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# Biochemical composition and antioxidant capacity of extracts from *Podophyllum hexandrum* rhizome

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## Abstract

**Background:** *Podophyllum hexandrum* Royle (*P. hexandrum*) is a perennial herb and widely used in clinic. The present study was designed to separate and identify the biochemical composition and antioxidant capacity of extracts from *P. hexandrum* rhizome.

**Methods:** The ethyl acetate and ethanol extracts from *P. hexandrum* rhizome were analyzed by GC-MS (gas chromatography–mass spectrometry), and the antioxidant capacity of the extracts and the components was tested by using the DPPH (2, 2-diphenylpicrylhydrazyl) and FRAP (Ferric reducing/antioxidant power) assays.

**Results:** The rhizome extracts had greater antioxidant capacity than the petiole extracts in DPPH and FRAP assays. About 16 kinds of main reactive oxygen components were identified in the extracts. Components of PADE (Phthalic acid, diisobutyl ester), BADE (1,2-Benzenedicarboxylic acid, diisooctyl ester), Polyneuridine, PODD (Podophyllotoxin, deoxy),  $\beta$ -Sitosterol and POD (Podophyllotoxin) showed the antioxidant capacity in some degree. PODD, POD, and Polyneuridine showed stronger antioxidant capacity with the  $IC_{50}$  and FRAP values of  $9.61 \pm 0.81$  and  $2923.98 \pm 21.89 \mu\text{M}$ ,  $9.98 \pm 0.24$  and  $2847.27 \pm 14.82 \mu\text{M}$ , and  $13.37 \pm 0.35$  and  $2404.32 \pm 36.88 \mu\text{M}$ , respectively, than the positive control ASA (Ascorbic acid) with the values of  $60.78 \pm 1.22$  and  $1267.5 \pm 30.24 \mu\text{M}$  ( $P < 0.01$ ).

**Conclusions:** PODD, POD, and Polyneuridine are very critical for the antioxidant capacity in the extract of *P. hexandrum* rhizome. These results provide useful biochemical basis and information for the potential use of this plant.

**Keywords:** *Podophyllum hexandrum*, Biochemical composition, Antioxidant capacity, GC-MS

## Background

*Podophyllum hexandrum* Royle (*P. hexandrum*), commonly named Himalayan Mayapple, is a perennial herb that grows in the Himalayan region and the southwest of China [1,2]. Since 1940 *P. hexandrum* resin has been used topically for various skin lesions such as warts and condylomas [3]. POD is a natural product mainly existing in *P. hexandrum* rhizome. It has been used in the treatment of genital infection to sterilize noncervical human papilloma virus [4]. Penile warts can be safely treated with 0.5–2.0% podophyllin self applied [5]. Goel et al. [6] reported a significant antitumour effect at

subtoxic, well-tolerated, sequential doses of aqueous extract of *P. hexandrum*. POD is also used as starting compound for the chemical synthesis of etoposide (VP16-213) and teniposide (VM-26), and its congeners and derivatives have pronounced biological activity as anticancer, antineoplastic and anti-HIV drugs [7-9]. Researchers have recently paid attention to the antioxidant activity of POD and its derivatives, such as GP7OH, GP7H, GP7 and VP16 [10]. So far, there is no specific study on the biochemical composition and the antioxidant capacity of *P. hexandrum* rhizome extracts. In our previous study, GC-MS showed that the ethyl ether extract of *P. hexandrum* seed contained some bioactive components, such as Hexanedioic acid, Oleic acid, and Octadecanoic acid [11].

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In the present study, we tested the antioxidant capacity of ethyl acetate and ethanol extracts in the rhizome and petiole of *P.hexandrum* by the DPPH and FRAP assays, separated and identified the biochemical compositions of the rhizome extracts by GC-MS, and then evaluated antioxidant capacity of some identified compositions with the parameters of IC<sub>50</sub> and FRAP value.

## Methods

### Plant materials

*P. hexandrum* was collected from a forest (2,100 m) in HuiChuan, WeiYuan of Gansu Province, China, after the plant fruit ripened in September 2011. The plant was dried in shade under room temperature. The species was identified by Professor Yanling Qi (Gansu Provincial Academy of Agricultural Sciences, Lanzhou, Gansu, P.R.China). A voucher specimen (No.0209069) was deposited in the herbarium of College of Agronomy, Gansu Agricultural University, Lanzhou, Gansu, P.R. China.

