sample of *A. uliginosa* flowers, leaves, stems and roots were converted into powder form and extracted with water macerated extraction procedures. The crude extracts were freeze-dried, and the extracts obtained were analysed using gas chromatography mass spectrometry. More than 80 chemical compounds were identified from the flowers, leaves, stems and roots of water extracts of *A. uliginosa*. The bioactive compounds that were identified include alkaloids, terpenoids, phenolic compounds and steroids. This study also revealed that the most abundant compound in both flowers and leaves were spilanthol, whereas linoleic acid and 2-Furancarboxaldehyde, 5-methyl- were identified as the highest compound for stems and roots; respectively. This finding suggested that water extracts of various parts of *A. uliginosa* are good sources of many bioactive compounds that might have pharmacological potential and high therapeutic value. Further experiments are needed to prove that all parts of the water extract of *A. uliginosa* do have therapeutic value.

Keywords: *Acmella uliginosa* (Sw.) Cass., gas chromatography mass spectrometry, water extract

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INTRODUCTION

Acmella uliginosa (Sw.) Cass. (*A. uliginosa*) or commonly known as "subang nenek" in Malaysia is a species of flowering plant which is indigenous and widely distributed in the tropics and sub-tropics regions especially in the West Indies, Venezuela,

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Brazil, Africa, Indonesia and Malaysia. "Subang nenek" does not specifically refer to *A. uliginosa*, but it is referred to a genus *acmella*. Genus *acmella* is commonly used by the Malay community in Malaysia to relieve pain which often associated with mouth ulcers, toothache, sore throat and stomachache. The alkaloid compound known as spilanthol or its IUPAC name is N-Isobutyl-2(E),6(Z),8(E)-decatrienamide was reported to be responsible for this property. The plant is known as the toothache plant due to the analgaesic effect of spilanthol. Spilanthol can be found not just in genus *acmella* such as *A. uliginosa*, *Acmella oleracea*, *Acmella ciliate* and *Acmella paniculata* but also in other plants such as *Heliopsis longipes* (Barbosa et al., 2016).

The use of genus *acmella* is not only limited to pain relief. It is also traditionally used to treat tuberculosis (Storey & Salem, 1997) and leucorrhoea (Hossan et al., 2010). In the present study, we have focused on *A. uliginosa*. Previous studies of *A. uliginosa* showed that this plant had various pharmacological properties such as antioxidant (Maimulyanti et al., 2016), anti-inflammatory (Paul et al., 2016) and antimicrobial properties (Lagnika et al., 2016). However, most of the previously reported studies focused on *A. uliginosa* flowers that were obtained using organic solvent including methanol, hexane, and ethyl acetate (Maimulyanti et al., 2016; Modak et al., 2017; Ong et al., 2011). The study of the other parts of *A. uliginosa* such as its stems and roots, however, is still lacking. The *in vitro* study of the methanolic extract of *A. uliginosa* flowers collected from West Bengal showed anti-inflammatory effect (Modak et al., 2017). The *in vivo* study of the methanolic extract of *A. uliginosa* flowers collected from Pahang, an East Coast state in Malaysia showed antinociceptive activity in chemical and thermal-induced nociception mice models (Ong et al., 2011). On the other hand, an *in vivo* study of water extract of *A. uliginosa* flowers revealed that there were anti-inflammatory and anti-arthritic properties in arthritic rats that ingested the extract (Paul et al., 2016). Furthermore, a previous study of oral toxicity had shown that water extract of *A. uliginosa* leaves had low oral toxicity compared to dichloromethane and methanol extracts of it on rats (Lagnika et al., 2016).

Water extract is preferable depending on the intended final applications of the extract, especially in pharmaceutical and food industries. In the present study, we report on the chemical constituents of the water extract from the flowers, leaves, stems and roots of *A. uliginosa* from Malaysia. As far as we know, it is the first report about the phytochemical analysis of the water extract of *A. uliginosa*.

