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## Abstract

**Background:** *G. divaricata* (L.) DC belongs to genus the *Gynura* Cass which is a kind of perennial herb that has good health protection efficacy and is especially used widely in medicine and functional food. It is one of the most famous traditional Chinese medicinal herbs and is usually used to cure bronchitis, pulmonary tuberculosis, kink cough, rheumatism, diabetes, and so on. It has a long history for the treatment of diabetes mellitus in the folk medicine. This review is aimed at gathering all information relating to *G. divaricata* and obtaining new insights for further studies on *G. divaricata*

**Materials and Methods:** PubMed, Springer Link, Web of Science, Science Direct and Research Gate were used for the literature search. The key search terms included *G. divaricata* isolation and identification, flavonoids and their combinations without language restriction. The period for the search is from year 1979 to 2016.

**Results:** The main chemical components were listed, and the folk application, the extraction and separation methods of main chemical components, pharmacological effects of *G. divaricata* were discussed, which further demonstrated the plant's value as health food and medicine.

**Conclusion:** The present review is of great significance to the development of new medicinal resources and health food of *G. divaricata*

**Key words:** *G. divaricata*, diabetes mellitus; chemical components; folk application; pharmacological effects

## Introduction

It is a well-known fact that traditional Chinese medicine (TCM) plays a significantly high role in the modern pharmaceutical industry. It has been used for the treatment of many diseases for several thousands of years because of its high pharmacological activity, few or no side-effects, and low toxicity. Numerous studies have confirmed that components isolated from TCM have many important biological activities such as antioxidant, anti-hyperglycaemic, anti-inflammatory and/or anticancer effects (Kirby and Schmidt, 1997; Li et al., 2009; Li et al., 2003; Yarosh et al., 2006; Shoemaker et al., 2005; Cai et al., 2004; Yang et al., 2015). With the development of theory and clinical practice, TCM has been proved to have a rich scientific connotation. A school of TCM has developed in global traditional medicine because of its reliable curative effect. Therefore, TCM has held and still holds an important position in primary healthcare in China and it is also appreciated in other countries because of its thousands-year-old tradition. In TCM, the so called a fairy grass, *G. divaricata*, has attracted a great deal of attention in recent years.

*G. divaricata* (L.) DC, a traditional Chinese medicinal herb, called "Bai Bei San Qi" in China (Chen et al., 2009a; Chen et al., 2009b), is a new source of food approved by the Minister of Health of the People's Republic of China in 2010 (Chen et al., 2014). It has been planted widely in the south of China (Jie et al., 2011). Furthermore, it is one of the

most famous traditional Chinese medicinal herbs and is usually used for the treatment of bronchitis, pulmonary tuberculosis, kink cough rheumatism, pertussis, sore eye, toothache, rheumatic arthralgia and diabetes (Chen et al., 2009b; Ye et al., 2009). *G. divaricata* is a medicinal and edible plant in folk medicine and the roots, stems and leaves can all be used as Chinese medicine. The literature has reported some alkaloids, flavonoids, phenolic acids, terpenoid, steroids, nucleosides, polysaccharides, fatty acid and phytosterols from the plant (Roeder et al., 1996; Hu et al., 2006; Li et al., 2008; Chen et al., 2003; Chen et al., 2009c). In recent years, some scholars have conducted a lot of research work on the medicinal plant and made some progress on the research of the antihypertensive, anti-hyperglycaemic and anti-tumour effects of *G. divaricata*. Now this review first describes the research progress of *G. divaricata* that summarises the main chemical components, the extraction and separation methods of main chemical components, pharmacological effects and applications thereof.

### **Cultivation and Production of *G. divaricata***

*G. divaricata* from Silk Biotechnology Laboratory (Soochow University, China) is shown in Figure 1. Bing-Qing Xu, the first author of this review, took the picture. It can be planted throughout the year; April to September is the best period to seed and transplant *G. divaricata*. However, the branch cutting of the plant is more widely used so far (Yang and Liu, 1998). As long as the soil is kept moist after cutting propagation, *G. divaricata* can survive for 15 days. In addition, the regeneration capacity of *G. divaricata* is so strong that it could be harvested several times after one-time cultivation. The mature stems from strongly maternal plant can be used as cuttings. Moreover, the stem cuttings need to be 15 cm long, with 5 to 10 leaves. The optimum growth temperature of *G. divaricata* is 20–30°C. It grows well in sunny places under the condition that soil is abundant in organic matter. To ensure the high yield and high quality of *G. divaricata*, plants need full sun and good drainage.