### Reagents and instrumentation

DPPH, 2, 4, 6-tris (2-pyridyl)-s-triazine (TPTZ), PADE, BADE, Polyneuridine, PODD,  $\beta$ -Sitosterol, and POD were purchased from Sigma Chemical Company (St. Louis, MO, USA). Ethyl acetate, ethanol, methanol, ASA, FeCl<sub>3</sub>·6H<sub>2</sub>O, and HCl were purchased from Guangfu Chemical Research Institute (Tianjin, P.R. China). UV-1810 (Beijing Persee General Instrument Co., Ltd, P.R.China) and Trace DSQ GC-MS (American Finnigan Company, USA) were used.

### Preparation of extracts

Rhizome and petiole were washed, dried and grinded to powder. Then the powder was weighted (20.00 g) and soaked in ethyl acetate and ethanol (500 mg/mL) for 10 months at room temperature in dark, and was then filtered, evaporated and condensed to dryness under nitrogen to obtain extracts.

### Antioxidant assays

Although there are numerous methods for determining the antioxidant capacity of soluble natural extracts and insoluble food components [12], no perfect system is available to help us know the "true" antioxidant capacity of a complex medium [13,14]. The DPPH and FRAP assays, despite their disadvantages, are still used by many researchers for rapid evaluation of antioxidant [15].

### DPPH assay

The free radical scavenging activity (FRSA) of DPPH was measured according to Ramadan *et al.* [16] and

Nencini *et al.* [17], which is one of the few stable and commercially available organic nitrogen radical assays [18,19]. Foti *et al.* [20] suggested it is an electron transfer reaction. The initial electron transfer occurs very quickly, while the subsequent hydrogen transfer occurs more slowly and depends on the hydrogen-bond accepting solvent [18,19]. This reaction has been measured by the decoloration assay where DPPH has an absorption band at 515 nm which disappears upon reduction by an antiradical compound [18,21]. The specific steps are as follows.

The extracts were diluted with 15% aqueous ethanol with concentration of 10 mg/mL, and then 50  $\mu$ L of the diluted extracts was added with 950  $\mu$ L of 10<sup>-4</sup> M DPPH methanol solution. Then the mixture was shaken and kept in dark for 30 min at room temperature. The decreased absorbance of DPPH solution was evaluated at 515 nm by a spectrophotometer UV-1018, and 500  $\mu$ M 15% aqueous ethanol ASA was tested as a positive control. The test was carried out in triplicate, and the capability to scavenge the DPPH radicals was calculated as:

$$\begin{aligned} \text{Scavenging effect(I\% , Percentage of inhibition)} \\ = [(A_0 - A)/A_0] \times 100 \end{aligned}$$

where A<sub>0</sub> and A were the absorbance of DPPH without and with sample, respectively.

### FRAP assay

The FRAP test was first introduced by Benzie *et al.* [22] for measuring the total antioxidant activity, which was initially developed to assay plasma antioxidant capacity but can also be used on other fluids. In the FRAP test, reductants (antioxidants) in the sample reduce ferric-tripyridyltriazine complex (Fe<sup>3+</sup>- TPTZ), in stoichiometric excess, to a blue ferrous form (Fe<sup>2+</sup>), with an increase in absorbance at 593 nm [17]. The specific steps were described by Tsao *et al.* [23] as follows.

The working FRAP reagent was prepared *ex tempore* by mixing 10 volumes of 300 mmol/L acetate buffer, pH 3.6, with 10 mmol/L TPTZ in 40 mmol/L HCl, and 20 mmol/L FeCl<sub>3</sub>·6H<sub>2</sub>O at 10:1:1 (v/v/v).

