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Characterization of N-Alkylamides of *Spilanthes acmella var calva* by Using High Performance Liquid Chromatography Electrospray Ionization Ion Trap Mass Spectrometry

Sajan L. Shyaula[1]*, Ankita Acharya[2], Muhammad Iqbal Choudhary[3], Syed Ghulam Musharraf[3], Rukesh Maharjan[3], and Arslan Ali[3]

[1]*Faculty of Science, Nepal Academy of Science and Technology, Khumaltar, Lalitpur, Nepal*
[2]*Central Department of Chemistry, Tribhuvan University, Kirtipur,Nepal*
[3]*H.E.J. Research Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi-75270, Pakistan*

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Abstract : *Spilanthes acmella var calva* popularly known as 'Marauti' in Nepal, are widely used by different communities as the food ingredients in their traditional cuisines. It displays a unique tingling sensation mainly due to N-alkylamides when applied to mucosal surfaces. *Spilanthes* served as a remedy for alleviating toothache, throat infections and other oral ailments. N-Alkylamide profilings of *Spilanthes* extracts were performed using a gradient reversed phase high performance liquid chromatography/ electrospray ionization ion trap mass spectrometry (HPLC/ESI-MS) method. A total of 33 peaks were analyzed and 16 N-alkylamides were identified. Seven compounds (1, 5, 6, 8, 15, 16 and 25) were N-isobutylamides and nine compounds (4, 7, 10, 11, 12, 14, 17, 18 and 20) were 2-phenylethylamides.

Keywords : *Spilanthes acmella,* N-Alkylamides, N-Isobutylamides, 2-Phenylethylamide, Ion trap

Introduction

Spilanthes popularly known as 'Marauti' in Nepal, is a native plant in tropics regions of Asia and South America. According to annonated check list of flowering plants of Nepal, 4 species namely *Spilanthes acmella, S. calva, S. oleracea* and *S. paniculata* occur in Nepal.[1] It is a member of Asteraceae family. It has large cylindrical discoid capitula which owe a unique golden yellow color with the red tip. Several species of *Spilanthes* are widely used by different communities as the food ingredient in their traditional cuisines. Extracts of *Spilanthes* are also used as a food-flavoring agent in the cuisines all over the world. It is also used as condiment, appetite stimulant and as flavouring and seasoning agents in green salads.[2] *Spilanthes* served as a remedy for alleviating toothache, throat infections and other oral ailments.[3,4] *Spilanthes* attained pharmacological importance owing to its ability to display a unique property that elicits a distinct tingling sensation when applied to mucosal surfaces. *Spilanthes* derived products, possessing analgesic, cleansing, detoxification and oral hygienic properties are also available in the market. Extracts of several *Spilanthes* species were later shown to exhibit broad spectrum of biological activities such as antioxidant, gastroprotective, anti-proliferative, immuno-modulatory, diuretic, vasorelaxant, anti-inflammatory, enzyme inhibitory, antimicrobial, insecticidal and larvicidal properties which had led to attraction by several pharmaceutical industries to launch a variety of heath care products.[5-8]

S. acmella contains amino acids, triterpenoids, phytosterol, myricyl alcohol, and alkaloids especially rich in N-alkylamides.[5-8] N-Alkylamides are unique in exhibiting strong pungent taste accompanied by tingling and sialagogic effects. There are many literatures that disclosed the biological importance of spilanthol which is one of the major active ingredients in *Spilanthes* plant. Apart from spilanthol, the arrays of pharmacologically active N-alkylamides were isolated from *Spilanthes*. They are basically fatty acid amides formed from a fatty acid chain and a decarboxylated amino acid, probably by a condensation

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*Reprint requests to Sajan L. Shyaula
https://orcid.org/0000-0002-9951-8090
E-mail: shyaulsajan@gmail.com

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reaction. Chemically distinct amine moieties found coupled to a variety of fatty acids that lead to occurrence of diverse N-alkylamides in plants.

The essential oil and low polar fractions of different *Spilanthes* species have been analyzed by GC-MS.[9,10] The fractions are predominant of hydrocarbons and terpene derivatives. Saponifications of extracts have been further carried prior to GC-MS analysis for fatty acid analysis.[11] However, HPLC/ESI-MS is considered an excellent technique for comprehensive analysis of the plant extracts. It has advantage over GC-MS for analysis of both polar and non-volatile constituents. Compounds were identified upon retention time, molecular weight and fragmentation patterns. N-alkylamide profiling of an ethanolic *Spilanthes* extract was performed using a gradient reversed phase high performance liquid chromatography/electrospray ionization ion trap mass spectrometry (HPLC/ESI-MS) method on an embedded polar column by Boonen et al. and Sharma et al.[12,13] Investigation of UV-B irradiation effect on identified N-alkylamides from *S. oleracea* extract was monitored by UHPLC-DAD-ESI-MS/MS.[14] Comparative analysis of bioactive N-alkylamides produced by tissue culture raised versus field plantlets of *S. ciliata* was determined using LC-Q-TOF (HRMS).[15] A liquid chromatography–electrospray ionisation–mass spectrometry (HPLC/ESI-MS) method was developed for rapid identification and quantification of the N-alkylamide spilanthol from *S. acmella*.[16] The chemical composition of leaves, flowers and stems of *Spilanthes* cultivated in hydroponic and conventional systems were investigated by LC-MS analysis.[17]

The extract of *S. acmella* has promising potential in the cosmetic industry, especially in oral health. Therefore, efforts are needed to explore, standardize, and validate traditional medicines for their potency, safety, and efficacy in order to bring them to market as the main line therapeutics and to establish a relation between pharmacology and chemistry of the plant. N-alkylamide profiling of *Spilanthes* extracts were performed using high performance liquid chromatography/ electrospray ionization ion trap mass spectrometry (HPLC/ESI-MS) method in this study. The aim of this study was to screen and characterize the bioactive phytochemicals, evaluating different solvents' efficiency for extraction of these bioactive phytochemicals from *S. acmella*. A total of 33 peaks were analyzed and 16 N-alkylamides were identified. The results of present study could be of significant scientific and practical importance for scientists and researchers for its claimed purpose.

Experimental

Collection of samples

The flowers of *Spilanthes acmella var calva* were collected from the Gulariya, Ghorahi Dang, Nepal at the heights of 700 m in the months of April 2022. Plant materials were authenticated by comparision with voucher specimen (Vo no. 14580) deposited at National Herbarium and Plant Laboratories, Godawari, Lalitpur, Nepal.