**Figure 1:** The whole plant, stem and leaf of *G. divaricata*.

### **Folk Application**

*G. divaricata* is a medicinal and edible plant in folk and the roots, stems, and leaves can all be used as medicine. It has antipyretic, cooling blood\*, promoting blood circulation, relieving pain, stanching bleeding and other effects (Liu et al., 2014). The plant has been used for the treatment of bronchitis, pulmonary tuberculosis, mastitis and other diseases for a long time. Some folk remedies gathered by us have been listed in Table 1. Furthermore, *G. divaricata* was found to have high contents of crude protein, crude fibre, vitamin C, minerals and so on, which demonstrates the plant's nutritional value as a vegetable (Yang and Guo, 2003). The fresh shoots of *G. divaricata* are

used to make soup in folk medicine and eaten once every two or three days, which can improve insomnia, stomach trouble, diabetes mellitus, hypertension and hyperlipaemia. The stems and leaves can also be brewed into tea for the treatment of diabetes. In addition, people of Zhuang in China are fond of making soup with tender branches and leaves of *G. divaricata* for eating. The combination of fresh branches and leaves of *G. divaricata* with pork is also used to make savoury dishes with crisp and tender taste and abundant nutrition.

**Table 1:** Folk Remedies of *G. divaricata* in China

Diseases	Methods of Taking Medicine	Ref
Hyperglycaemia/skin ulcer	Mix and mash <i>G. divaricata</i> and pouzolzia by the ratio of 1:1, apply cover in trouble place	<i>a</i>
Bruises	Mix and mash <i>G. divaricata</i> and schizonepeta by the ratio of 1:1, apply cover in trouble place	<i>a</i>
Fracture	With appropriate amount of <i>G. divaricata</i> roots Paojiu to drink	<i>b</i>
Burns	Mash fresh <i>G. divaricata</i> leaves and add appropriate amount of white sugar to turn into a paste, and then apply cover in troubled place	<i>b</i>
Bronchitis/ pulmonary tuberculosis	10–15 g <i>G. divaricata</i> roots are decocted in water for oral administration	<i>b</i>
Pertussis	10–15 g <i>G. divaricata</i> stems and moderate brown sugar to boil an egg for eating	<i>b</i>
Rheumatism	15–25 g <i>G. divaricata</i> leaves and moderate sugar to boil an egg for eating	<i>c</i>

*a*: The Guangxi Zhuang Autonomous Region Institute of traditional Chinese Medicine. Annals of Guangxi Medicinal plants. The Guangxi Zhuang Autonomous Region Institute of traditional Chinese Medicine, 1959.

*b*: Revolutionary Committee of Yunnan Province Health Bureau. Yunnan Chinese herbal medicine. People's Publishing Press of Yunnan, 1971.

*c*: Kunming Military Region. Yunnan herbal election. Tianjin People Printing Factory, 1970.

### Chemical Constituents

The rich medicinal value of *G. divaricata* attracts more and more scholars' attention so that they began to study the chemical composition of the plant. Therefore, the chemical constituents of this plant were summarized. At present, studies have shown that *G. divaricata* mainly contains alkaloids, flavonoids, phenolic acids, terpenoids, polysaccharides, aliphatic compounds, and so on.

Two kinds of pyrrolizidine alkaloids from *G. divaricata* have been separated (Roeder et al., 1996): integerrimine and usaramine. Alkaloids of the pyrrolizine family are widespread in nature, occurring in over 150 plant species and nearly 70 genera (Mattocks, 1986). Extensive pharmacological investigations of the pyrrolizidine alkaloids have shown that many are acute hepatotoxins, while others exhibit carcinogenic properties (White et al., 1992).

Chen et al. have separated 25 compounds from *G. divaricata* including niacin, pedatisectine G, 2-(1',2',3',4'-tetrahydroxybutyl)-6-(2'',3'',4''-trihydroxybutyl)-pyrazine, 5-hydroxypicolinic acid, 5-hydroxypyridine-2-carboxylic acid methyl ester, uridine, adenosine, stigmaterol-5-O-β-D-glucoside, dibutyl terephthalate, stigmaterol, daucosterol, and so on (Chen et al., 2010a; Chen et al., 2010b).

In addition, several flavonoid compounds have been separated from *G. divaricata* leaves by Wang et al. and they are quercetin, kaempferol and their derivatives including quercetin-3-O-β-D-rutinoside, quercetin-3-O-β-D-glucoside, kaempferol-3-O-β-D-rutinoside, kaempferol-3,7-di-O-β-D-glucoside and so on (Wan et al., 2011a).

Li et al. have also reported the isolation of n-eicosane, friedelin, daucosterol, tetracosanol, palmitic acid and other compounds from *G. divaricata* (Li et al., 2008).

Eleven phenolic acids, characterised by spectrometric methods, were separated from the EtOAc extract of the

aerial parts of *G. divaricata* (Chen et al., 2014). They were 3-O-caffeoylquinic acid (chlorogenic acid), 3-O-p-coumaroylquinic acid, 3-O-feruloylquinic acid, methyl 3-O-caffeoylquinic acid, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid, 4,5-dicaffeoylquinic acid, methyl 3,4-dicaffeoylquinic acid, methyl 3,5-dicaffeoylquinic acid, methyl 4,5-dicaffeoylquinic acid and ethyl 3,4-dicaffeoylquinic acid. All these compounds, except 3,5-dicaffeoylquinic acid (Wan et al., 2011a), were separated for the first time from this plant.