The 300 $\mu$ L FRAP reagent and the 10 $\mu$ L standard samples (FeSO<sub>4</sub>·7H<sub>2</sub>O, 500  $\mu$ M) or test samples (10 mg/mL 15% aqueous ethanol) were added and mixed well. The reaction temperature was 37°C and the absorbance readings were taken at 593 nm immediately and 4 min later using a spectrophotometer UV-1018. And 500  $\mu$ M 15% aqueous ethanol ASA was tested as a positive control. All tests were carried out in triplicate. The FRAP value of the test samples was

calculated on the basis of  $500 \mu\text{M Fe}^{2+}$  ( $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ ) as follows:

$$\text{FRAP value } (\mu\text{M}) = \frac{\text{Absorbance}_{593\text{nm}} \text{ test sample}}{\text{Absorbance}_{593\text{nm}} \text{ standard sample}} \times 500 (\mu\text{M})$$

where  $\text{Absorbance}_{593\text{nm}}$  was the absorbance of the sample minus the absorbance of the blank at the 4th minute.

### GC-MS analysis

The samples were the rhizome extracts of ethyl acetate and ethanol (500 mg/mL). Trace DSQ GC-MS from American Finnigan Company was used. The GC conditions were: 1 N NOWAX quartz capillary column:  $30 \text{ m} \times 0.32 \text{ cm} \times 0.25 \text{ mm}$ ; column temperature:  $50\text{--}190^\circ\text{C}$ ; procedure temperature:  $5^\circ\text{C}/\text{min}$ ; carrier gas: He; vaporizer temperature:  $280^\circ\text{C}$ ; the MS conditions were: Ion source: EI; temperature:  $200^\circ\text{C}$ ; ionizing voltage: 70 eV; electric current of collection:  $300 \mu\text{A}$ ; electric current of emission: 1 mA; resolution: 600; mass: 10–600.

### Evaluation of antioxidant assays

Reagents PADE, BADE, Polyneuridine, PODD,  $\beta$ -Sitosterol, POD, and including the positive control ASA were dissolved in 15% aqueous ethanol and tested at the same concentration of  $500 \mu\text{M}$ . Antioxidant capacity was expressed  $\text{IC}_{50}$  ( $\mu\text{M}$ ) and FRAP ( $\mu\text{M}$ ) and the test methods were mentioned above (as the DPPH and FRAP assays).

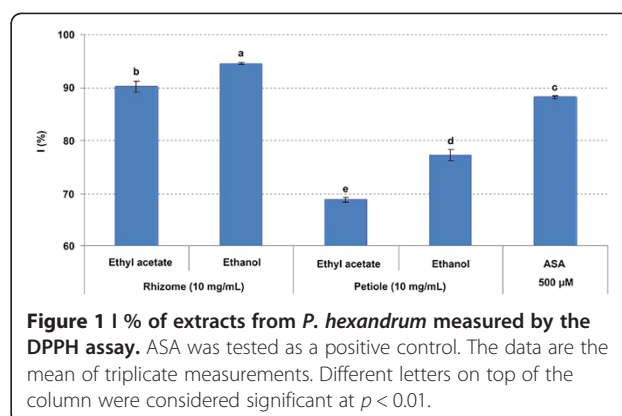
### Statistical analysis

The results were presented as mean  $\pm$  SD of triplicate determinations. Statistical analysis was performed by SPSS 11.5. One-way analysis of variance (ANOVA) was utilized to evaluate differences.

## Results

### Antioxidant capacity of rhizome and petiole extracts

FRSAs from the rhizome and petiole extracts of *P. hexandrum* expressed as I % are showed in Figure 1, and the antioxidant capacity expressed as FRAP value is reported in Figure 2. FRSAs of ethyl acetate and ethanol extracts were higher from the rhizome ( $90.17 \pm 1.11$  and  $94.52 \pm 0.17\%$ , respectively) than from the petiole ( $68.75 \pm 0.52$  and  $77.23 \pm 1.01\%$ , respectively). The FRAP values showed the same trend as FRSAs, and the FRAP values of ethyl acetate and ethanol extracts from the rhizome were  $1784.09 \pm 52.07$  and  $2079.55 \pm 34.09 \mu\text{M}$ , and the values of the petiole were  $420.45 \pm 85.79$  and  $886.36 \pm 68.18 \mu\text{M}$ . In both DPPH and FRAP assays,  $500 \mu\text{M}$  ASA was tested as a positive control, and the values of I% and FRAP value were  $88.25 \pm 0.24\%$  and  $1267.5 \pm 30.24 \mu\text{M}$ , respectively. The statistical

