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Original Article

Phytochemical constituents of *Centotheca lappacea* (L.) Desv. determined with online DPPH assay coupled to LC-ESI-QTOF-MS and headspace GC-MS

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Abstract

Centotheca lappacea **(**L.**)** Desv**.** or **"***hee* yum**"** grass has been used in Thailand for postpartum care. This study focused on analyses of elements, antioxidants and volatile compounds from *C. lappacea*. Wavelength dispersive X-ray fluorescence showed the presence of silicon in this plant. Liquid chromatography–electrospray ionization-quadrupole-time of flight-mass spectrometer coupled with DPPH assay was used for separation, detection and identification of antioxidants in *C. lappacea* in one run. Twelve antioxidants including *C*-and *O*-glycosides of luteolin and apigenin were identified. Moreover, a headspace gas chromatography-mass spectrometer was used for analyses of volatile compounds by direct burning of the plant material, which is similar to how smoke is made during a traditional treatment. Nineteen and twenty-five compounds were found from the roots and the aerial parts of *C. lappacea*, respectively. In conclusion, antioxidants and volatile constituents after *C. lappacea* has been burnt are reported here for the first time.

Keywords: *Centotheca lappacea*, antioxidants, LC-ESI-QTOF-MS, DPPH, headspace GC-MS

1. Introduction

Centotheca lappacea (L.) Desv. is a member of the family of Poaceae (Gramineae). It is a perennial plant mainly distributed in Thailand, India, China, and Africa. In Thailand, it is known as ya hee yum or repair grass, and has been used in traditional medicine for postpartum care. It has helped to heal wounds and tears on the labia, and tighten the respective muscles. Traditionally, the dried plant material is burned in a smoke pot to fumigate the patient's labia area for 10-30 minutes during the postpartum treatment. They also drink *C. lappacea* tea because of the belief that it creates "balance" inside and outside of their body. The plant powder is also used

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to disinfect and heal open wounds (Kamoltham, Manosroi, Chankhampan, Manosroi, & Manosroi, 2018; Kamoltham, Rungsimakan, Supan, & Rodsienglump, 2017).

A phytochemical study on the aerial part of *C. lappacea* revealed the presence of phenolic compounds, flavonoids, fatty acids and phytosterols. Also, 4-coumaric acid and 5,7,4'-trimethoxyflavone were isolated from the aerial part using column chromatography (Kamoltham *et al.*, 2017). In addition, vitamins and minerals, in particular silicon that contributes to collagen synthesis (Martin, 2013; Seaborn, & Nielsen, 2002), were found in significant amounts in *C. lappaceae*. According to Kamoltham *et al.* (2018), the ethanolic extract of *C. lappacea* showed antioxidant activity in a DPPH assay, metal chelation, lipid peroxidation and tyrosinase inhibitory activity. It also showed strong collagenase inhibition and estrogenic activity by increasing MCF-7 cell proliferation (Kamoltham *et al.*, 2018) . However, the compounds responsible for such activities have not yet been reported.

High performance liquid chromatography (HPLC) coupled with mass spectrometry (MS) is a recent method applied to the identification of biologically active compounds from complex samples. Liquid chromatography–electrospray ionization-quadrupole-time of flight-mass spectrometer (LC-ESI-QTOF-MS) coupled with a DPPH assay has been developed and demonstrated as a useful technique to characterize antioxidants in plant extracts (Nuengchamnong, Krittasilp, & Ingkaninan, 2009). The HPLC system provides information on individual compounds, while the molecular structure and molecular weight of target compounds are obtained using mass spectrometry. This technique was applied to separate and identify antioxidants from *C. lappacea.* Moreover, a headspace gas chromatography-mass spectrometer (GC-MS) was used to analyze the chemical constituents of *C. lappacea* after directly heating at a high temperature, to provide information about the chemical constituents of the smoke used in traditional treatment.

2. Materials and Methods

2.1 Plant materials and chemicals

Dried *C. lappacea* was obtained from Chaophraya Abhaibhubejhr Hospital, Prachin Buri, Thailand. The voucher specimen (Collection no. 004358) was deposited at Faculty of Science, PNU herbarium at Naresuan University. Gallic acid, quercetin, trolox and 1,1 diphenyl-2-picrylhydrazyl (DPPH) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile was purchased from Labscan, (Bangkok, Thailand). Ultrapure water was prepared by using a Millipore MilliQ Integral 3 Water Purification System (Millipore, Bedford, MA). Formic acid (analytical grade) was obtained from Merck (Darmstadt, Germany).

2.2 Sample preparation

The dried aerial part and roots of *C. lappacea* were powdered using a grinder and passed through a 60-mesh size sieve prior to elemental analysis. For the antioxidant evaluation, the plant powder was macerated with 50% and 95% ethanol at room temperature. The extracts were filtered and dried under reduced pressure. The water extract was prepared by infusing plant powder in hot water and drying by lyophilization.

For LC-ESI-QTOF-MS coupled with DPPH assay, 50% ethanolic extract of the aerial part was diluted with methanol to obtain a concentration of 20 mg/ml and filtered by 0.2 µm syringe filter before injection.

2.3 Elemental analysis using X-ray fluorescence

Elemental analysis of the powder of the aerial part and roots of *C. lappacea* were conducted using Bruker S8 Tiger model wavelength dispersive X-ray fluorescence (WDXRF), at the Thailand Institute of Nuclear Technology, Thailand.