Chou et al. reported some polysaccharides of this plant through chromatographic methods including  $\beta$ -D-fructofuranose, sucrose, nystose, 1-kestose and 1(F)- $\beta$ -fructofuranosylnystose (Chou et al., 2012).

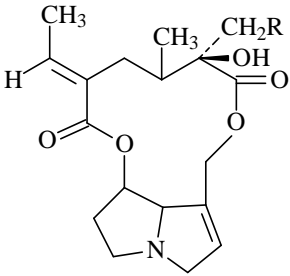
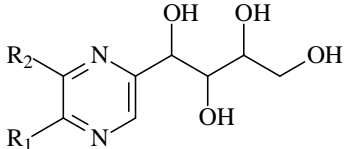
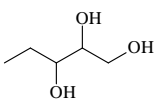
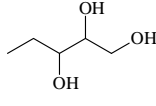
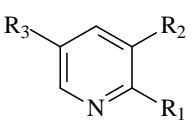
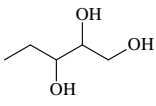
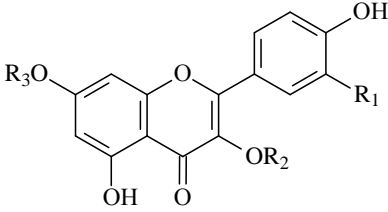
Twenty-four chemical components of volatile oil in *G. divaricata*, which are shown in Table 2, have been described by Qin et al. with the gas chromatography–mass spectrometry (GC-MS) method (Qin et al., 2006). The main components of volatile oil are cubenol (19.78%), spathuleno (12.24%), delta-cadinene (11.79%), cedrene (6.38%), beta-caryophyllene (5.77%), gamma-elemene (4.93%), phytol (4.88%), alpha-caryophyllene (4.77%), perilla aldehyde (3.65%), beta-farnesene (1.97%), ledol (1.78%), n-hexadecanoic acid (1.47%), copaene (1.22%) and 2-undecanone (0.91%).

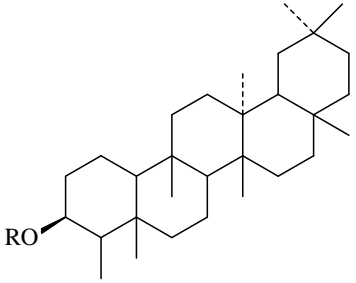
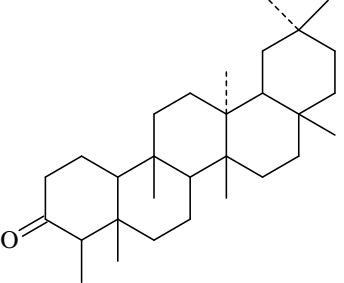
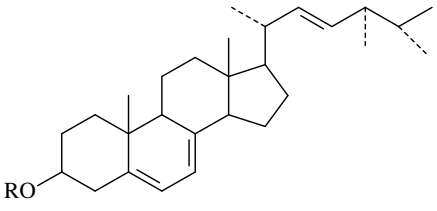
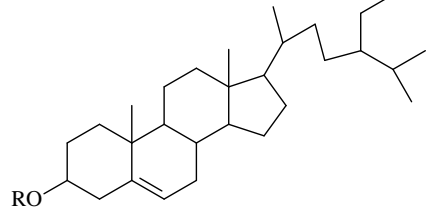
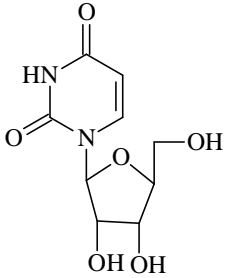
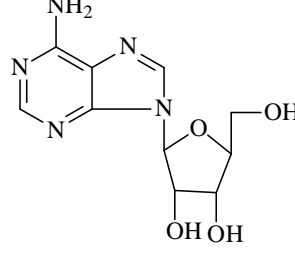
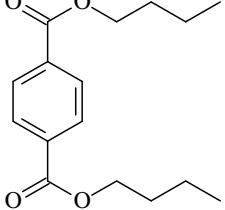
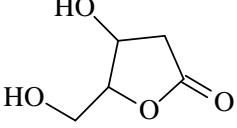
In summary, the classification and molecular structures of the main compounds in *G. divaricata* can be seen in Table 3.