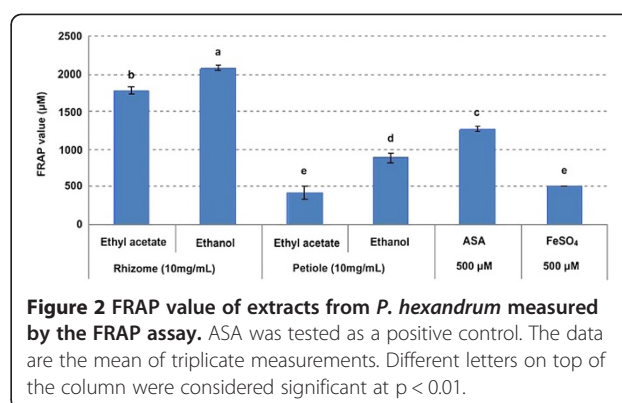


analyses showed that the rhizome extracts had greater antioxidant capacity than the petiole extracts and the ethanol extracts had greater antioxidant capacity than the ethyl acetate extract both in DPPH and FRAP assays ( $p < 0.01$ ).

### Separation and identification of rhizome extracts

In order to further study the biochemical compositions in *P. hexandrum* rhizome, the extracts of the ethyl acetate and ethanol were separated and identified by GC-MS. In total, About 16 kinds of reactive oxygen were identified. The compound retain time (RT), name, molecular formula, molecular weight, area and percentage of area (POA) were listed in Table 1 (the ethyl acetate extract) and Table 2 (the ethanol extract), and the compound structures were presented in Figure 3 (the ethyl acetate extract) and Figure 4 (the ethanol extract).

The ethyl acetate and ethanol extracts contained five common components: PADE, BADE, PODD,  $\beta$ -Sitosterol, POD, and the POA of the five components in the ethyl acetate and ethanol extracts were 2.77 and 1.23, 2.25 and 7.05, 20.59 and 20.97, 1.61 and 1.22, 67.41 and 22.73%, respectively. The Polyneuridine also showed the high POA with 28.01% in the ethanol extract.



**Figure 2** FRAP value of extracts from *P. hexandrum* measured by the FRAP assay. ASA was tested as a positive control. The data are the mean of triplicate measurements. Different letters on top of the column were considered significant at  $p < 0.01$ .

**Table 1 Separation and identification of ethyl acetate extract from *P.hexandrum* rhizome by GC-MS**

No.	RT	Name	Molecular Formula	molecular weight	Area	POA (%)
1	16.56	D-Allose	C <sub>6</sub> H <sub>10</sub> O <sub>5</sub>	162	68174247.68	1.85
2	20.24	Phthalic acid, diisobutyl ester (PADE)	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	278	101990836.36	2.77
3	22.44	1-Octadecanol	C <sub>18</sub> H <sub>38</sub> O	270	5668336.18	0.15
4	22.96	Oleic Acid	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282	57474673.65	1.56
5	23.17	Octadecanoic acid	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	284	15872716.84	0.43
6	26.29	1,2-Benzenedicarboxylic acid, diisooctyl ester (BADE)	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	390	82848910.41	2.25
7	30.56	2,2'-Benzylidenebis(3-methylbenzofuran)	C <sub>24</sub> H <sub>20</sub> N <sub>2</sub> O	352	18407733.56	0.50
8	35.04	Campesterol	C <sub>28</sub> H <sub>48</sub> O	400	8076867.21	0.22
9	35.89	Podophyllotoxin, deoxy (PODD)	C <sub>22</sub> H <sub>22</sub> O <sub>7</sub>	398	758866242.2	20.59
10	37.19	β-Sitosterol	C <sub>29</sub> H <sub>50</sub> O	414	59476314.62	1.61
11	38.05	Naphtho[2,3-c]furan-1(3H)-one,4-(3,4-dimethoxyphenyl)-3a,4,9,9a-tetrahydro-6,7-dimethoxy-, [3aR-(3a?4?9a?)-]	C <sub>22</sub> H <sub>24</sub> O <sub>6</sub>	384	24682996.63	0.67
12	40.77	Podophyllotoxin (Podofilox) (POD)	C <sub>22</sub> H <sub>22</sub> O <sub>8</sub>	414	2484773776	67.41