MATERIALS AND METHODS

Sample of *Acmella uliginosa*

Acmella uliginosa sample was collected from Kelantan, Malaysia. The plant was confirmed by a botanist from Forest Research Institute Malaysia (FRIM), Dr. Fadzureena Jamaludin. A voucher specimen was then deposited in the Herbarium of FRIM, under code sample SBID 035/19.

Preparation of Water Extract of *Acmella uliginosa*

Water extract of *A. uliginosa* was prepared based on the methods described by Lagnika et. al (2016) with some modification. The whole parts of *A. uliginosa* were cleaned, separated and dried in an oven (40°C) for a day and were ground into a fine powder. The extraction was performed under mechanical agitation with distilled water (3:7 w/v) in a glass bottle for one day. The mixture was filtered and dried using a freeze dryer. The dried extract was stored in amber glass and was kept in the freezer (-20°C).

GC-MS Conditions and Parameters

The phytochemical analyses of various parts of water extracts of *A. uliginosa* were carried out using Hewlett Packard 6890 Gas Chromatograph equipped with 5973N Mass Selective Detector (Agilent Technologies, USA). The column was fused silica capillary, HP-5 column (30 m x 0.25 mm i.d x 0.25 µm film thickness). The carrier gas was helium at a flow rate of 1.0 ml/min. The column temperature was programmed from 50°C (held for 2 min) to 280°C (held for 10 min) at a rate of 20°C/min. The injection and interface temperatures were set at 250°C and 280°C, respectively. The electron ionisation was fixed at 70eV. The samples (1 µl) were injected in splitless mode and analysed in MS full scan mode (*m/z* 40-650). Acquisition of data was performed using Chemstation software. Blank solvent (water) was dried and treated similarly as the sample and used as a control.

Identification of Chemical Constituents

The compounds in *A. uliginosa* were identified by matching their mass spectra with the National Institute of Standards and Technology (NIST02) and Wiley275 libraries (≥ 80% matching). The percentage of the compound was calculated from the summation of the peak areas of *A. uliginosa* compounds.

RESULTS AND DISCUSSION

The GC-MS analyses of flowers, leaves, stems and roots of the water extracts of *A. uliginosa* exhibited the presence of various interesting compounds as listed in Table 1. The GC-MS chromatograms obtained are given in Figures 1-4. More than 80 chemical compounds were identified from the water extract of *A. uliginosa*. Generally, GC-MS analyses of water extracts of *A. uliginosa* showed the differences in chemical constituents between the plant's parts. A total of 34 chemical compounds were identified in the flowers and leaves, 30 chemical compounds in the stems and 32 chemical compounds in the roots.

These chemical compounds were grouped into few biologically active classes which were alkaloids, phenolic compounds, terpenoids and steroids. In this study, alkaloids are the major biologically active class of the water extract of *A. uliginosa* was obtained from flowers, leaves and roots. The alkaloids that were found in this study were spilanthol and N-(2-Phenylethyl) (2E,6Z,8E)-decatrienamide. However, spilanthol was the only alkaloid that was identified in all parts of the water extract

Table 1
Chemical compounds of water extracts of Acmella uliginosa *(Sw.) Cass. flowers, leaves, stems and roots*