Chemicals and equipments

Analytical grade solvents (Fisher Scientific) were used for extraction and HPLC grade solvents were used for chromatographic analysis (Aldrich-Sigma, USA). The separation and analysis of metabolites were performed on a hyphenated HPLC/ESI-MS/MS. The HPLC system employed consisted of an analytical HPLC (Thermo/ Dionex) coupled to amazon speed ion trap low-resolution mass spectrometer (Bruker Daltonics). Nitrogen was used as the nebulizing gas at a pressure of 20 psi and a flow rate of 6 L/min. The heated capillary and the voltage were maintained at 180°C and 4.5 kV, respectively. Full scan MS of the compounds were measured from *m/z* 100 to 3000 amu. Collision-induced fragmentation experiments were performed in the ion trap using helium as the collision gas. The collision energy was set at 100%. MS data were acquired in both the positive and negative ionization mode. MS/MS data were acquired in full-scan mode and auto-MS^n mode was used to acquire the fragments. The data was acquired in compass hystar and trap control. The acquired data were analyzed with Bruker compass data analysis (4.2).

Chromatographic separations were carried out on Agilent porosil EC-18 MS column (2.7 µm × 1.8 × 50 mm). The mobile phase comprised two solvents, namely, 0.1% Formic acid in ACN (Solvent A) and 0.1% Formic acid in H_2O (solvent B). In a gradient program which was initially 95:5 (B:A) and changed to 80:20 (B:A) at 1 min, 70:30 (B:A) at 1 min, 60:40 (B:A) at 3 min, 50:50 (B:A) at 8 min, 40:60 (B:A) at 10 min, 20:80 (B:A) at 13 min, 10:90 (B:A) at 16 min, and finally 95:5 (B:A) at 23 min. The flow rate was 0.3 mL/min, and the sample injection volume was 10 µL.

Extraction and fractionation

S. acmella flowers (1.8 kg) were crushed into the powder before extraction. These powdered samples were extracted by percolation in methanol to get the crude methanolic extract (AME 211.21 g). The crude extract was suspended in Milli-Q water and then further successively fractionated in different solvents by the solvent-solvent extraction process. Separation of compounds based on their acidity or basicity by exploiting their solubility in different solvents was applied. The acid-base extraction of alkaloids was one of the effective, selective and versatile methods commonly applied in the plant matrix. First it was extracted with ethyl acetate at acidic condition (pH 3 by adding CH_3COOH) to obtain 88.84 g extract (AETHA). Then the suspension was basified by adding NH_4OH to maintain pH 10 and extracted with ethyl acetate to obtain basic extract 3.18 g (AETHB). The suspension was then neutralized (pH 7) and extracted with *n*-butanol to obtain 23.44 g (ABU).

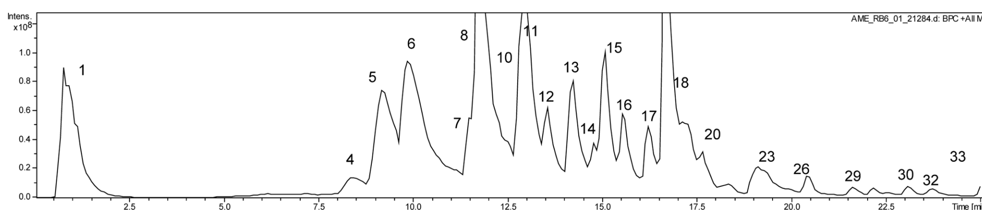

Figure 1. Chromatogram of crude methanolic extract of *S. acmella*.

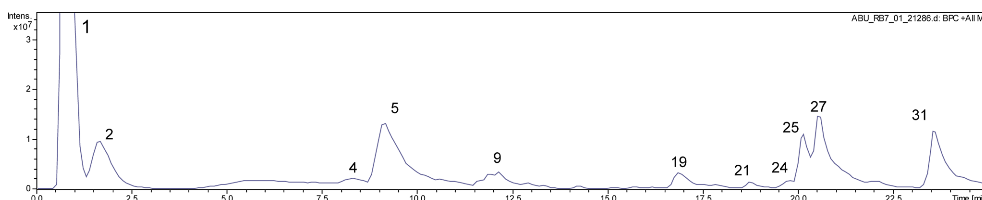

Figure 2. Chromatogram of butanolic fraction of *S. acmella*.

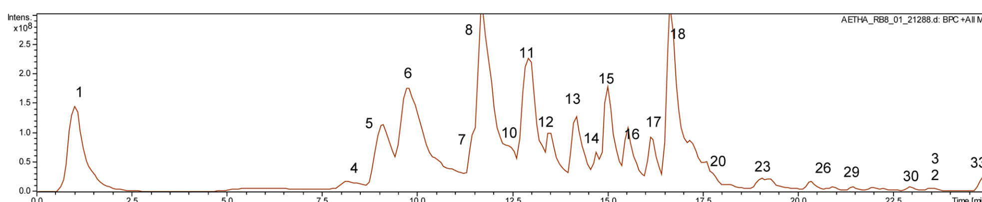

Figure 3. Chromatogram of acidic dichloromethane fraction of *S. acmella*.

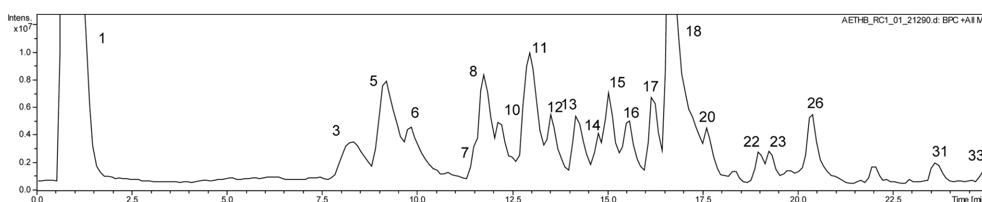

Figure 4. Chromatogram of basic dichloromethane fraction of *S. acmella*.