### Evaluation of antioxidant capacity

Figure 5 and 6 show antioxidant capacity of the six components identified from the extracts by using the DPPH and FRAP assays at the same concentration of 500 μM. The IC<sub>50</sub> and FRAP values of PADE, BADE, Polyneuridine, PODD, β-Sitosterol, and POD were 22.49 ± 0.60 and 1062.5 ± 23.39 μM, 16.32 ± 0.67 and 1025.00 ± 9.24 μM, 13.37 ± 0.35 and 2404.32 ± 36.88 μM, 9.61 ± 0.81 and 2923.98 ± 21.89 μM, 32.43 ± 0.81 and 891.37 ± 22.14 μM, 9.98 ± 0.24 and 2847.27 ± 14.82 μM, respectively. Both of the tested statistics of IC<sub>50</sub> and FRAP values showed that PODD, POD and Polyneuridine had greater antioxidant capacity than the positive control ASA (60.78 ± 1.22 and 1267.5 ± 30.24 μM) (*p* < 0.01).

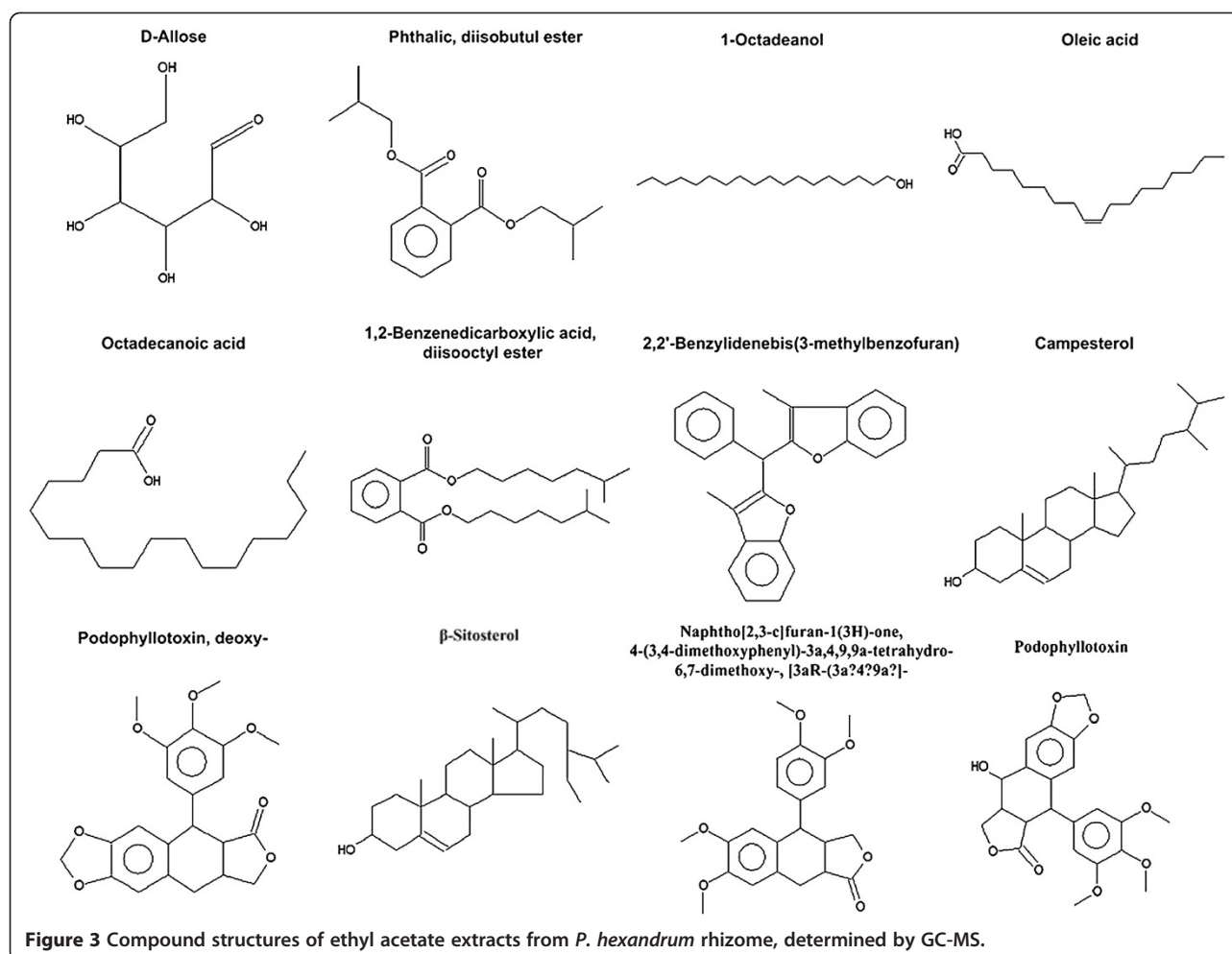
### Discussion

*Podophyllum* contained 4–20% of podophyllum resin, which is the source of POD [24,25]. Giri *et al.* [3] reported that Podophyllotoxin is commonly extracted from *P.hexandrum* that contains 6–12% of resin, of which the concentration of podophyllotoxin is around 40%. Chawla *et al.* [26] reported that polyphenols and lignans were rich in *P.hexandrum* rhizome, which revealed several bioactivities of direct relevance to radioprotection.

In this work, about 16 kinds of reactive oxygen components were identified by GC-MS, some of which have been preliminarily studied for antioxidant capacity or identified from other plants. Zhang *et al.* [27] and Jin *et al.* [28] reported that POD derivatives had strong

**Table 2 Separation and identification of ethanol extract from *P.hexandrum* rhizome by GC-MS**

No.	RT	Name	Molecular formula	Molecular weight	Area	POA (%)