Class of compounds		Compounds name	Composition (%)			
			Flowers	Leaves	Stems	Roots
Alkaloids	1.	Spilanthol	6.08	8.42	2.74	3.71
	2.	N-(2-Phenylethyl)(2E,6Z,8E)-decatrienamide	1.43			
Phenolics	3.	Benzaldehyde	0.03	0.02		
	4.	Mequinol		0.37		
	5.	4-vinyl-2-methoxy-phenol		1.68		
	6.	Eugenol		0.17		
	7.	Benzeneacetyldehyde			0.86	
	8.	p-Vinylguaiacol			1.65	
Steroids	9.	Cholesterol	0.21			
	10.	Stigmasterol	1.52	2.00	1.66	2.34
	11.	Ergost-5-en-3-ol,(3β)-		0.46		
	12.	Ergost-5-en-3-ol,(3β,5α)-		0.15		
	13.	β-Sitosterol		2.49		
	14.	δ-8(14)-stigmastenol		0.61		
	15.	Campesterol			0.17	
	16	Dihydrochondrillasterol	0.09		0.09	
	17.	γ -Sitosterol				0.75
Terpenoids	18.	Cyperene	0.12			
	19.	Caryophyllene	0.10		1.02	0.42
	20.	β-Cubebene	0.07	0.06		
	21.	Germacrene D	0.20	0.41		
	22.	Caryophyllene oxide	1.69	1.65	0.55	0.97
	23.	Phytol	0.55	1.84		
	24.	Squalene	0.64	0.63		
	25.	α-Amyrin	0.60			0.21
	26.	12-Oleanen -3-yl-acetate(3α)	0.31			
	27.	α- Amyrenyl acetate	0.50			
	28.	Lupenyl acetate	0.24			
	29.	Trans-β-Caryophyllene		1.47		
	30.	Neophytadiene		0.75	0.78	
	31.	α-Cubebene			0.55	0.34
	32.	β-Amyrin			0.10	
	33.	Sitostenone				0.08
Fatty Acids	34.	Lauric acid	0.31	0.28	1.54	0.32
	35.	Myristic acid	0.45		0.93	
	36.	Palmitic acid	2.66	3.33	3.32	4.32
	37.	Linoleic acid	5.80		5.30	
	38.	Stearic acid			1.70	1.80
	39.	Margaric acid				0.39
	40.	Linolenic acid				3.68
	41.	Eicosanoic acid				0.22
	42.	Behenic acid				0.38

Table 1 *(continue)*

Class of compounds		Compounds name	Composition (%)			
			Flowers	Leaves	Stems	Roots
	43.	Vitamin E	0.65	0.12	0.21	0.24
	44.	γ-Tocopherol		0.04		
	45.	β-Monolinolein	3.81	1.72	0.38	0.74
	46.	2-Monolinolenin		3.92		
	47.	Lauric acid, methyl ester	0.11		0.24	
	48.	Palmitic acid, methylester	1.54	2.59	0.91	0.99
	49.	Linoleic acid, methylester	3.75	2.01	0.55	0.58
	50.	9,12,15-Octadecatrienoic acid, methyl ester		3.72		
	51.	Stearic acid, methylester	1.20	4.39		
	52.	Linolenic acid				0.42
	53.	Eicosanoic acid, methyl ester	3.02			
	54.	Tricosanoic acid, methyl ester		0.60		
	55.	2-Cyclopentene-1,4-dione	0.02		0.37	0.06
	56.	Butyrolactone	0.08			0.06
	57.	1(3,6,6-trimethyl-1,6,7,7a-tetrahydrocyclopenta(c) pyran-1-yl ethanone	0.14			
	58.	Looplure	0.89			
	59.	1-Pentadecene	1.63	0.95		1.05
	60.	1,13-Tetradecadiene	0.69			
	61.	Butan-4-olide		0.12		
	62.	1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl-		0.12		
Others	63.	1-Undecanol		0.14		
	64.	9,12,15-Octadecatrien-1-ol		7.05		
	65.	Silikonfett se30 (grevels)		0.31		
	66.	2-Furanmethanol			0.48	0.12
	67.	Benzyl alcohol				0.41
	68.	Butanoic acid, 4-hydroxy			0.19	
	69.	1,2-cyclopentanedione			1.28	
	70.	Cis-7-Dodecen-1-yl acetate			0.74	
	71.	1-Nonadecene			2.88	
	72.	*Spiro*[*2.4*]*heptane, 1,5-dimethyl-6-methylene-*			0.26	
	73.	Tricosanoic acid, methyl ester			0.07	
	74.	Lignoceric acid, methyl ester			0.26	
	75.	Furfural				0.76
	76.	2(3H)-Furanone, 5-methyl-				0.05
	77.	2-Cyclopenten-1-one, 2-hydroxy-				0.13
	78.	2-Furancarboxaldehyde, 5-methyl-				0.40
	79.	2-Furancarboxaldehyde, 5-(hydroxymethyl)-				11.48
	80.	1,11-Dodecadiene				0.66
	81.	2-Tridecanone				0.40
	82.	Cyclononasiloxane, octadecamethyl-				0.95
	83.	Nonanoic acid, 9-(3-hexenylidenecyclopropylidene)-, 2-hydroxy-1-(hydroxymethyl)				0.31