2.4 Antioxidant activity by DPPH assay

The antioxidant activity of *C. lappacea* was determined using a method adopted from Brand-Williams, Cuvelier, and Berset (1995). Serial dilutions at concentrations of 0.1 - 10,000 µg/ml of 50**%** ethanolic extracts were mixed with 0.1 mM DPPH and incubated for 30 min in dark at room temperature**.** The absorbance was read at 517 nm**.** Antioxidant activity was calculated as percentage inhibition using equation 1, while IC_{50} values were determined from the percentage inhibition versus the log concentration plot**.** The data are presented as mean ± standard deviation of three replicates**.** Trolox was used as a positive control.

$$\% \text{ inhibition} = \frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}} \times 100$$

2.5 LC–ESI–QTOF-MS coupled with DPPH assay

The online LC-ESI-QTOF-MS coupled with DPPH assay was used as reported previously by Nuengchamnong, Krittasilp, and Ingkaninan (2011). The instrument was composed of HPLC coupled to MS, and a continuous flow of eluent from the analytical column was split into the MS detector (Line 1) and into the reactor coil where DPPH reaction took place (Line 2). Line 1 specifications were; HPLC system 1260 infinity model (Agilent Technologies, Waldbronn, Germany) coupled with an Agilent 6540 Q-TOF MS spectrometer (Agilent Technologies, Singapore) equipped with electrospray ionization interface (ESI). The chromatographic separation was achieved using a Phenomenex Luna C18 (2) column 150 x 4.6 mm i.d. (Phenomenex, Torrance, USA) protected by a C18 guard column. The mobile phase consisted of solvent A (0.1% v/v formic acid in water) and solvent B (0.1% v/v formic acid in acetonitrile). The linear gradient elution was started by 5% B then increased to 90% over 30 min with a post run of 5 min. The column temperature was set at 35°C and the injection volume was 20 µl with a flow rate of 500 µl/min. The MS operating condition was set as: drying gas (N_2) temperature 350°C with flow rate 10 l/min, nebulizer pressure 30 psig, capillary 3500, fragmenter voltage 100, skimmer 65 V and octapole RFPeak 750 V. The full scan mass spectra over the *m/z* range 100 –1000 amu were acquired in negative ion mode.

The on-line antioxidant activity (line 2) was determined using an HPLC equipped with a 1260 infinity cap

pump (Agilent Technologies, Waldbronn, Germany), and home-made knitted reaction coil PEEK tubing with an inner diameter of 180 µm and volume of 100 µL. 0.1 mM DPPH was set at a flow rate of 200 µl/ min and the change in color was recorded as positive peak at wavelength 515 nm using a UV–vis detector (1260 VWP Agilent Technologies, Waldbronn, Germany). The system was operated at 25°C. The split ratio of the eluent between the MS and DPPH lines was 8:2. Agilent Chem station for UV-vis Rev.B.04.03 was used for data analysis. 50% ethanolic extract of the aerial part of *C. lappacea* was used for analysis and quercetin and gallic acid at concentrations of 100 µg/ml were added to the sample to mark an antioxidant peak with the MS peak. Tentative identifications of active compounds were obtained by comparing the retention time and mass spectra of the sample with the Chemspider public data base and with previous reports.

2.6 Headspace GC-MS analysis

The analysis was done using Agilent 7890 B gas chromatography coupled to an Agilent 5977A mass spectrometer with an Agilent Model 7697 headspace auto-sampler equipped with a 1 ml sample loop. A 0.1 g sample of the dried powders of the aerial parts and of roots of *C. lappacea* was placed in a 20 ml vial for each and they were tightly closed. An Agilent 19091S-433 HP 5 MS (30 m x 0.25 mm ID, 0.25 µm film thickness) capillary column was used for separation. The temperatures of the headspace oven, loop and transfer line were set at 180°C, 190°C and 225°C, respectively. The vial was heated to 180°C, pressurized for 0.1 min at 15 psi and equilibrated for 20 min until injection. Loop equilibrium time and injection duration were set as 0.05 and 0.5 min.

The injector temperature was set at 250°C and the injection split mode ratio was 1:10. The GC oven temperature was initially programmed at 40°C and held for 3 min, then increased by 10°C/min to 120°C, which was held for 3 min and then increased again at the rate of 15°C/min to 240°C, which was held for a period of 5 min. This gave a total GC run time of 27 min. Helium (99.995%) was used as the carrier gas at a flow rate of 2.0 ml/min during the analysis. A series of n-alkanes (C_{10} - C_{40}) obtained from CPAchem (Stara Zagora, Bulgaria) was used at the same condition mentioned above to calculate the Kovates retention indices (KI).

The MS operating condition was set in positive electron ionization mode with 70 eV electron energy. It was operated in the SIM /SCAN mode from *m/z* 50 to 500 amu. The mass (MS) source and quadrupole temperature were set at 230°C and 150°C. Each compound was identified by comparing the retention time and *m/z* to the reference in the National Institute of Standards and Technology (NIST) mass spectral database and by retention index matching.

3. Results and Discussion

3.1 Elemental analysis of *C. lappacea*

The trace elements, including silicon in the aerial parts and roots of *C. lappacea*, were determined. As shown in Table 1, *C. lappacea* contained a number of essential elements, such as potassium (5.5% w/w), calcium (2.8% w/w) and silicon (3.14% w/w). These elements play important roles in the maintenance of human health (Siddiqui, Bawazeer, & Scaria Joy, 2014). Silicon, which is the most abundant element in this plant and its family, increases collagen synthesis and promotes wound healing (Seaborn, & Nielsen, 2002) and has anti-inflammatory activity (Nielsen, 2010). Therefore, the presence of this element at a high content in the water extract (0.72 % w/w) and in the powder might explain the effects of *C. lappacea* in postpartum care. Moreover, higher percentages (w/w) of macro-elements were found in the aerial part than in the roots. This is the first report of an elemental analysis of the roots, while that of the aerial part is comparable with the study of Kamoltham *et al.* (2017).