Results and discussions

Chromatograms of *S. acmella* extracts in different solvents, recorded by using UPLC-ESI-MS/MS method, were presented in Figure 1-4. The obtained peaks of identified N-alkylamides are labeled numerically in accordance with their retention times (Table 1). The untargeted metabolites were identified using a UPLC-ESI-MS/MS method to compare and characterize the main compounds present in *S. acmella*. The compounds were characterized according to the retention time (t_R) and tandem mass fragmentation pattern. The mass information with chromatographic data and other details related to the proposed compounds in ESI$^+$ mode of different fractions are summarized in Table 1. Similarly the mass spectrometric data in ESI$^-$ mode of different fractions eluted at different retention time are shown in Table S1. A total of 33 peaks were analyzed and 16 N-alkylamides metabolites were tentatively identified, seven (1, 5, 6, 8, 15, 16 and 25) were N-isobutylamides and nine (4, 7, 10, 11, 12, 14, 17, 18 and 20) were 2-phenylethylamide (Figure 5). The MS/MS spectra of compounds eluted at different retention time in ESI$^+$ mode with its fragmentation ions were presented in supplementary Figure S1-S33. The compounds peaks at *m/z* 238 $[M+H]^+$ and 236 $[M+H]^+$ eluted at 1.01 and 9.18, min respectively, were common to all fractions.

N-Alkylamides are a promising group of naturally occurring bioactive metabolites. Identification of N-alkylamides can be revealed by different characteristic fragments of signature ions formed by collision induced fragmentation at similar fragmentation sites (Figure S1-S33). In isobutyl amide, a major N-alkylamide fragment often formed by dissociation of the C-N bond is the acyllium ion, with *m/z* value indicative for the amount of carbon atoms in the alkyl chain (Figure 6). Similarly fragments formed by cleavage of C-C bond, R(CO)-N bond, C-N bond and NC-R bond

Table 1. Chromatographic and mass spectrometric data of *S. acmella*

Ref	t_R (min)	mw	Measured *m/z*		Product ions (*m/z*) and relative intensity %	Identification	Samples			
							AME	ABU	AETHA	AETHB
				$[2M+H]^+$						
1	1.01	237	238		165 (100), 147 (30), 137(45), 123 (65)	(6Z,8E)-N-isobutylundeca-6,8-dienamide (**1**)	+	+	+	+
2	1.71	235	236		163 (100), 137 (20)	-		+		
3	8.24	273	274		256 (100), 230 (5)	-				+
4	8.45	267	268		250 (30), 240(30), 222(20), 147(50), 121(40), 105 (100)	3-(hexa-3,5-diyn-1yl)-N-phenethyloxirane-2-carboxamide (**4**)	+	+	+	
5	9.18	235	236	471	163(100), 137 (20)	(2E,6Z,8E)-N-isobutylundeca-2,6,8-trienamide (**5**)	+	+	+	+
6	9.87	229	230	459	174 (30), 157(20), 131(100), 117 (20)	(2E,4Z)-N-isobutylundeca-2,4-dien-8,10-diynamide (**6**)	+		+	+
7	11.48	299	300		244(5), 232(5), 218 (10), 204(8), 190(5), 161(25), 122(95), 105 (100)	(8E,10E)-N-phenethyldodeca-8,10-dienamide (**7**)	+		+	+
8	11.79	221	222	443	149 (10), 123(100), 166 (5)	(2E,6Z,8E)-N-isobutyldeca-2,6,8-trienamide (spilanthol) (**8**)	+		+	+
9	12.15	431	432		415(100), 397(5), 295(5), 119 (5)	-		+		
10	12.53	277	278		174(5), 157(20), 131(100), 105 (80)	(2E,4E)-N-phenethylundeca-2,4-dien-8,10-diynamide (**10**)	+		+	+
11	12.96	289	290		272(100), 148 (5), 122(40), 105(35)	(E)-9-hydroxy-N-phenethyldec-2-enamide (**11**)	+		+	+
12	13.56	269	270		242 (5), 216 (5), 148(60), 123 (100), 105(80)	(2E,6E,8E)-N-phenethyldeca-2,6,8-trienamide (**12**)	+		+	+
13	14.24	247	248	495	175(25), 149(100), 142(55), 107(25)	-	+		+	+
14	14.78	271	272		148 (35), 122(75), 105 (100), 151(25)	(6E,8E)-N-phenethyldeca-6,8-dienamide (**14**)	+		+	+
15	15.09	225	226		170(100), 153 (20), 107(5)	(E)-N-isobutyldec-2-enamide (**15**)	+		+	+
16	15.54	249	250		194(40), 177(15), 167(100), 149(10), 135(15), 109(25)	(5E,7E,9E)-N-isobutyldodeca-5,7,9-trienamide (**16**)	+		+	+
17	16.23	273	274		272(100), 216(5), 153(55), 122(50), 105(50)	(E)-N-phenethyldec-7-enamide (**17**)	+		+	+
18	16.68	287	288		246(5), 232(15), 218(15), 204(10), 190(10), 167(5), 148(20), 122(100), 105(90)	(E)-N-phenethylundec-8-enamide (**18**)	+		+	+
19	16.88	278	279		205(50), 149(100)	-		+		
20	17.66	301	302		246(5), 232(15), 218(15), 204(10), 179(50), 161(20), 148(15), 122(100), 105(95)	(E)-N-phenethyldodec-8-enamide (**20**)	+		+	+
21	18.75	279	280		263(100), 245(80)	-		+		

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Table 1. (Continued) Chromatographic and mass spectrometric data of *S. acmella*

Ref	t_R (min)	mw	Measured *m/z*	Product ions (*m/z*) and relative intensity %	Identification	Samples			
						AME	ABU	AETHA	AETHB
22	18.97	355	356	337(100), 263(70), 245(60),	-				+
23	19.14	308	309	277(30), 259(75), 241(70), 179(100)	-	+		+	+
24	19.73	282	283	265(100), 247 (55)	-		+		
25	20.17	255	256	200(10), 186(5), 172(10), 158(10), 144(10), 130(15), 116(30) 102(100)	N-isobutyldodecanamide (**25**)		+		
26	20.41	312	313	293(40), 279(100), 257(25), 109(25)	-	+		+	+
27	20.52	281	282	265(99), 247 (100)	-		+		+
28	20.93	608	609	591(100), 531(40)	-			+	
29	21.63	592	593	533(100), 565(5)	-	+		+	
30	23.10	292	293	261(95), 243(100)	-	+			
31	23.63	283	284	341(100)	-		+		+
32	23.76	622	623	605(100), 574(5), 545(35)	-	+		+	
33	25.02	606	607 1213	575(5), 547(100)	-	+		+	+