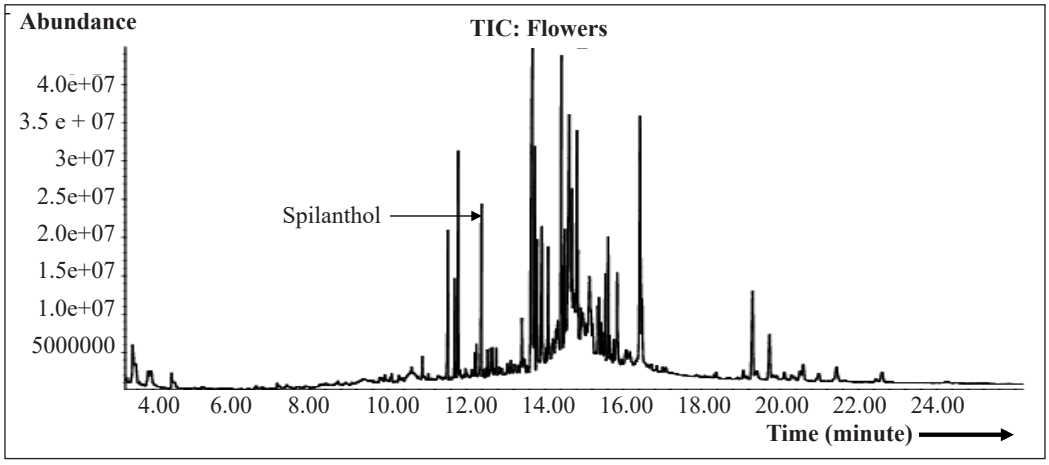

Figure 1. Chromatogram of water extract of *Acmella uliginosa* (Sw.) Cass. flowers

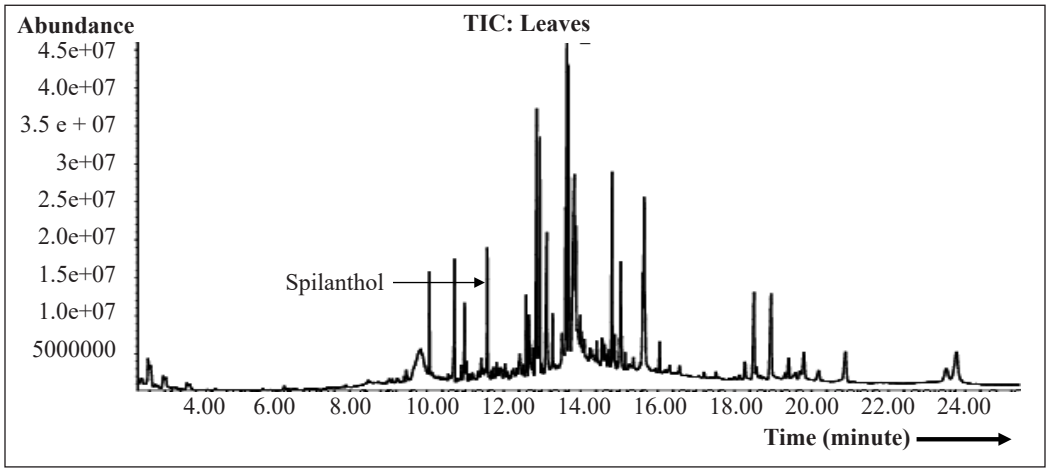

Figure 2. Chromatogram of water extract of *Acmella uliginosa* (Sw.) Cass. leaves