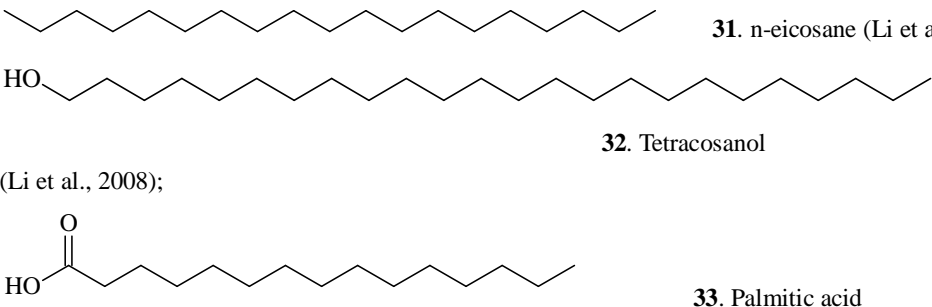
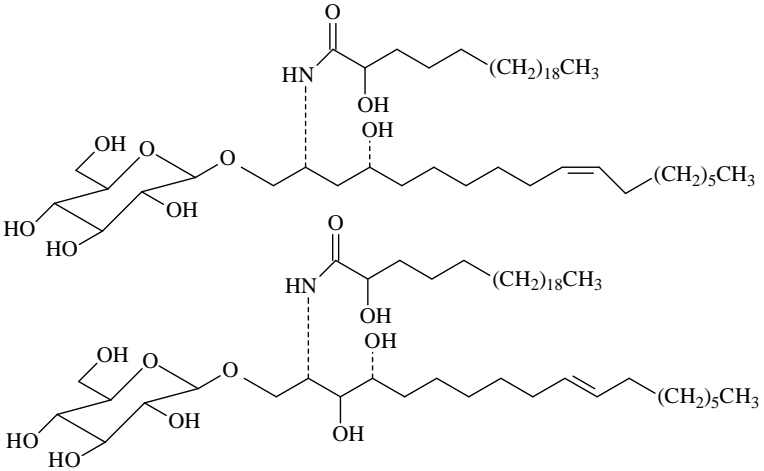
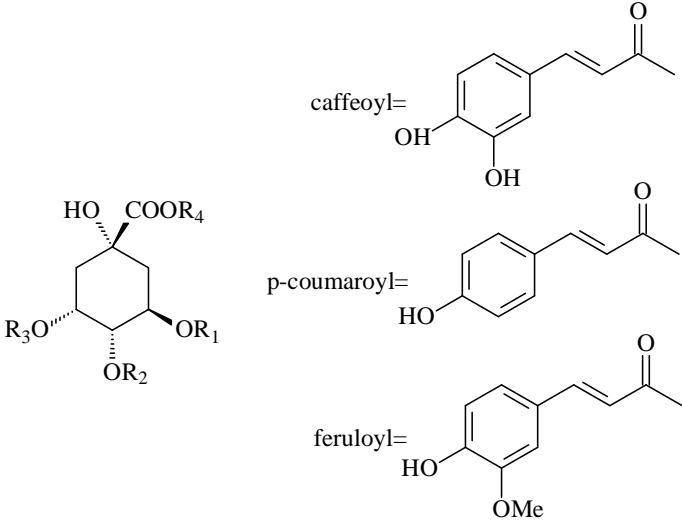
**Table 2:** Chemical components and their relative contents of volatile oil in *G. divaricata*

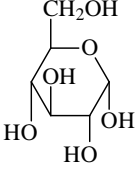
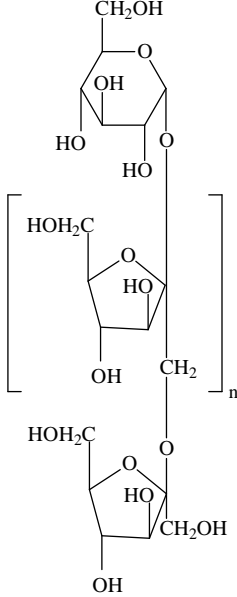
Number	Compound Name	Relative percentage/%
1	Eucalyptol	0.27
2	1-Undecene	0.21
3	Perilla aldehyde	3.65
4	2-Undecanone	0.91
5	Alpha-Cubebene	0.22
6	Copaene	1.22
7	Naphthalene,2,3,4,4a,5,6-hexahydro-1,4a-dimethyl-7-(1-methylethyl)	0.42
8	beta-Caryophyllene	5.77
9	beta-Farnesene	1.97
10	alpha-Caryophyllene	4.77
11	alpha-Farnesene	0.6
12	Cedrene	6.38
13	Gamma-Elemene	4.93
14	Delta-cadinene	11.79
15	Phytol	4.88
16	Spathulenol	12.24
17	Ledol	1.78
18	Cubenol	19.78
19	Aromadendrene oxide	0.54
20	Octadecane,1-chloro	0.47
21	Bicyclo(3,1,0)hexane-6-methanol,2-hydroxy-1,4,4- trimethyl	0.29
22	2-undecanone,6,10-dimethyl	0.81
23	1-decanol,2-hexyl	0.72
24	n-hexadecanoic acid	1.47