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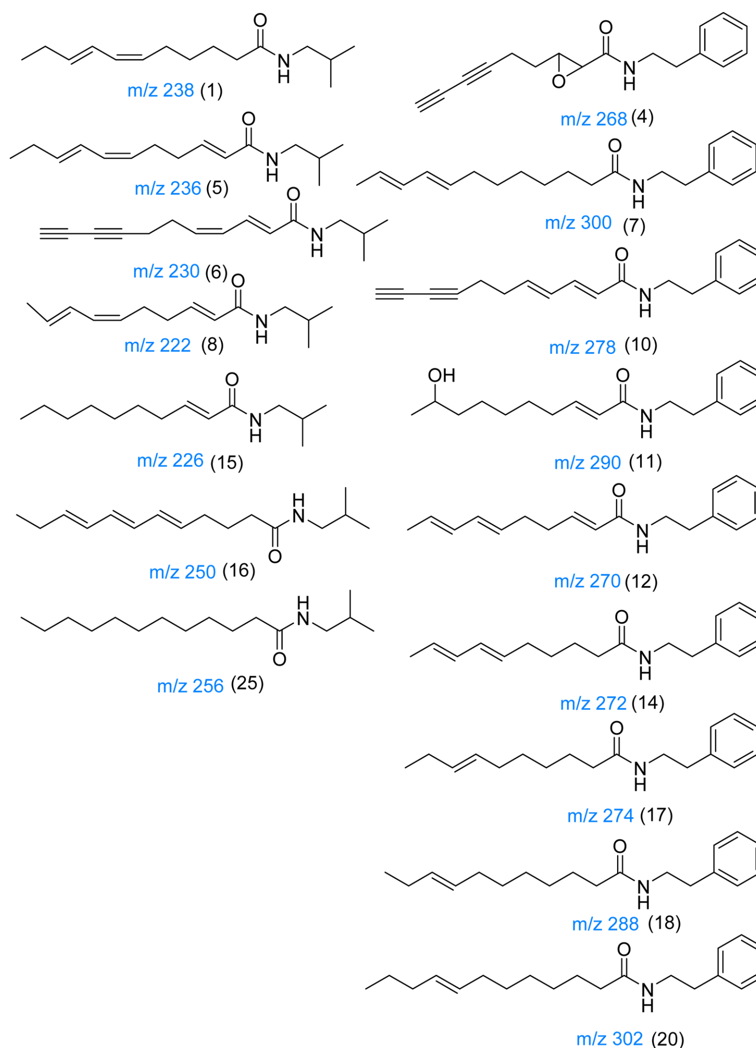

Figure 5. Structures of N-alkylamides from *S. acmella* determined by LC MS.

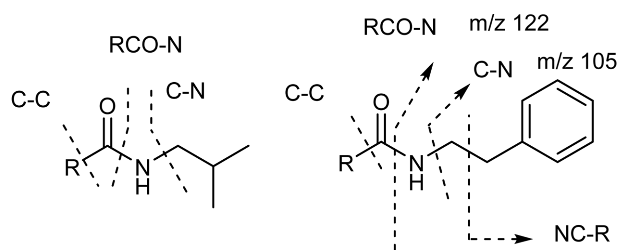

Figure 6. Major characteristic fragmentation pathways.

were observed in the fragmentation patterns. In phenyl ethyl amide (N-alkylamides), characteristic ion peaks at *m/z* 122 and *m/z* 105 for 2-phenylethan-1-aminium and phenylethan-1-ylium, respectively confirms the phenyl ethyl moiety.

LC-MS analysis was also performed in negative modes. The chromatograms in negative ionization mode are shown in supplementary figures (Figure S34-S37). The numbers of metabolites detected in ESI^- mode are considerably different than in ESI^+ mode (Figure S38-S68).

Mass Fragmentation of (6Z,8E)-N-isobutylundeca-6,8-dienamide (1), (2E,6Z,8E)-N-isobutylundeca-2,6,8-trienamide (5), (2E,6Z,8E)-N-isobutyldeca-2,6,8-trienamide (8) and (E)-N-isobutyldec-2-enamide (15)

(6Z,8E)-N-isobutylundeca-6,8-dienamide (**1**), *m/z* 238 $[M+H]^+$ eluted at 1.10 min, (2E,6Z,8E)-N-isobutylundeca-2,6,8-trienamide (**5**) *m/z* 236 $[M+H]^+$ eluted at 9.18 min, (2E,6Z,8E)-N-isobutyldeca-2,6,8-trienamide (**8**) (Spilanthol) *m/z* 222 $[M+H]^+$ eluted at 11.79 min and (E)-N-isobutyldec-2-enamide (**15**) *m/z* 226 $[M+H]^+$ eluted at 15.09 min showed similar fragmentation patterns. Fragmentation of amide bond give peak at *m/z* 165 for compound **1**, *m/z* 163 for compound **5**, *m/z* 149 for compound **8** and *m/z* 153 for compound **15** (Figure 7). Compound **1** differs from **5** by one unsaturated double bond and Compound **8** differs from