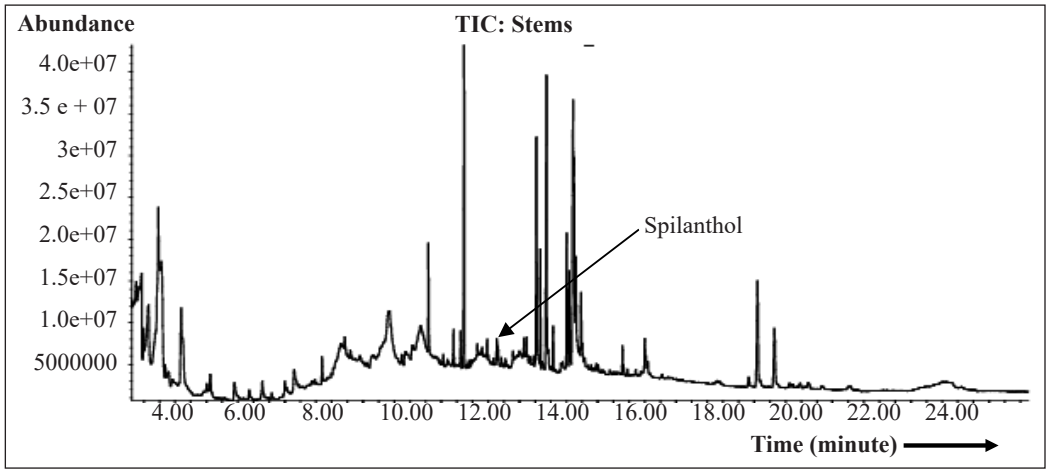

Figure 3. Chromatogram of water extract of *Acmella uliginosa* (Sw.) Cass. stems

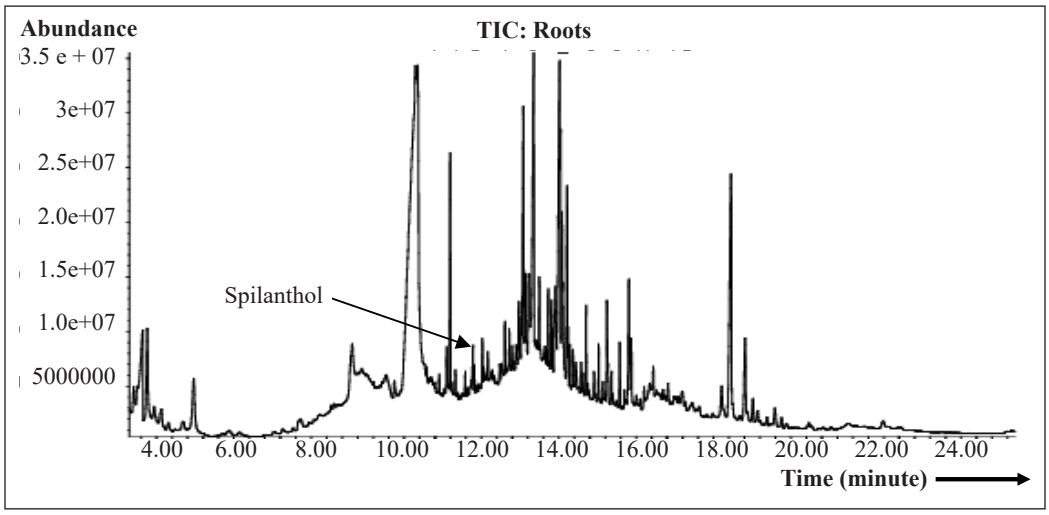

Figure 4. Chromatogram of water extract of *Acmella uliginosa* (Sw.) Cass. roots

of *A. uliginosa*. The highest percentage of alkaloids was found in leaves (8.42%), followed by flowers (6.08%), while lower in the root (3.71%) and stems (2.74%). The terpenoids were the main chemical class that were identified from leaves (6.81%).