Table 1. Elemental analysis of aerial part, root and water extracts of *C. lappacea* by wavelength dispersive X - Ray Fluorescence (WDXRF)

Element	Aerial part (% w/w)	Root Part (% w/w)	Water extracts (% w/w)
Potassium	5.05	0.12	22.05
Silicon	3.14	0.44	0.72
Calcium	2.80	0.99	4.96
Chlorine	0.86	ND	3.68
Sulfur	0.38	0.05	1.05
Manganese	0.09	< 0.01	0.1
Phosphorous	0.56	0.10	1.33
Magnesium	0.14	0.03	0.54
Iron	0.03	0.02	ND
Zinc	0.08	<0.01	0.04
Rubidium	0.03	ND	0.07
Strontium	0.03	ND	0.03
Titanium	0.02	ND	ND

ND: not detected
*Remark: - Light elements such as carbon (C) and hydrogen (H) are not detected by XRF

3.2 Antioxidant activity by DPPH assay

Ethanolic extract of the aerial part of *C. lappacea* showed antioxidant activity in a DPPH assay (Kamoltham *et al.*, 2018) but the active compounds have not been previously reported. In our study, the antioxidant activity of the water extract and the 50% and 95% ethanolic extracts of the aerial part and of the roots of *C. lappacea* were analyzed using DPPH assay. The results show that the 50% ethanolic extract of aerial part (IC_{50} = 240 ± 40 µg/ml) had a higher activity than either the water extract (IC_{50} =370 ± 60 µg/ml) or the 95% ethanolic extract, (IC_{50} = 360 ± 130 µg/ml). Therefore, 50% v/v ethanolic extract of the aerial part was selected for further studies**.**

3.3 Determination of antioxidant compounds by LC-ESI-QTOF-MS coupled with DPPH assay

50 % ethanolic extract of the aerial part of *C. lappacea* was injected into online LC-ESI-QTOF-MS coupled with DPPH assay. As a result, 12 compounds that exhibited antioxidant activity were identified by comparing the retention time of each peak from the antioxidant activity detector (line 2) with the retention times of peaks from a mass detector (line 1). Gallic acid and quercetin were added to the extract to mark

the delay times of the two detectors. The delay between retention times of the peak from the MS detector and the corresponding peak from antioxidant activity detector was calculated as 0.6 min. Negative ionization mode was used as it gave more fragmentation when compared to the positive mode. The online antioxidant activity trace and the total ion current (TIC) chromatogram are shown in Figure 1. The negative molecular ions, together with their fragmented ions and the tentative identification of the compounds are listed in Table 2. The ESI-MS/MS spectra of the antioxidant compounds can be seen in the supplementary data.

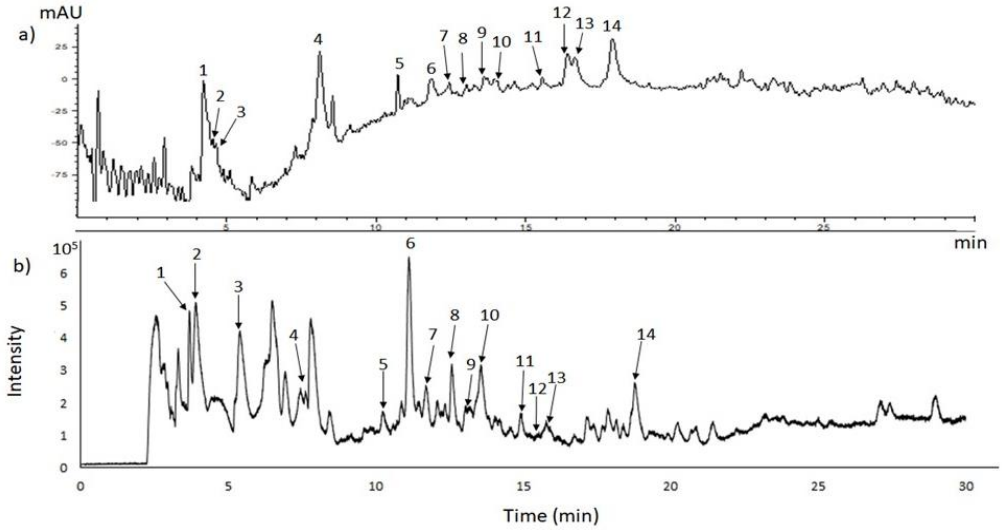

Figure 1. Separation of antioxidant compounds from 50% ethanol extract of *C. lappacea* performed by LC-ESI-QTOF-MS coupled with DPPH assay. (a) Chromatogram from antioxidant activity detector set at 515 nm. (b) The total ion current (TIC) output from the LC-ESI-QTOF-MS in negative mode

Table 2. Tentative identification of compounds from the 50% ethanolic extract of *C. lappacea* using LC-ESI-QTOF-MS coupled to DPPH assay