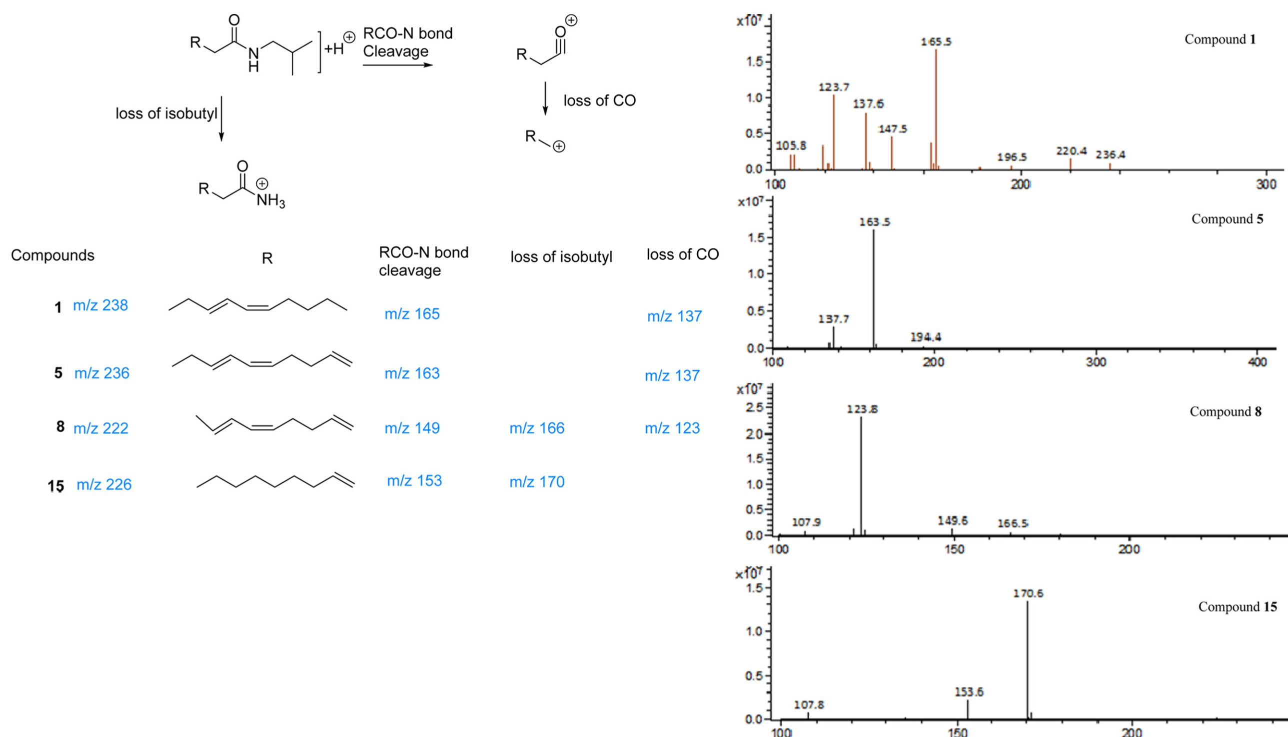

Figure 7. Major fragmentation pathway of compounds **1, 5, 8** and **15** in ESI-MS/MS.

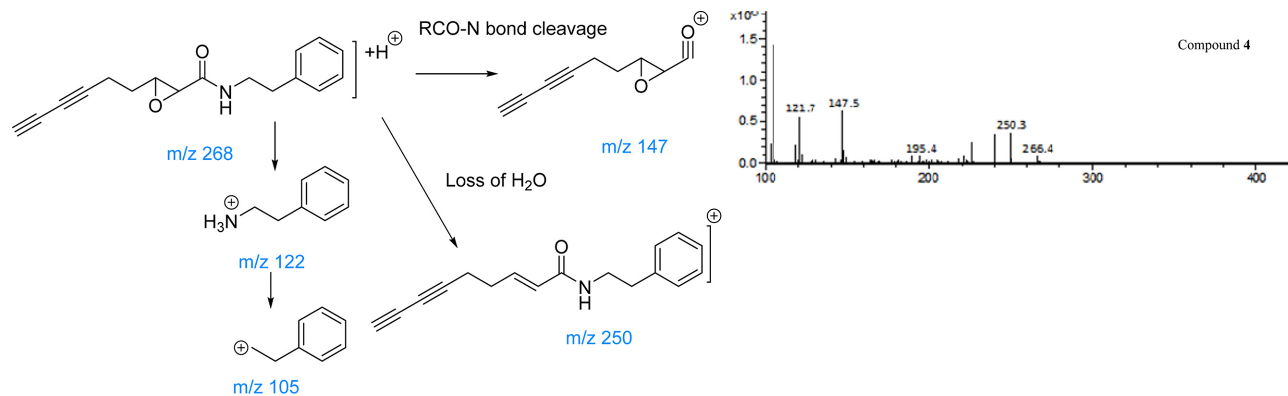

Figure 8. Fragmentation pathway of compound **4** in ESI-MS/MS.

15 by two unsaturated double bonds.[12] The 14 amu mass (extra CH_2 group) difference in compound **5**, in each fragment ions in comparison to compound **8** (spilanthol) confirmed it as homologous series of spilanthol, one of the most abundant compound from *Spilanthes*.[12] Further loss of carbonyl group showed peak at *m/z* 137 for compound **1** and **5** and *m/z* 123 for compound **8**. A peak at *m/z* 166 for compound **8** and peak at *m/z* 170 for compound **15** were observed by loss of isobutyl moiety.

Mass Fragmentation of 3-(hexa-3,5-diyn-1yl)-N-phenethyloxirane-2-carboxamide (4)

3-(hexa-3,5-diyn-1yl)-N-phenethyloxirane-2-carboxamide (**4**) at t_R 8.45 min was assigned to the peaks *m/z* 268 $[M+H]^+$ (Figure 8). It was reported by Savic et al. 2021.[14] N-phenyl ethyl group can be confirmed by peaks at *m/z* 122 and *m/z* 105 by cleavage of amide and consecutive loss of NH_3. The peak at *m/z* 147 was observed due to C-N bond cleavage of amide. Loss of water molecule give peaks at *m/z* 250.

Mass Fragmentation of (2E,4Z)-N-isobutylundeca-2,4-dien-8,10-diynamide (6)

(2E,4Z)-N-isobutyl 2,4-undecadiene 8,10-diynamide (**6**) at t_R 9.87 min was assigned to the peaks *m/z* 230 $[M+H]^+$

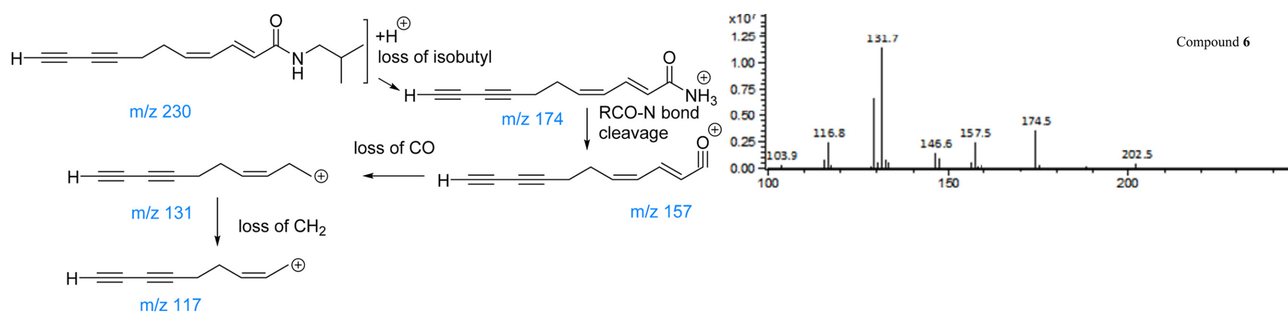

Figure 9. Fragmentation pathway of compound **6** in ESI-MS/MS.