Out of 83 identified chemical compounds, only nine chemical compounds were present in the water extracts of all parts of the plant which were spilanthol, stigmasterol, caryophyllene oxide, lauric acid, vitamin E, β-monolinolein, palmitic acid, palmitic acid methyl ester and linoleic acid methyl ester. All these chemical compounds are known to have a range of biological properties. Spilanthol is known to possess local analgaesic effect and various biological properties such as neuroprotective, antioxidant, antimutagenic, anticancer, antimicrobial and anti-inflammatory activities (Barbosa et al., 2016). A recent study of stigmasterol indicated that this steroid has antiangiogenic (Michelini et al., 2016) and antinociceptive properties (Walker et al., 2017). While the study of caryophyllene oxide showed that this terpenoid compound had anticancer and analgaesic properties (Fidyt et al., 2016).

Flowers

GC-MS analysis of water extract of *A. uliginosa* flowers showed that 34 chemical compounds were identified, and the main biologically active classes were alkaloids (7.51%) and terpenoids (5.02%). This finding was in agreement with a previous study of n-hexane extract of *A. uliginosa* flowers obtained from Indonesia (Maimulyanti & Prihadi, 2016). Both studies indicated that alkaloids and terpenoids were the main biologically active classes identified in *A. uliginosa* flowers. Interestingly, the major compound identified from *A. uliginosa* flowers in both regions was spilanthol. However, a study by Maimulyanti and Prihadi (2016) found that the percentage of spilanthol was 37.80%, whereby the percentage of the spilanthol in the

current study was only 6.08%. Other than spilanthol, N-(2-Phenylethyl) (2E,6Z,8E)-decatrienamide was also identified in both studies. The main terpenoid identified in the present study was caryophyllene oxide (1.69%), whereby α-Pinene was the main terpenoid identified from the n-hexane extract of *A. uliginosa* flowers (Maimulyanti & Prihadi, 2016). Only two terpenoids were identified from both studies which were caryophyllene and β-Cubebene. Other minor biologically active classes found in the present study were steroids (1.82%) and phenolic compounds (0.03%). Steroids were not identified in the n-hexane extract of *A. uliginosa* flowers obtained from Indonesia. Phenolic compounds were found in both studies; however, the compounds differed.

Leaves

The results of water extract of *A. uliginosa* leaves showed that 34 chemical compounds were identified and spilanthol was the main compound (8.42%), followed by 9,12,15-octadecatrien-1-ol (7.05%). Alkaloid (8.42%), terpenoids (6.81%) and steroids (5.71%) were found to be the major biologically active classes in the water extract of *A. uliginosa* leaves. Steroids have been identified to have the highest percentage in the leaves compared to other parts. Similar to the water extract of *A. uliginosa* flowers, alkaloid and terpenoids were the main identified bioactive classes in water extract of *A. uliginosa* leaves. This finding is in agreement with the previously published study on chemical compounds of the n-hexane and ethyl acetate extracts of *A. uliginosa* leaves from Indonesia (Maimulyanti et al., 2016). Study by Maimulyanti et al. (2016) found that alkaloids and terpenoids were the main biologically active classes identified in both organic solvent extracts. Spilanthol was the major alkaloid identified in both studies, whereby caryophyllene oxide was the main terpenoid identified in the current study and caryophyllene epoxide was the main terpenoid identified in the n-hexane and ethyl acetate extracts of *A. uliginosa* leaves (Maimulyanti et al., 2016). This finding contradicted with the methanol extract of *A. uliginosa* leaves from Indonesia (Maimulyanti et al., 2016) and Nigeria (Uraku, 2016). Both phytochemical studies on the methanol extracts of *A. uliginosa* leaves from both regions showed that alkaloids, phenolics and steroids were not found. Terpenoid was found in the methanol extract of *A. uliginosa* from Indonesia but was absent in the methanol extract of *A. uliginosa* from Nigeria. Neophytadiene was the only identified terpenoid in the methanol extract of *A. uliginosa* from Indonesia. In the present study, the following terpenoids β-Cubebene, germacrene D, caryophyllene oxide, phytol, squalene, trans-β-Caryophyllene and neophytadiene were found in the water extracts of *A. uliginosa* leaves. This findings were contradicted with the previous study of phytochemical screening of water extract of *A. uliginosa* leaves from West Africa (Lagnika et al., 2016). A study by Lagnika et al. (2016) found that terpenoids, as well as alkaloids, were not identified in water extract of *A. uliginosa* leaves.