**Table 3:** The main compounds identified from *G. divaricata* (L.) DC

Alkaloids	<p>pyrrolizidine alkaloids</p>  <p>1. R=H: Integerrimine (Roeder et al., 1996);                  2. R=OH: Usaramine (Roeder et al., 1996);</p>	<p>Pyrazine alkaloids</p>  <p>3. <math>R_1 = </math>, <math>R_2 = H</math>: Pedatisectine G (Chen et al., 2010b);                  4. <math>R_1 = H, R_2 = </math>:                  2-(1',2',3',4'-tetrahydroxybutyl)-6-(2'',3'',4''-trihydroxybutyl)-pyrazine (Chen et al., 2010b);</p>
	<p>Pyridine carboxylic acid alkaloids</p>  <p>5. <math>R_1 = </math>, <math>R_2 = COOH</math>: Niacin (Chen et al., 2010b);                  6. <math>R_1 = COOH, R_2 = H, R_3 = OH</math>: 5-hydroxypicolinic acid (Chen et al., 2010b);                  7. <math>R_1 = COOCH_3, R_2 = H, R_3 = OH</math>: 5-hydroxypyridine-2-carboxylic acid methyl ester (Chen et al., 2010b);</p>	
Flavonoids	 <p>8. <math>R_1 = OH, R_2 = R_3 = H</math>: Quercetin (Wan et al., 2011a);                  9. <math>R_1 = OH, R_2 = \beta\text{-D-Glu}, R_3 = H</math>: Quercetin-3-O-<math>\beta\text{-D-glucoside}</math> (Wan et al., 2011a);                  10. <math>R_1 = OH, R_2 = \beta\text{-D-Glu}(6 \rightarrow 1) \alpha\text{-L-Rha}, R_3 = H</math>:                  Quercetin-3-O-<math>\beta\text{-D-rutinoside}</math> (Wan et al., 2011a);                  11. <math>R_1 = R_2 = R_3 = H</math>: Kaempferol (Wan et al., 2011a);                  12. <math>R_1 = H, R_2 = \beta\text{-D-Glu}, R_3 = H</math>: Kaempferol-3-O-<math>\beta\text{-D-glucopyranoside}</math>                  (Wan et al., 2011a);                  13. <math>R_1 = H, R_2 = \beta\text{-D-Glu}(6 \rightarrow 1) \alpha\text{-L-Rha}, R_3 = H</math>: Kaempferol-3-O-rutinoside (Wan et al., 2011a);                  14. <math>R_1 = H, R_2 = R_3 = \beta\text{-D-Glu}</math>: Kaempferol-3,7-di-O-<math>\beta\text{-D-glucoside}</math> (Wan et al., 2011a);                  15. <math>R_1 = H, R_2 = Rob, R_3 = \beta\text{-D-Glu}</math>: Kaempferol-3-O-robinobioside-7-O-<math>\beta\text{-D-glucoside}</math> (Wan et al., 2011a);                  16. <math>R_1 = H, R_2 = \beta\text{-D-Glu}(6 \rightarrow 1) \alpha\text{-L-Rha}, R_3 = \beta\text{-D-Glu}</math>:                  Kaempferol-3-O-rutinoside-7-O-<math>\beta\text{-D-glucoside}</math> (Wan et al., 2011a);                  17. <math>R_1 = H, R_2 = Rob, R_3 = H</math>: Kaempferol-3-O-robinobioside (Wan et al., 2011a);                  18. <math>R_1 = H, R_2 = \beta\text{-D-Gal}, R_3 = H</math>: Kaempferol-3-O-<math>\beta\text{-D-galacoside}</math> (Wan et al., 2011a);                  19. <math>R_1 = H, R_2 = (6''\text{-O-acetyl})\text{-}\beta\text{-D-Glu}, R_3 = H</math>: Kaempferol-3-O-(6''-O-acetyl)-<math>\beta\text{-D-glucoside}</math> (Chen et al., 2010a);</p>	

<p><b>Triterpenoids</b></p>	<div style="display: flex; justify-content: space-around;"> <div style="text-align: center;">  <p><b>20.</b> R=H: Epifriedelinol (Hu et al., 2006);  <b>21.</b> R=Ac: Acetyl epifriedelinol (Hu et al., 2006);</p> </div> <div style="text-align: center;">  <p><b>22.</b> Friedelin (Li et al., 2008);</p> </div> </div>
<p><b>Steroidal</b></p>	<div style="display: flex; justify-content: space-around;"> <div style="text-align: center;">  <p><b>23.</b> R=H: Stigmasterol (Chen et al., 2010a);  <b>24.</b> R=β-D-Glu: stigmasterol-5-O-β-D- glucoside (Chen et al., 2010b);</p> </div> <div style="text-align: center;">  <p><b>25.</b> R=H: β-sitosterol (Chen et al., 2010a);  <b>26.</b> R=β-D-Glu: Daucosterol (Chen et al., 2010a);</p> </div> </div>
<p><b>Nucleoside</b></p>	<div style="display: flex; justify-content: space-around;"> <div style="text-align: center;">  <p><b>27.</b> Uridine (Chen et al., 2010b);</p> </div> <div style="text-align: center;">  <p><b>28.</b> Adenosine (Chen et al., 2010b);</p> </div> </div>
<p><b>Esters</b></p>	<div style="display: flex; justify-content: space-around;"> <div style="text-align: center;">  <p><b>29.</b> Dibutyl terephthalate (Chen et al., 2010b);</p> </div> <div style="text-align: center;">  <p><b>30.</b> 3-hydroxy-4-hydroxymethyl-γ- butyrolactone (Chen et al., 2010b);</p> </div> </div>