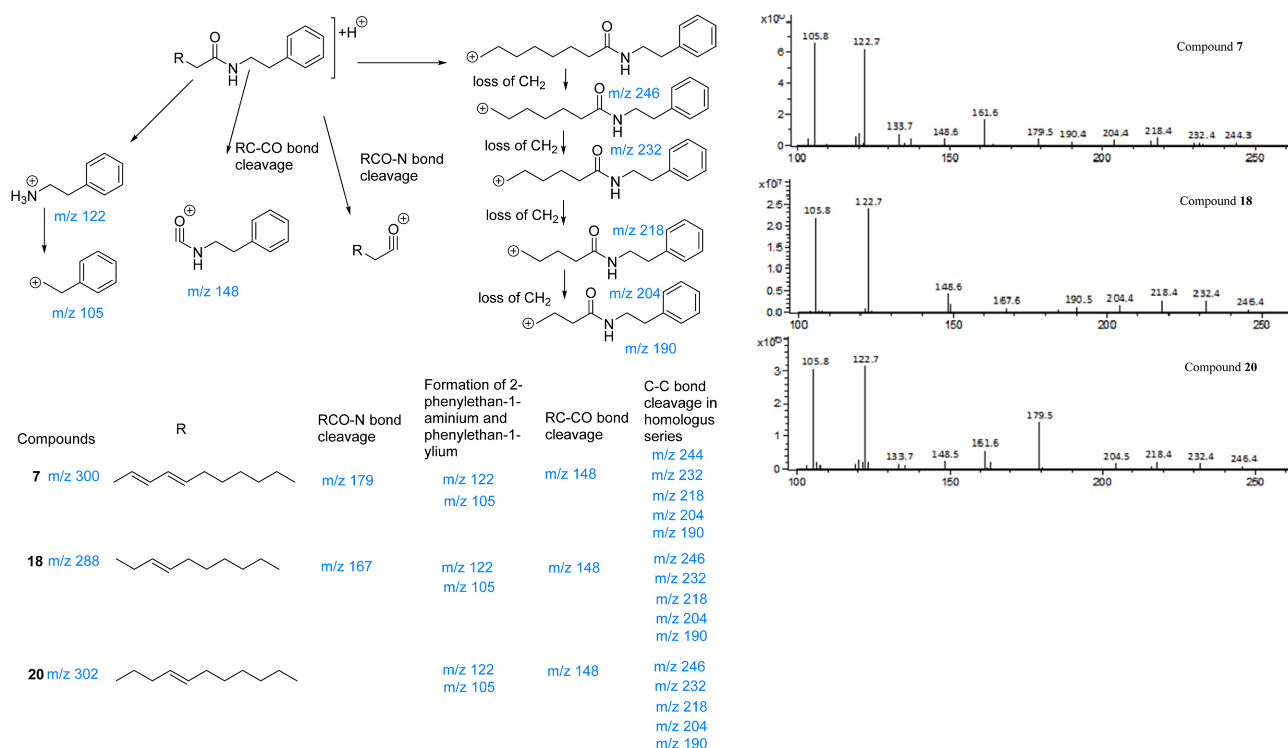

Figure 10. Fragmentation pathway of compounds **7, 18** and **20** in ESI-MS/MS.

(Figure 9).The loss of isobutyl group gives peak at *m/z* 174, cleavage of amide bond gives peak at *m/z* 157 and further loss of CO group gives peak at *m/z* 131.[14]

Mass Fragmentation of (8E,10E)-N-phenethyldodeca-8,10-dienamide (7) and (E)-N-phenethylundec-8-enamide (18) and (E)-N-phenethyldodec-8-enamide (20)

(8E,10E)-N-phenethyldodeca-8,10-dienamide (**7**) *m/z* 300 $[M+H]^+$ was eluted at 11.48 min (Figure 10). (E)-N-phenylethylundec-8-enamide (**18**) *m/z* 288 $[M+H]^+$ and (E)-N-phenethyldodec-8-enamide (**20**) *m/z* 302 $[M+H]^+$ were eluted at 16.68 min and 17.66 min . Compound **20** showed similar fragmentation pattern as that of compound **18** and it differs by extra CH_2 unit. The characteristic peaks at *m/z* 122 and *m/z* 105 were observed for N-phenyl ethyl group. Cleavage of amide bond gives peak at *m/z* 179 in compound 7 and at *m/z* 167 in compound **18**. The position of double bond can be ascertained by homologous series of mass peaks *m/z* at 190, 204, 218, 232 and 246. Cleavage of C-C bond adjacent to carbonyl group gives peak at *m/z* 148.

Mass Fragmentation of (2E,4E)-N-phenethylundeca-2,4-dien-8,10-diynamide (10)

(2E,4E)-N-phenylethylundeca-2,4-dien-8,10-diynamide (**10**) was eluted at 12.53 min with *m/z* 278 $[M+H]^+$ (Figure 11). Loss of phenyl ethyl group gives peak at *m/z* 174, consecutive loss of ammonia give peaks at *m/z* 157 and loss of CO group gives peak at *m/z* 131. The mass peak at *m/z* 105 was assigned to tropilium ion.