Stems

The water extract of *A. uliginosa* stems revealed the presence of 30 compounds; the main group was fatty acids (12.79%). The main identified compound was linoleic acid (5.30%) and followed by palmitic acid (3.32%). Spilanthol (2.74%) was the least compound identified in the water extract of *A. uliginosa* stems compared to the other parts. Similar to the water extract of *A. uliginosa* leaves and stems, spilanthol was the only alkaloid identified. The other bioactive groups that had been identified were terpenoids (3.00%), phenolic compounds (2.51%) and steroids (1.92%). The major terpenoid identified was caryophyllene (1.02%), followed by neophytadiene (0.78%). The main phenolic compound identified was p-Vinylguaiacol (1.65%), whereby stigmasterol was the main steroid identified in the water extract of *A. uliginosa* stems. Until present, no phytochemical analysis of the *A. uliginosa* stems has been attempted before. However, study on the other species of *Acmella* which is *Acmella oleraceae* Murr. (Asteraceae) showed that terpenoids were not found in the methanolic extract of its stems (Abeysiri et al., 2013).

Roots

The results obtained from GC-MS analysis of water extract of *A. uliginosa* roots showed that 32 compounds were identified. Similar to the water extract of *A. uliginosa* stems, fatty acid was the main identified class (11.11%). The main identified chemical compounds in the water extract of *A. uliginosa* roots was 2-Furancarboxaldehyde, 5-methyl- (11.48%), followed by palmitic acid (4.32%), and spilanthol (3.71%). The 2-Furancarboxaldehyde, 5-methyl- was only identified in water extract of *A. uliginosa* roots. The other bioactive groups that were identified in the water extract of *A. uliginosa* roots were steroids (3.09%) and terpenoids (2.02%). Similar to *A. uliginosa* stems, no previous study on the *A. uliginosa* roots had been done so far. A previous study of the other genus *acmella* which is *Spilanthes acmella* Murr. collected in India showed that the phenolic content was present in the roots (Tanwer et al., 2010). This finding is contradicted with the current study as in our study, the phenolic compound was not identified in the roots.

CONCLUSION

The detection of chemical constituents in the water extracts of various parts of *A. uliginosa* showed the presence of bioactive compounds such as alkaloids, phenolic compounds, steroids and terpenoids. Both flowers and leaves were mostly composed of alkaloids, while the stems and roots were characterised by higher amounts of fatty acids. These compounds are known for their bioactivities. This study revealed that the water extracts of *A. uliginosa* were good sources of many bioactive compounds and it is suggested that all parts of water extract of *A. uliginosa* may have pharmacological potential and high therapeutic value. Further experiments are needed to prove that all parts of water extract of *A. uliginosa* do have therapeutic effects.

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CONFLICT OF INTEREST

Authors declare that there is no conflict of interest regarding the publication of this article.

STATEMENT OF HUMAN AND ANIMAL RIGHTS

This article does not contain any studies with human or animal subjects performed by any of the authors.

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