<p style="writing-mode: vertical-rl; transform: rotate(180deg);"><b>Long-chain aliphatic hydrocarbons</b></p>	 <p><b>31.</b> n-eicosane (Li et al., 2008);</p> <p><b>32.</b> Tetracosanol (Li et al., 2008);</p> <p><b>33.</b> Palmitic acid (Li et al., 2008);</p>
<p style="writing-mode: vertical-rl; transform: rotate(180deg);"><b>Ceramides</b></p>	 <p><b>34.</b> 1-O-β-D-glucopyranosyl-(2S,3S,4R,10Z)-2-[(2' R)- 2'-hydroxylignocenoyl]-10-octadecene-1,3,4-triol (Chen et al., 2009a; Chen et al., 2009c);</p> <p><b>35.</b> 1-O-β-D-glucopyranosyl-(2S,3S,4R,10E)-2-[(2' R)- 2'-hydroxylignocenoyl-amino]-10-octadecene-1,3,4-triol (Chen et al., 2009a; Chen et al., 2009c);</p>
<p style="writing-mode: vertical-rl; transform: rotate(180deg);"><b>Acids: Phenolic</b></p>	 <p>caffeoyle=</p> <p>p-coumaroyl=</p> <p>feruloyl=</p>

	<p>36. R<sub>1</sub>=caffeoyl, R<sub>2</sub>=R<sub>3</sub>=R<sub>4</sub>=H: 3-O-caffeoylquinic acid (Chen et al., 2014);</p> <p>37. R<sub>1</sub>=p-coumaroyl, R<sub>2</sub>=R<sub>3</sub>=R<sub>4</sub>=H: 3-O-p-coumaroylquinic acid (Chen et al., 2014);</p> <p>38. R<sub>1</sub>=feruloyl, R<sub>2</sub>=R<sub>3</sub>=R<sub>4</sub>=H: 3-O-feruloylquinic acid (Chen et al., 2014);</p> <p>39. R<sub>1</sub>=caffeoyl, R<sub>2</sub>=R<sub>3</sub>=H, R<sub>4</sub>= Me: methyl 3-O-caffeoylquinic acid (Chen et al., 2014);</p> <p>40. R<sub>1</sub>=H, R<sub>2</sub>=R<sub>3</sub>= caffeoyl, R<sub>4</sub>=H: 4,5-dicaffeoylquinic acid (Chen et al., 2014);</p> <p>41. R<sub>1</sub>=R<sub>3</sub>= caffeoyl, R<sub>2</sub>=H, R<sub>4</sub>=H: 3,5-dicaffeoylquinic acid (Chen et al., 2014);</p> <p>42. R<sub>1</sub>=R<sub>2</sub>= caffeoyl, R<sub>3</sub>=R<sub>4</sub>=H: 3,4-dicaffeoylquinic acid (Chen et al., 2014);</p> <p>43. R<sub>1</sub>=H, R<sub>2</sub>=R<sub>3</sub>= caffeoyl, R<sub>4</sub>= Me: methyl 4,5-dicaffeoylquinic acid (Chen et al., 2014);</p> <p>44. R<sub>1</sub>=R<sub>3</sub>= caffeoyl, R<sub>2</sub>=H, R<sub>4</sub>=Me: methyl 3,5-dicaffeoylquinic acid (Chen et al., 2014);</p> <p>45. R<sub>1</sub>=R<sub>2</sub>= caffeoyl, R<sub>3</sub>=H, R<sub>4</sub>=Me: methyl 3,4- dicaffeoylquinic acid (Chen et al., 2014);</p> <p>46. R<sub>1</sub>=R<sub>2</sub>= caffeoyl, R<sub>3</sub>=H, R<sub>4</sub>=Et: ethyl 3,4- dicaffeoylquinic acid (Chen et al., 2014)</p>
Polysaccharides	<div style="display: flex; justify-content: space-around;"> <div style="text-align: center;">  <p>47. β-D-fructofuranose (Chou et al., 2012);</p> </div> <div style="text-align: center;">  <p>48. n=0: sucrose (Chou et al., 2012);</p> <p>49. n=1: 1-kestose (Chou et al., 2012);</p> <p>50. n=2: nystose (Chou et al., 2012);</p> <p>51. n=3: 1(F)-β- D-fructofuranosylnystose (Chou et al., 2012)</p> </div> </div>

### Isolation, Purification and Determination

TCM consists of multicomponent systems with mostly unknown ingredients. In order to study the chemical composition, various separation techniques have been employed for the analysis of active components from TCM. These techniques include thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), liquid chromatography–mass spectroscopy (LC-MS), gas chromatography, GC-MS, capillary electrophoresis, etc (Liu et al., 2008; Stalikas, 2007). At present, many researchers have reported some chemical composition of *G. divaricata* using these separation techniques by treating it with ethanol, petroleum ether, chloroform, n-hexane, EtOAc and n-BuOH. There are some extraction steps (Chen et al., 2014; Wan et al., 2011a).