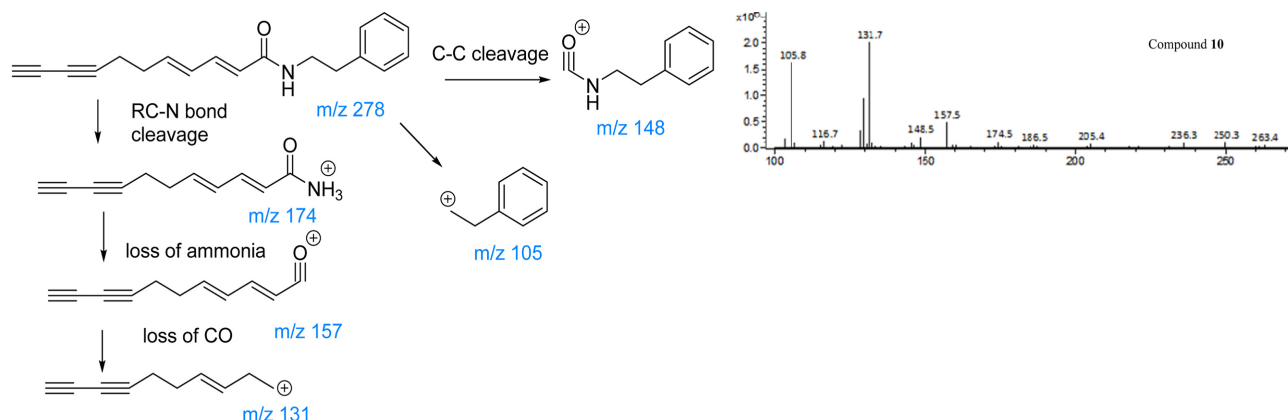

Figure 11. Fragmentation pathway of compound **10** in ESI-MS/MS.

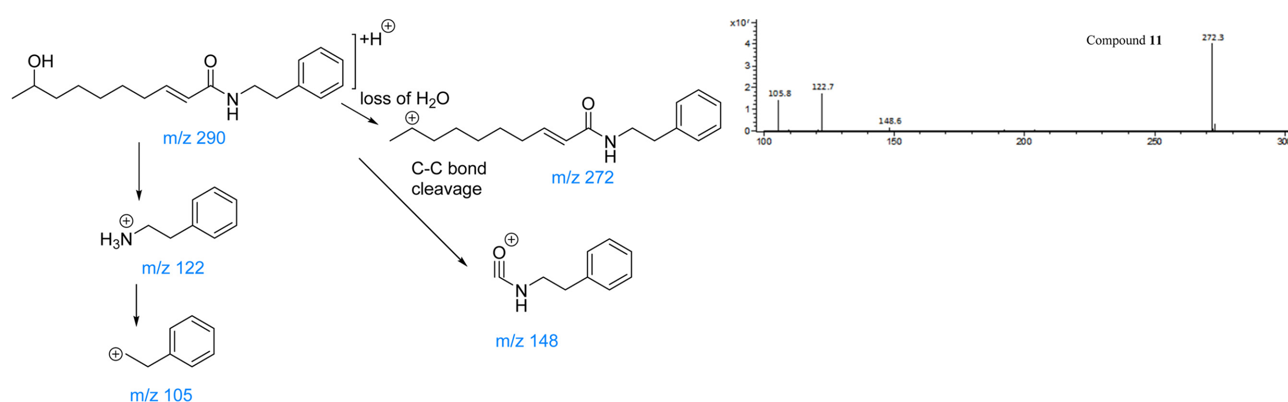

Figure 12. Fragmentation pathway of compound **11** in ESI-MS/MS.

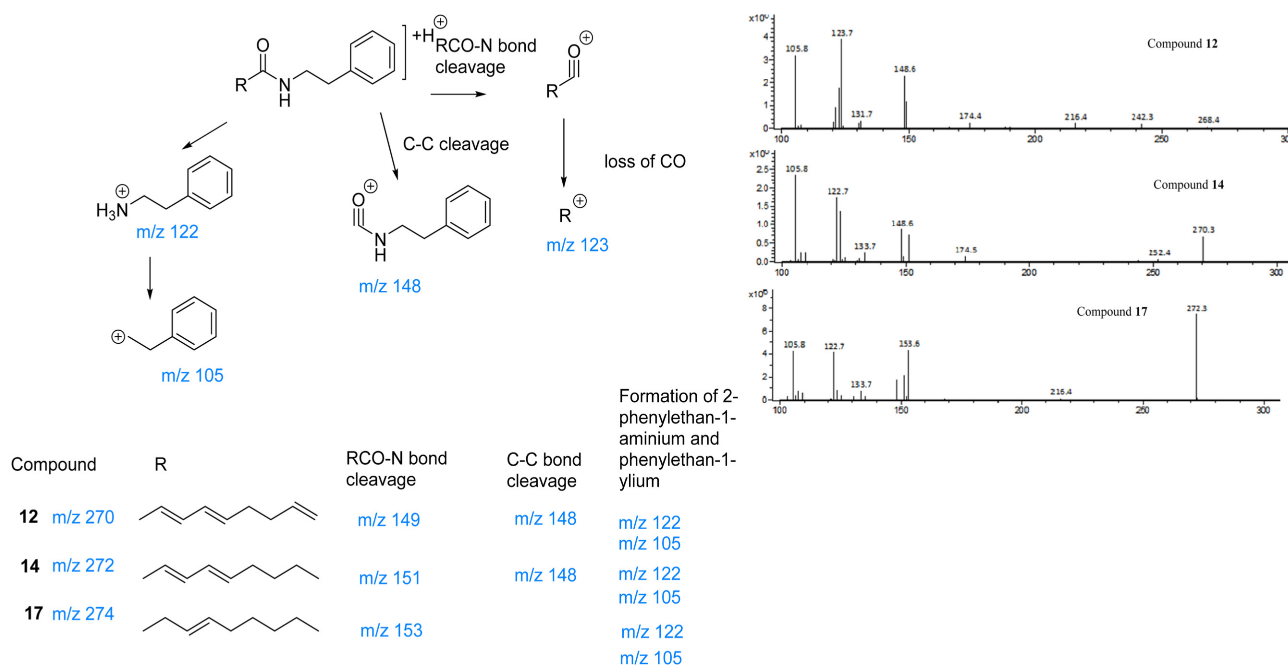

Figure 13. Fragmentation pathway of compounds **12, 14** and **17** in ESI-MS/MS.