Compound no	t_R (min)	$[M-H]^-$ (m/z)	Error (ppm)	ESI-MS (*m/z*) MS/MS	Formula	Tentative identification
1	4.2	133.0196	-4.16	115, 71	$C_4H_6O_5$	Malic acid
2	4.5	191.0269	-	173, 111	$C_6H_8O_7$	Isocitric acid/ citric acid
3	4.6	191.0261	-	-	$C_6H_8O_7$	Isocitric acid/ citric acid
4	8.0	169.0198*	-2.68	125	$C_7H_6O_5$	Gallic acid
5	10.6	579.1410	9.42	561, 519, 489, 459, 429, 399, 369	$C_{26}H_{28}O_{15}$	Luteolin 6*C*-deoxyarabinoside-8*C*-glucoside
6	11.8	593.1566	-9.11	503, 473, 429, 357, 327, 309	$C_{27}H_{30}O_{15}$	Luteolin -*O*-rhamnoside-8*C*-glucoside
7	12.3	533.1351	-9.5	503, 515,473, 443, 413, 383, 353	$C_{25}H_{26}O_{13}$	Apigenin-6, 8- di-C-arabinoside
8	13.5	563.1533	-9.9	503, 473, 399, 381, 357, 327, 309	$C_{26}H_{28}O_{14}$	Luteolin -*O*-rahmnoside-6*C*-arabinoside
9	13.9	547.1516	-10.8	457, 383, 311, 293, 117, 59	$C_{26}H_{28}O_{13}$	Apigenin-*O*-rahmnoside-6*C*-arabinoside
10	14.2	163.0458	-7.0	119, 93	$C_9H_8O_3$	*p*-Coumaric acid
11	15.5	577.1633	-12.2	515, 473, 457,413, 311, 293	$C_{27}H_{30}O_{14}$	Apigenin-*O*- rhamnoside-6*C*-glucoside
12	16.2	571.2073	-11.0	469, 427, 325, 221, 179, 99, 57	$C_{26}H_{36}O_{14}$	unidentified
13	16.5	549.2125	-2.5	447, 405, 343, 303, 261, 179, 125, 99, 57	$C_{26}H_{30}O_{13}$	unidentified
14	17.7	301.0409*	-9.8	273, 245, 227, 201, 178, 151, 121, 93, 65	$C_{15}H_{10}O_7$	Quercetin

*Compounds used as delay time markers

Compound 1, at a retention time of 4.2 min to antioxidant activity detector, showed molecular ions $[M-H]^-$ at *m/z* 133.0196 corresponding to the molecular formula of $C_4H_6O_5$. It fragmented to *m/z* 115 and 71 parts by losing 18 ($-H_2O$) and 44 ($-CO_2$) Da, respectively. From the fragmentation pattern and the report of Fernandez-Fernandez *et al.* (2010), compound 1 was tentatively identified as malic acid. Compounds 2 (t_R 4.5 min) and 3 (4.6 min) showed the same molecular ions at *m/z* 191.0269 $[M-H]^-$ and fragmentation ions at *m/z* 111 but different retention times. This suggests that the two compounds were isomers with the molecular formula of $C_6H_8O_7$. Based on these results, and on the database (Http://Chemspider.com), compounds 2 and 3 are tentatively identified as isocitric and citric acid. Peak 4 (t_R =8.0 min) showed *m/z* 169.0198 $[M-H]^-$ which typically represents gallic acid ($C_7H_6O_5$) that was the internal standard added to the sample to mark the delay time between the MS and UV-vis detectors.

Compound 5 (t_R =10.6 min) showed $[M-H]^-$ ions at *m/z* 579.1417 corresponding to the molecular formula $C_{26}H_{28}O_{15}$. Fragment ions at *m/z* 369 [luteolin + $C_4H_3O_2]^-$ and 399 (luteolin + $C_5H_5O_3$) were similar with the pattern of di-substituted (deoxy pentose and hexose) luteolin glycosides reported by Geng *et al.* (2016) and Wang *et al.* (2019). Additional ions at *m/z* 519 $[M-H-60 (-C_2H_4O_2)]^-$, *m/z* 489 $[M-H-90 (-C_3H_6O_3)]^-$ and *m/z* 459 $[M-H-120 (-C_4H_8O_4)]^-$ (Figure S1a) gave information about the position of the substituents on luteolin (Ferreres, Gil-Izquierdo, Andrade, Valentao, & Tomas-Barberan, 2007; Ferreres *et al.*, 2011; Singh *et al.*, 2015). Based on the above information and by comparing with the literature, compound 5 was tentatively identified as luteolin 6*C*-deoxyarabinoside-8*C*-glucosides.

Compound 6 (t_R =11.8 min) showed $[M-H]^-$ ions at *m/z* 593.1673 which corresponds to the molecular formula of $C_{27}H_{30}O_{15}$. Fragmented ions at *m/z* 309 [luteolin+C_3H_5-$H_2O]^-$, *m/z* 327 [luteolin + $C_3H_5]^-$ and *m/z* 357 [luteolin + $C_4H_7O]^-$ together with ions at *m/z* 503 $[M-H-90]^-$ and *m/z* 473 $[M-H-120]^-$ (Figure S1b) revealed the presence of mono *C*-hexose substituent on luteolin, which is in agreement with prior literature (Singh *et al.*, 2015). Additional fragment ion at *m/z* 429 $[M-H-164 (-C_6H_{12}O_5)]^-$ revealed the presence of *O*-substituted rhamnose on this molecule (Ferreres *et al.*, 2007; Parveen *et al.*, 2014). However, we could not confirm the position of rhamnose linkage from the data. Based on the above information and literature, compound 6 could be tentatively identified as luteolin *O*-rhamnoside-8*C*-glucoside.