### Flavonoids

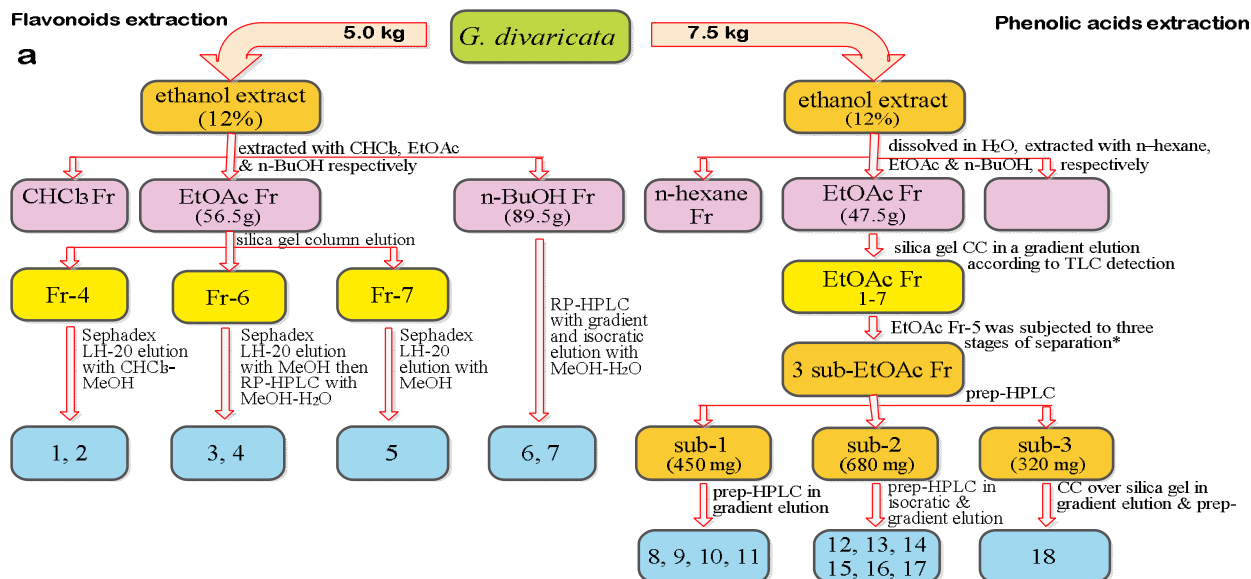
Several flavonoid compounds have been isolated from *G. divaricata* (Wan et al., 2011a). As shown in Figure 2a, 5 kg of the *G. divaricata* powder was extracted twice with 60% ethanol. The extracts were combined and evaporated to dryness. The dry extract was subjected to liquid–liquid extraction with chloroform, EtOAc and n-BuOH, and afford a chloroform fraction (CHCl<sub>3</sub>Fr; 31.2 g), a EtOAc fraction (EtOAc Fr; 56.5 g) and a n-BuOH fraction (n-BuOH Fr; 89.5 g), respectively. The EtOAc Fr was chromatographed using flash column on a silica gel step-gradient eluted with different ratios of chloroform-methanol, and the eluted fractions were combined on their TLC pattern to yield 8 fractions in which fractions- 4, -6 and -7 were used for further separation. The fraction-4 was chromatographed on a Sephadex LH-20 column eluted with chloroform-methanol to yield compounds quercetin (1) and kaempferol (2). The fraction-6 was also chromatographed on a Sephadex LH-20 column elution with methanol and further chromatographed on an RP-ODS column gradient elution with methanol-water gave compounds kaempferol-3-O-β-D-glucopyranoside (3) and 3,5-dicaffeoylquinic acid (4). The fraction-7 was also chromatographed on a Sephadex LH-20 column elution with methanol yields rutin (5). The n-BuOH Fr was chromatographed using flash RP-ODS column gradient elution with methanol-water and the eluted fractions were combined on their HPLC pattern to yield 4 fractions. The methanol-water fraction was further chromatographed using flash RP-ODS column and isocratic elution with methanol-water gave compounds kaempferol-3,7-di-O-β-D-glucopyranoside (6) and kaempferol-3-O-rutinoside-7-O-β-D-glucopyranoside (7). The other few constituents of n-BuOH Fr were isolated and identified as 13 compounds including quercetin, kaempferol and caffeoylquinic derivatives by HPLC-DAD-ESI-MS method.

The separation process mentioned above is a conventional method. Jie et al. (2011) developed an efficient separation process of the flavonoids from *G. divaricata* crude extracts (Jie et al., 2011). They employed HPD-100 macroporous resins to enrich the flavonoids from *G. divaricata* under the optimal conditions. After treatment with HPD-100 resin, the flavonoids content was increased more than 6-fold, with a recovery yield of 85%. Compared to traditional separation methods of the flavonoids in *G. divaricata*, this purification method is better because of its lower cost, higher efficiency. Therefore, it may provide scientific references for the massive flavonoids production from *G. divaricata* extracts for its medicinal use.