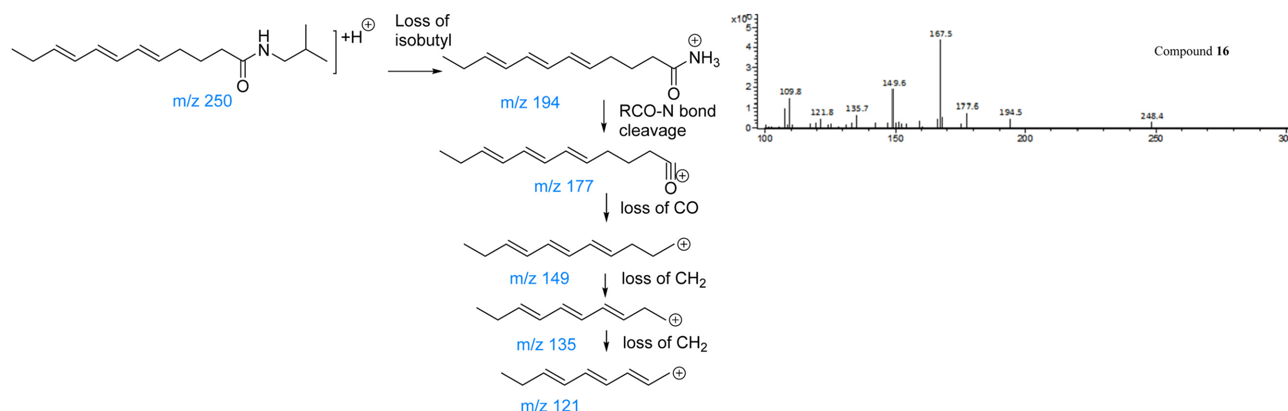

Figure 14. Fragmentation pathway of compound **16** in ESI-MS/MS.

Mass Fragmentation of (E)-9-hydroxy-N-phenethyldec-2-enamide (11)

(E)-9-hydroxy-N-phenylethyldec-2-enamide (**11**) *m/z* 290 $[M+H]^+$ eluted at 12.96 min (Figure 12). N-phenyl ethyl group was confirmed by characteristic peaks at *m/z* 122 and *m/z* 105. The loss of H_2O gives peak at *m/z* 272 which confirms the presence of hydroxyl group. The peak at *m/z* 148 was observed by cleavage of C-C bond adjacent to carbonyl group.

Mass Fragmentation of (2E,6E,8E)-N-phenethyldeca-2,6,8-trienamide (12), (6E,8E)-N-phenethyldeca-6,8-dienamide (14) and (E)-N-phenethyldec-7-enamide (17)

(2E,6E,8E)-N-phenethyldeca-2,6,8-trienamide (**12**), (6E,8E)-N-phenethyldeca-6,8-dienamide (**14**) and (E)-N-phenethyldec-7-enamide (**17**) were eluted at 13.56 min, 14.78 min and 16.23 min with *m/z* 270 $[M+H]^+$, *m/z* 272 $[M+H]^+$ and *m/z* 274, respectively (Figure 13). N-phenyl ethyl group was confirmed by characteristic peaks at *m/z* 122 and *m/z* 105. Cleavage of amide bond gives peak at *m/z* 149, *m/z* 151 and *m/z* 153.The difference of 2 amu was due to more unsaturated double bond in compound **12** from compound **14** and **17**. Further loss of carbonyl group gives peak at *m/z* 123 in compound **12**. Cleavage of C-C bond adjacent to carbonyl group gives peak at *m/z* 148. The mass peak at *m/z* 216 assists for position of double bond in compound **17**.

Mass Fragmentation of (5E,7E,9E)-N-isobutyldodeca-5,7,9-trienamide (16)

(5E,7E,9E)-N-isobutyldodeca-5,7,9-trienamide (**16**) at t_R 15.54 min was assigned to the peak *m/z* 250 $[M+H]^+$ (Figure 14). Loss of isobutyl gave peak at *m/z* 194 and further cleavage of C-N bond gave peak at *m/z* 177. The characteristic mass fragments of homologous series at *m/z* 121, *m/z* 135 and *m/z* 149 were fragments from long hydrocarbon chains.

Mass Fragmentation of N-isobutyldodecanamide (25)

N-isobutyldodecanamide (**25**) with mass peak at *m/z* 256

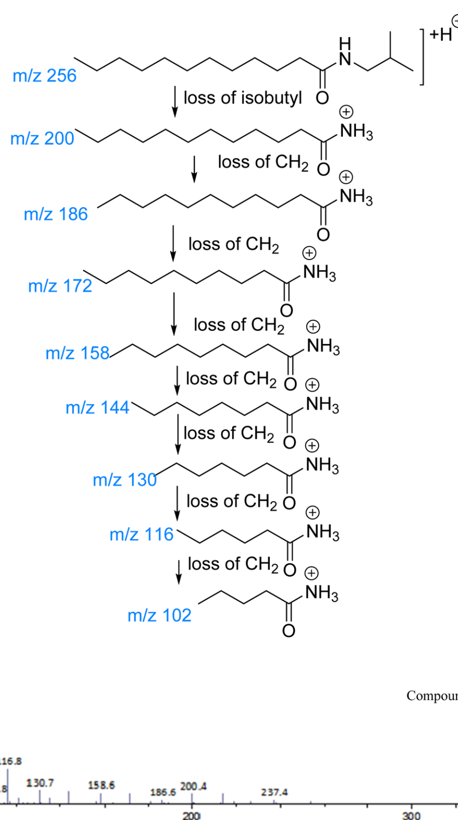

Figure 15. Fragmentation pathway of compound **25** in ESI-MS/MS.

$[M+H]^+$ was eluted at 20.17 min (Figure 15). The loss of isobutyl group gives peak at *m/z* 200. The characteristic peaks for homologous series at *m/z* 186, 172, 158, 144, 130, 116 and 102 confirms long chain hydrocarbon of N-isobutyldodecanamide.

Conclusion

This study, based on high performance liquid chromatog-

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raphy/ electrospray ionization ion trap mass spectrometry (HPLC/ESI-MS) reveals the structure–fragmentation relationship of sixteen metabolites belonging to N-isobutylamides and 2-phenylethylamide. Compounds **4**, **6** and **8** have been previously reported and other N-alkylamides are purposed on the basis of fragmentation analysis for the first time from *S. acmella*.[12,14] N-Alkylamides are a promising group of naturally occurring bioactive metabolites mainly in *S. acmella*. In isobutyl amide, the acyllium ion formed by dissociation of the C-N bond gives *m/z* value indicative for the amount of carbon atoms in the alkyl chain. The fragmentation pattern presented in this paper would serve as a platform for future exploration and characterization of N-alkylamides. The identification of metabolites were assured on the basis of retention time, mass spectra and proposed fragmentation pattern at low resolution. A high resolution spectrometer would have been more suitable for further studies. Further conclusive studies are required to ascertain the mechanism of action of N-alkylamides in their pharmacological activities and to promote the usage of *Spilanthes* species as the functional foods and in therapeutics.

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