Compound 7 (t_R= 12.3 min) showed $[M-H]^-$ ions at *m/z* 533.1351 corresponding to the molecular formula of $C_{25}H_{26}O_{13}$. The fragmentation pattern fitted to that of apigenin di-*C* substituted glycoside with prominent fragment ions at *m/z* 353 [apigenin + $C_4H_3O_2$] and 383 [apigenin + $C_5H_5O_3$]. The fragment ions at *m/z* 473 $[M-H-60]^-$, *m/z* 443 $[M-H-90]^-$, and *m/z* 413 $[M-H-120]^-$ (Figure S1c) might be from pentose sugar (arabinose) substituent on 6*C* and 8*C* positions of the aglycon (Singh *et al.*, 2015). Therefore, compound 7 could be tentatively identified as apigenin 6,8-di-*C*-arabinoside.

Compound 8 (t_R = 13.5) showed $[M-H]^-$ ions at *m/z* 563.1533 which was 30 Da less than compound 6. Fragment ions at *m/z* 309 [luteolin + C_3H_5-$H_2O]^-$ and *m/z* 327 [luteolin + $C_3H_5]^-$ revealed compound 8 might be mono-*C* substituted glycoside of luteolin. Fragment ions at *m/z* 503 $[M-H-60]^-$ and *m/z* 473 $[M-H-90]^-$ (Figure S1d) indicated the presence of pentose sugar substituent on the 6*C* position of the parent compound. Moreover, the presence of a prominent peak at *m/z* 399 $[M-H-164]^-$ probably resulted from the cleavage of deoxy hexose sugar (rhamnose) from the molecule. By comparing the above information with the literature, compound 8 could be tentatively identified as luteolin *O*-rhamnoside-6*C*-arabinoside. The position of rhamnose on arabinose could not be confirmed from this study.

Compound 9 (t_R =13.9) showed $[M-H]^-$ ions at *m/z* 547.1516 where 17 Da (-OH) was less than compound 8 which revealed apigenin as aglycone. A fragment ion at *m/z* 293 [apigenin + C_3H_5-$H_2O]^-$ and *m/z* 311 [apigenin + $C_3H_5]^-$ indicated that the molecule might be mono substituted apigenin glycoside. Fragment ions at *m/z* 457 $[M-H-90]^-$ and *m/z* 383 $[M-H-164]^-$ (Figure S1e) might have resulted from the loss of $C_3H_6O_3$ and $C_6H_{12}O_5$ groups from the molecule, respectively. Therefore, from the above spectral data, compound 9 could be tentatively assigned as apigenin-*O*-rhamnoside-6*C*-arabinoside.

Compound 10 (t_R = 14.2) showed $[M-H]^-$ ions at *m/z* 163.0458 and intense peak at *m/z* 119 $[M-H-44]^-$ which revealed the loss of CO_2 from the parent ion. The fragmentation pattern was similar to that of Ferreres *et al.* (2011), so compound 10 was tentatively identified as *p*-coumaric acid.

Compound 11 (t_R = 15.5) showed $[M-H]^-$ions at *m/z* 577.1663 which were 30 Da greater than compound 9 were fragmented at *m/z* 311 [aglycone+ $C_3H_5]^-$, *m/z* 473 $[M-H-104 (-C_4H_8O_3)]^-$, *m/z* 415 $[M-H-162 (-C_6H_{10}O_5)]^-$ and *m/z* 353 $[M-H-(104+120)]^-$ (Figure S1f). The molecular and fragment ions were similar with a literature report (Parveen *et al.*, 2014). Therefore, compound 11 was tentatively identified as apigenin-*O*-rhamnoside-6*C*-glucoside.

Compounds 12 (t_R = 16.2) and 13 (t_R = 16.5) showed molecular ions at *m/z* 571.2073 and *m/z* 549.2125, respectively. Compound 12 fragmented at *m/z* 469 and *m/z* 427 whereas compound 13 fragmented at *m/z* 447 and *m/z* 405 where there were 22 Da difference between their fragment ions. However, it was difficult to elucidate their structure only by the above spectral information. More information acquired by other spectroscopic techniques is needed.

Compound 14 (t_R = 17.7) had molecular ion at *m/z* 301.0409 and fragmented as prominent peaks at *m/z* 178 and *m/z* 151 revealing that the compound was quercetin that was mixed in a sample as a marker for the two detectors.

The antioxidant activities of flavonoid glycosides have been well-documented. Their activities depend on the type of aglycone, type of glycosylation and number of sugars attached to their structures, which also affect their solubility and stability (Kumar, & Pandey, 2013; Xiao, Capanoglu, Jassbi, & Miron, 2016). The *C* and *O* flavonoid glycosides present in *C. lappacea* might be used as markers in further studies of this plant.

3.4 Headspace GC-MS analysis of *C. lappacea*

C. lappacea has long been used in Thailand to treat women after giving birth. The post-partum women were fumed with *C. lappacea* smoke during the treatment. It is believed that this will heal wounds and tighten stretched muscles after delivery (Kamoltham *et al.*, 2018). Therefore, in this study the volatile compounds from the burnt raw materials were studied using headspace GC-MS at a high temperature.

As shown in Tables 3 and 4 and Figures 2A and 2B, 19 compounds from the roots and 25 compounds from the aerial part were detected and tentatively identified. The identification was performed by comparing retention times and fragmentation patterns with those in a database of the National Institute of Standards and Technology (NIST) library together with Kovats index. The matching score with that of the NIST library was accepted within the range 650 - 990 for identification, when also the peaks showed more than 0.5% area of the total area in the whole chromatogram.