1	14.25	d-Gala-l-ido-octonic amide	C <sub>8</sub> H <sub>17</sub> NO <sub>8</sub>	255	651876.57	0.75
2	16.94	Sucrose	C <sub>12</sub> H <sub>22</sub> O <sub>11</sub>	342	2221794.92	2.55
3	20.75	Phthalic acid, diisobutyl ester (PADE)	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	278	1067190.30	1.23
4	21.12	9-Hexadecenoic acid	C <sub>16</sub> H <sub>30</sub> O <sub>2</sub>	254	577281.13	0.66
5	21.81	n-Hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256	4151490.41	4.77
6	22.04	Hexadecanoic acid, ethyl ester	C <sub>18</sub> H <sub>36</sub> O <sub>2</sub>	284	2383067.34	2.74
7	22.81	Retinol	C <sub>20</sub> H <sub>30</sub> O	286	660693.34	0.76
8	23.64	9,12-Octadecadienoic acid, ethyl ester	C <sub>20</sub> H <sub>36</sub> O <sub>2</sub>	308	2368466.98	2.72
9	23.82	Octadec-9-enoic acid	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282	1549676.99	1.78
10	26.52	Hexadecanoic acid, 2-(octadecyloxy) ethyl ester	C <sub>36</sub> H <sub>72</sub> O <sub>3</sub>	552	1112593.91	1.28
11	26.78	1,2-Benzenedicarboxylic acid, diisooctyl ester (BADE)	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	390	6141357.57	7.05
12	35.99	Podophyllotoxin, deoxy (PODD)	C <sub>22</sub> H <sub>22</sub> O <sub>7</sub>	398	18255949.66	20.97
13	37.60	β-Sitosterol	C <sub>29</sub> H <sub>50</sub> O	414	1063814.09	1.22
14	39.00	Ethyl iso-allocholate	C <sub>26</sub> H <sub>44</sub> O <sub>5</sub>	436	673221.09	0.77
15	40.01	Polyneuridine	C <sub>21</sub> H <sub>24</sub> N <sub>2</sub> O <sub>3</sub>	352	24387006.54	28.01
16	40.23	Podophyllotoxin (Podofilox) (POD)	C <sub>22</sub> H <sub>22</sub> O <sub>8</sub>	414	19787459.99	22.73