As shown in Table 3, furfural, 2- methoxy-4-vinylphenol, vanillin, coumaran and palmitic acid were the most abundant compounds in the roots of *C. lappacea*. There have been no reports previously on the chemical constituents of the roots. Coumaran and palmitic acid were also found in the aerial part of these plants (Table 4). The presence of

Table 3. Tentative identification of volatile compounds from the root of *C. lappacea* using headspace GC-MS

No.	Compound	t_R (min)	KI	Theoretical index	Match	Molecular weight	Formula	Fragmentation	%area
1	Acetic acid	2.47	-	662	972	60.0	CH_3COOH	-	19.6
2	Furfural	4.65	-	835	910	96.0	$C_5H_4O_2$	94.8	7.6
3	4-Cyclopentene-1,3-dione	5.71	-	880	882	96.0	$C_5H_4O_2$	67.8, 53.8	1.0
4	2(5H)-Furanone	6.49	-	916	939	84.0	$C_4H_4O_2$	54.9	1.3
5	2,5-Furandione, 3-methyl-	6.91	-	NF	933	112.0	$C_5H_4O_3$	-	1.6
6	Benzaldehyde	7.19	-	961	926	106.0	C_7H_6O	77.0, 51.0	0.8
7	2-Furancarboxaldehyde, 5-methyl-	7.26	-	966	944	110.0	$C_6H_6O_2$	80.8, 52.8	1.4
8	Furan, 2-pentyl-	7.79	-	996	728	138.1	$C_9H_{14}O$	80.8, 72.8, 59.8	1.7
9	1H-Pyrrole-2-carboxaldehyde	8.24	1020	1018	821	95.0	C_5H_5NO	94.8, 83.9, 65.8	1.5
10	2(3H)-Furanone, dihydro-3-hydroxy-4,4-dimethyl-, (+/-)-	8.66	1045	1032	898	130.0	$C_6H_{10}O_3$	70.8, 56.8	2.0
11	Endo-Borneol	10.74	1172	1166	937	154.1	$C_{10}H_{18}O$	121.0, 110.0, 94.9, 66.9	2.1
12	Coumaran	11.59	1218	1219	861	120.0	C_8H_8O	90.8, 64.8	11.0
13	2-Methoxy-4-vinylphenol	13.54	1311	1315	904	150.0	$C_9H_{10}O_2$	134.9, 106.8, 76.9	2.8
14	Vanillin	15.45	1403	1394	923	152.0	$C_8H_8O_3$	136.9, 108.9, 80.8	3.4
15	2,4-Di-tert-butylphenol	17.11	1529	1525	927	206.1	$C_{14}H_{22}O$	191.0, 56.9	0.9
16	2-Pentadecanone	18.99	1708	1698	885	226.2	$C_{15}H_{30}O$	84.9, 70.9, 57.9	0.7
17	Pentadecanal	19.13	1723	1715	877	226.2	$C_{15}H_{30}O$	198.9, 109.0, 95.9, 81.9	1.2
18	Palmitic acid	21.02	1968	1964	934	256.2	$C_{16}H_{32}O_2$	213,0, 185.0, 157.0, 129.0	6.4
19	Oleic Acid	22.17	2144	2140	858	282.2	$C_{18}H_{34}O_2$	256.1, 129.0, 96.9, 82.9	1.4

NF- not found

Table 4. Tentative identification of volatile compounds from the aerial part of *C. lappaceae* using headspace GC-MS

No	Compound	tR (min)	KI	Theoretical index	Match	Molecular weight	Formula	Fragmentation	% area
1	Acetic acid	2.47	-	662	981	60.0	CH_3COOH	-	11.6
2	Propanoic acid	3.14	-	740	908	73.9	$C_3H_6O_2$	66.9, 56.9	1.2
3	Methylpyrazine	4.43	-	826	916	94.0	$C_5H_6N_2$	85.9, 66.9, 52.9	1.1
4	Furfural	4.65	-	835	930	96.0	$C_5H_4O_2$	-	1.9
5	2(5H)-Furanone	6.48	-	916	944	84.0	$C_4H_4O_2$	54.9	1.1
6	Benzaldehyde	7.19	-	961	941	106.0	C_7H_6O	76.8, 50.8	1.5
7	Benzene acetaldehyde	8.71	1048	831	876	120.0	C_8H_8O	90.9	1.1
8	2,6-Dihydroxypyridine	9.35	1087	NF	742	111.0	$C_5H_5NO_2$	82.9, 67.9	1.2
9	1-Pyrrolidinecarboxaldehyde	9.40	1090	NF	794	99.0	C_5H_9NO	70.9, 59.9	1.2
10	Methyl imidazole-4-carboxylate	9.90	1121	NF	692	126.0	$C_5H_6N_2O_2$	94.9	1.6
11	Camphor	10.40	1151	1145	883	152.1	$C_{10}H_{16}O$	107.9, 94.9, 80.9, 67.9	0.7
12	Coumaran	11.59	1219	1219	831	120.0	C_8H_8O	90.9, 64.6	5.5
13	Methylethylmaleimide	11.82	1229	1265	875	139.0	$C_7H_9NO_2$	123.8, 95.9, 66.9, 52.9	1.1
14	2-Methoxy-4-vinylphenol	13.54	1311	1315	858	150.0	$C_9H_{10}O_2$	134.9, 106.9, 76.9	2.2
15	Vanillin	15.45	1403	1394	897	152.0	$C_8H_8O_3$	136.9, 108.9, 80.8	1.3
16	2,4-Di-tert-butylphenol	17.11	1529	1525	903	206.1	$C_{14}H_{22}O$	191.0	2.5
17	Dihydroactinidiolide	17.36	1548	1532	922	180.1	$C_{11}H_{16}O_2$	152.0, 136.9, 110.9	1.6
18	Neophytadiene	20.12	1845	1840	909	278.2	$C_{20}H_{38}$	137.0, 122.9, 108.9, 94.9, 81.9, 67.9	3.2
19	2-Pentadecanone,6,10,14 trimethyl	20.17	1851	1847	936	268.2	$C_{18}H_{36}O$	123.9, 108.9, 94.9, 84.9, 70.9, 57.9	1.0
20	Neophytadiene	20.30	1869	1840	853	278.2	$C_{20}H_{38}$	136.9, 122.9, 108.9, 94.9, 81.9, 67.9	1.5

Table 4. Continued.