antioxidative activities. Ng *et al.* [29] revealed that PODD could inhibit lipid peroxidation in brain and kidney homogenates, and the compound had antioxidant effects. Besides antioxidant potential,  $\beta$ -Sitosterol exhibited a protective action against DMH-induced depletion of antioxidants, such as catalase, superoxide dismutase, and glutathione peroxidase [30]. Zhao *et al.* [31] identified PADE from traditional Chinese medicines of pungent flavor and cool nature by GC-MS. Lin *et al.* [32] isolated BADE from the volatile components of *Eclipta prostrata* by GC-MS. The six components PADE, BADE, Polyneuridine, PODD,  $\beta$ -Sitosterol, and POD presented high POA (percentage of area) in the extracts, and the highest POAs of PODD, POD and Polyneuridine were 20.97, 67.41 and 28.01% in the rhizome, respectively, these results suggest that the components PODD, POD and Polyneuridine are very critical for the antioxidant capacity.

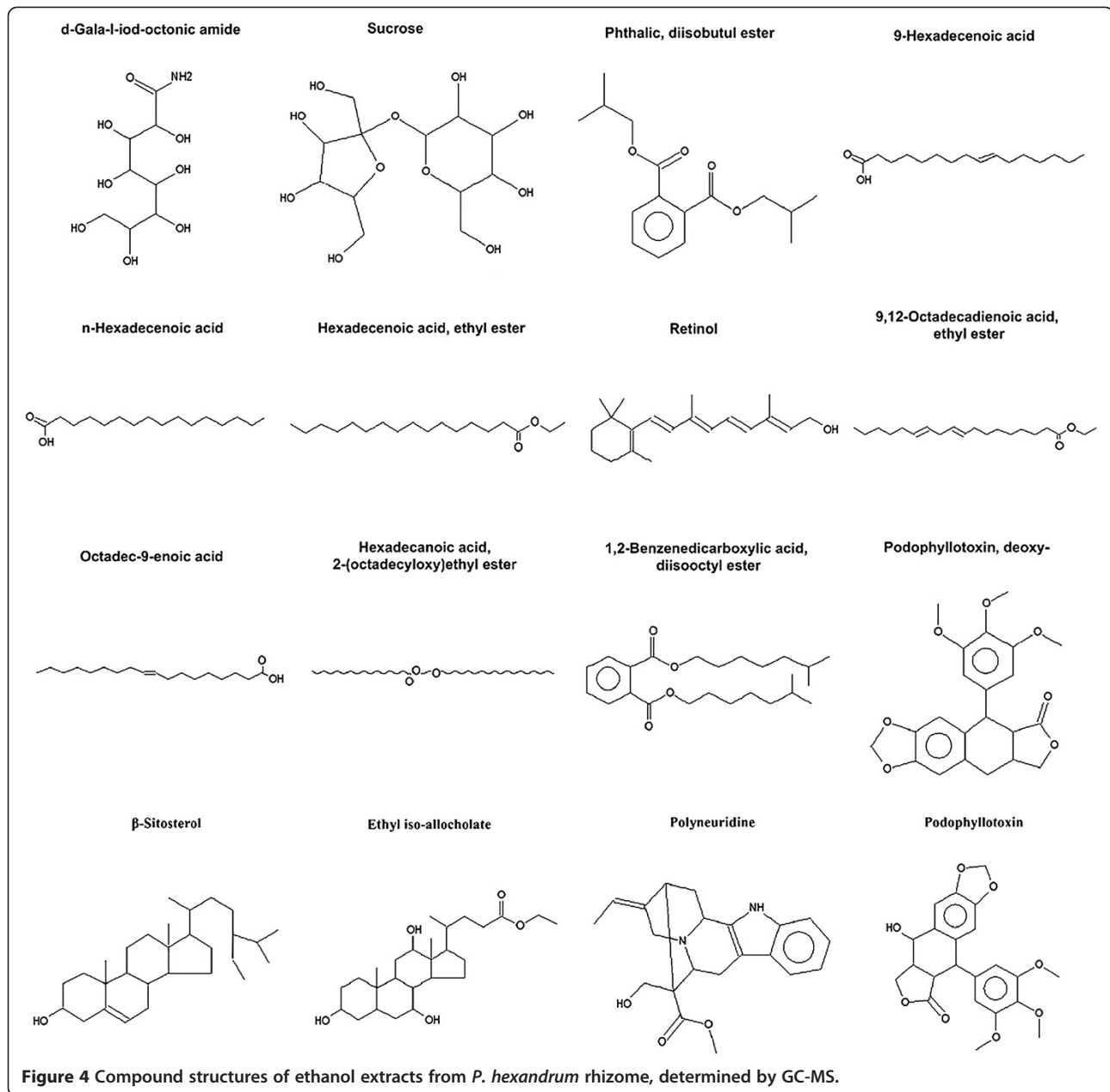
Although the POAs of other components in the ethyl acetate and ethanol extracts are relatively small, antioxidant capacity was also reported in other plant extracts,