No	Compound	tR (min)	KI	Theoretical index	Match	Molecular weight	Formula	Fragmentation	% area
21	Neophytadiene	20.43	1888	1840	869	278.2	$C_{20}H_{38}$	136.9, 122.9, 108.9. 94.9, 81.9, 67.9	-
22	3-Methyl-2-(3,7,11-trimethyldodecyl) furan	20.73	1929	1931	725	292.1	$C_{20}H_{36}O$	135.9, 108.9, 94.9, 80.9, 68.9	0.9
23	Palmitic acid	21.02	1969	1964	936	256.2	$C_{16}H_{32}O_2$	213.1, 185.1, 157.0,129.0	8.2
24	Phytol	22.01	2118	2122	889	296.3	$C_{20}H_{40}O$	136.9, 122.9, 110.9, 94.9, 81.9	4.86
25	Oleic acid	22.17	2141	2140	764	282.2	$C_{18}H_{34}O_2$	256.2, 129.0, 96.9, 82.9	1.31

NF- not found

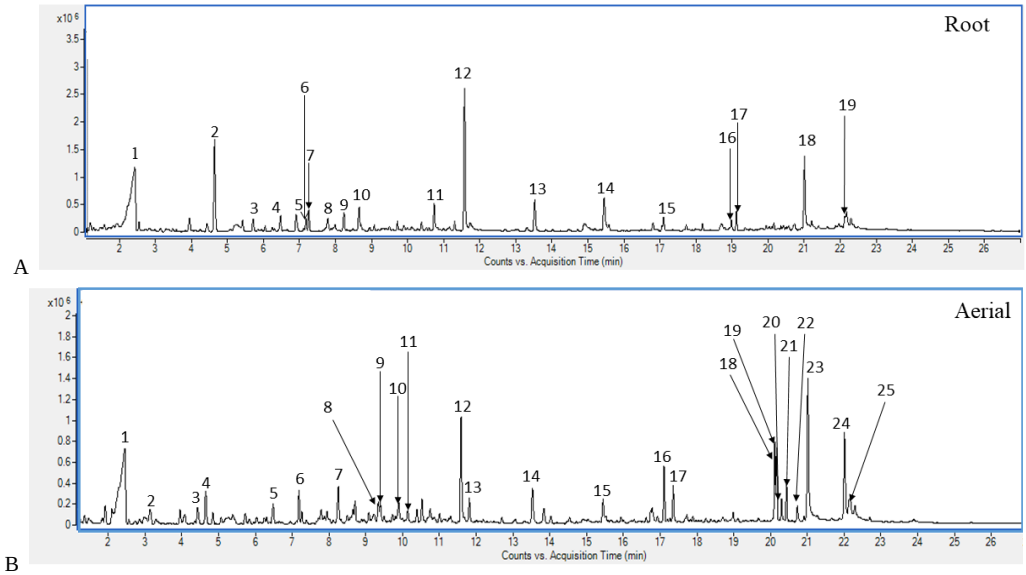

Figure 2. TIC from headspace GC-MS analysis of A) root part, and B) aerial part of *C. lappacea*. The peak numbers correspond to those in Tables 3-4.

phytol, palmitic acid, vanillin and oleic acid in the aerial part is in agreement with the report by Kamoltham *et al.* (2017). Other compounds found in this plant were identified and are reported here for the first time.

Coumaran and its derivatives have shown anticancer, antitubercular and anti-HIV activities (Ilya, Kulikova, Van der Eycken, & Voskressensky, 2018) and have been reported in many plants (Wu, Xu, & Huang, 2015). These compounds were found in both the aerial part and the roots and might play an important role in the biological activities of this plant. Palmitic acid showed antitumor activities *in-vivo* in mice. Phytol, the other abundant compound in the aerial part showed antioxidant, anti-inflammatory, immune modulating and antimicrobial effects (Lee, Woo, & Lee, 2016).

4. Conclusions

An on-line LC-ESI-QTOF-MS coupled to a DPPH assay enabled identification of 12 antioxidant compounds from the aerial part of *C. lappacea*. The results support the use of this herb as a food supplement. In addition, headspace GC-MS analysis revealed the volatile constituents of *C. lappacea* after burning it, which as constituents of the smoke from *C. lappacea* are used in Thai traditional medicine.