such as D-allose [33], Dibutyl phthalate [34,35], n-Hexadecanoic acid [33-36], Oleic acid [36,37], Octadecanoic acid [37], Octadec-9-enoic acid [32], Campesterol [38,39], Retinol [40]. So far, both PADE and BADE have not been reported on the antioxidant capacity. Other active components might be present in the extracts and should be under further investigation.

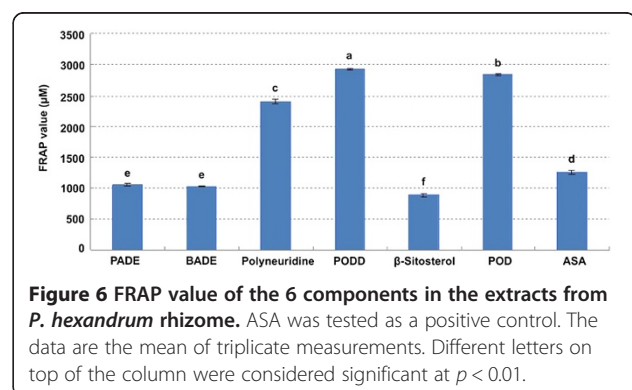
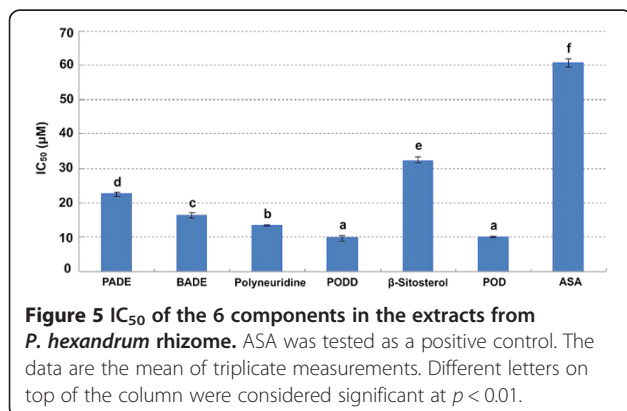
### Conclusions

The rhizome extracts had greater antioxidant capacity than the petiole extracts in DPPH and FRAP assays. About 16 kinds of reactive oxygen components were identified by GC-MS in the extracts of *P. hexandrum*, this was the first to report PADE and BADE from a plant product and further study the antioxidant capacity of the identified components PADE, BADE, Polyneuridine, PODD,  $\beta$ -Sitosterol, and POD. The results of tested data and statistical analysis proved that the extracts from *P. hexandrum* had strong antioxidant capacity, and the PODD, POD and Polyneuridine played an important role. Based on the above data it can be recommended as





**Figure 4** Compound structures of ethanol extracts from *P. hexandrum* rhizome, determined by GC-MS.



an alternative plant material of antioxidant and radical scavenging activity.

#### Competing interests

The authors declare that they have no competing interests.

#### Authors' contributions

MFL designated the study, performed the statistical analysis and drafted the manuscript. LLZ and TTL participated in the extraction and the antioxidant assays. DLY did the isolation and structure elucidation part and helped in manuscript editing. WL has made substantial contribution to conception and design, and revising the manuscript for intellectual content. All authors read and approved the final manuscript.

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