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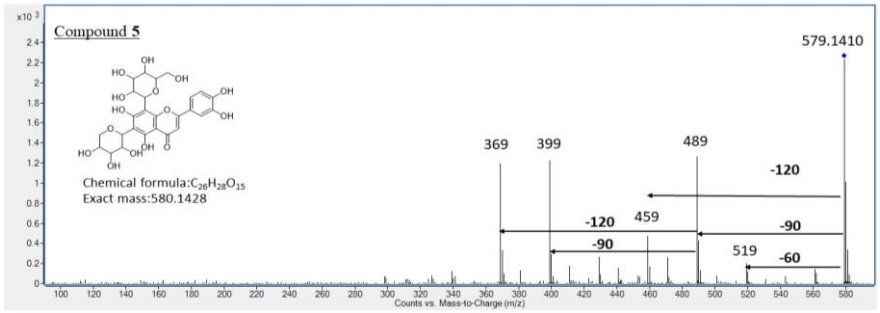
Appendix



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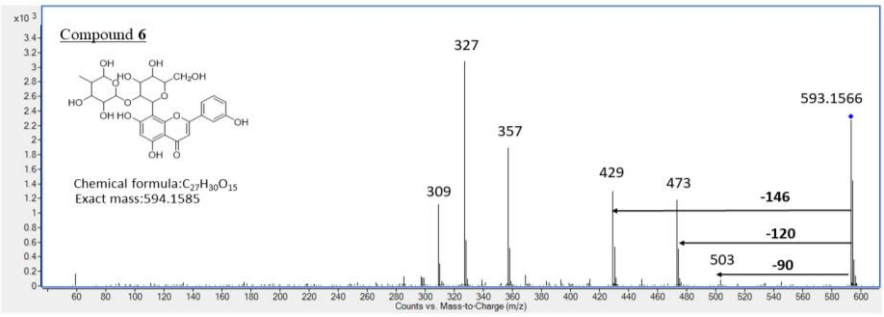

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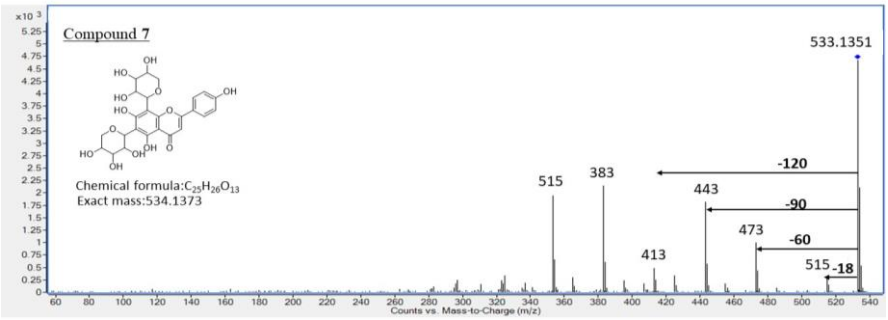

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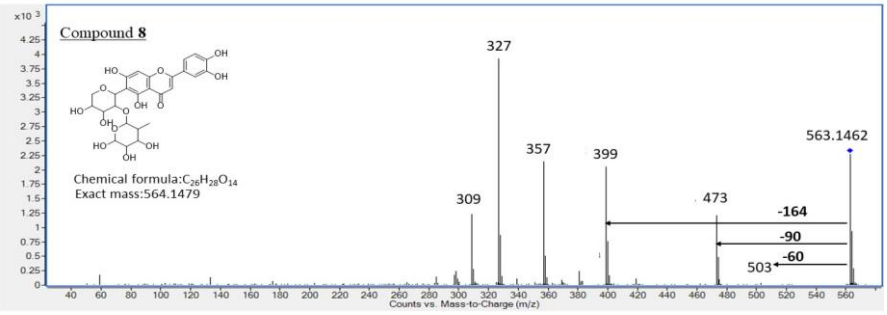

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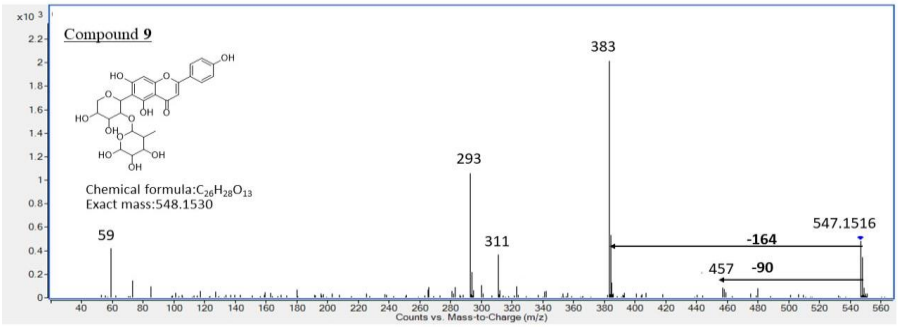

e)

f)

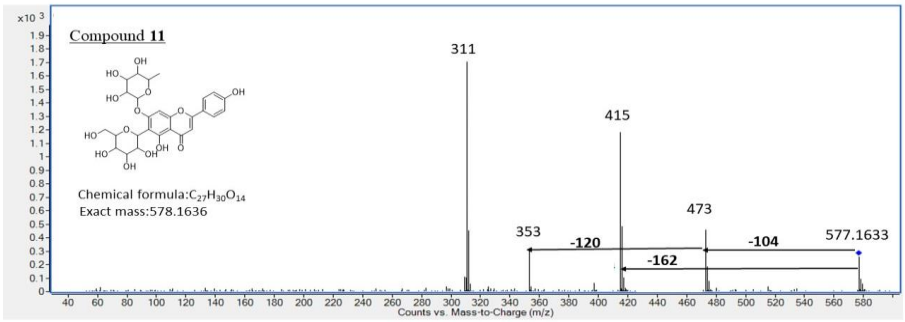

Figure S1. ESI-MS/MS spectra of antioxidant compounds: a) compound **5**; b) compound **6**; c) compound **7**; d) compound **8**; e) compound **9** and f) compound **11**