### Phenolic Acids

Chen et al. have separated some phenolic acids from *G. divaricata* (Chen et al., 2014). As shown in Figure 2b, 7.5 kg of dry *G. Divaricata* aerial parts were extracted twice with 80% ethanol at 70°C to acquire 900 g of crude extract after evaporation to dryness. The crude extract was dissolved in water (4.5 L) and partitioned with n-hexane, EtOAc and n-BuOH successively. The different fractions were concentrated to afford an n-hexane fraction (n-hexane Fr), an EtOAc fraction (EtOAc Fr; 47.5 g) and an n-BuOH fraction (n-BuOH Fr), respectively. The EtOAc Fr was subjected to silica gel column chromatography eluting with a gradient solvent system (n-hexane/EtOAc/acetone). EtOAc Fr 1–7 were obtained according to their TLC profiles. EtOAc Fr-5 (11.3 g) was further separated by three steps (column chromatography over MCI gel; automatic flash chromatography system on a reversed-phase column elution; Sephadex LH-20 gel column elution) to afford three subfractions (sub-EtOAc Fr). The concentrated sub-EtOAc Fr were purified by prep-HPLC. Sub-EtOAc Fr-1 (sub-1, 450 mg) was purified by prep-HPLC in linear gradient mode and afford compound 8 (5.0 mg), 9 (1.2 mg), 10 (0.5 mg) and 11 (1.7 mg). Sub-EtOAc Fr-2 (sub-2, 680 mg) was purified by prep-HPLC in isocratic mode and then in linear gradient mode to afford compound 13 (44.3 mg), 13 (38.2 mg), 14 (26.3 mg), 15 (13.6 mg), 16 (9.4 mg) and 17 (10.5 mg). Sub-EtOAc Fr-3 (sub-3, 320 mg) was subjected to column chromatography over silica gel eluted by a gradient system and additionally purified by prep-HPLC in isocratic mode

to afford compound 18 (1.0 mg). So far, there have been no reports in the literature about whether there was any new methods of improving the techniques to yields better quantities of these phenolic acids. Thus, there is still a lot of research to be done.



**Figure 2:** Extraction and isolation steps of flavonoids and phenolic acids from *G. divaricata*. Compound 1, quercetin; compound 2, kaempferol; compound 3, kaempferol-3-O- $\beta$ -D-glucopyranoside; compound 4, 3,5-dicaffeoylquinic acid; compound 5, rutin; compound 6, kaempferol-3, 7-di-O- $\beta$ -D-glucopyranoside; compound 7, kaempferol-3-O-rutinoside-7-O- $\beta$ -D-glucopyranoside; Compound 8, 3-O-caffeoylquinic acid; compound 9, 3-O-p-coumaroylquinic acid; compound 10, 3-O-feruloylquinic acid; compound 11, methyl 3-O-caffeoylquinic acid; compound 12, 4,5-dicaffeoylquinic acid; compound 13, 3,5-dicaffeoylquinic acid; compound 14, 3,4-dicaffeoylquinic acid; compound 15, methyl 4,5-dicaffeoylquinic acid; compound 16, methyl 3,5-dicaffeoylquinic acid; compound 17, methyl 3,4-dicaffeoylquinic acid; compound 18, ethyl 3,4-dicaffeoylquinic acid. CHCl<sub>3</sub> Fr, chloroform fraction; EtOAc Fr, EtOAc fraction; n-BuOH Fr, n-BuOH fraction; n-hexane Fr, n-hexane fraction. CC, column chromatography; Prep-HPLC, prepared HPLC. EtOAc Fr-5 was subjected to three stages of separation\*: first MCI gel CC, second automatic flash chromatography system on a reversed-phase column elution, and third Sephadex LH-20 prep column elution.

## Bioactivities and Pharmacological Functions

### Hypoglycaemic Activity

The ethanol extract of *G. divaricata* aerial parts was reported to show hypoglycaemic activity in vivo; the flavonoid compounds were the active components (Wan et al., 2011a; Akowuah et al., 2002; Zhang and Tan, 2000). Growing evidence indicates the hypoglycaemic activity of flavonoid compounds in *G. divaricata* (Wu et al., 2011; Babu et al., 2013), including quercetin, isoquercitrin, rutin, and kaempferol-3-O- $\beta$ -D-rutinoside. Moreover, polysaccharides from this plant have been shown to exert an antidiabetic effect (Deng et al., 2011). Chou et al. found that these hypoglycaemic components of *G. divaricata* are fructooligosaccharides, including  $\beta$ -D-fructofuranose, sucrose, 1-kestose, nystose, and 1(F)- $\beta$ -D-fructofuranosyl nystose (Chou et al., 2012). In addition, an investigation suggests that polysaccharide from the plant exerted an antidiabetic effect partly via inhibiting the increased intestinal disaccharidase activities of diabetic rats (Deng et al., 2011). Protein Tyrosine Phosphatase 1B (PTP1B) is a promising target for the

treatment of type 2 diabetes, which is thought to be a negative regulator of insulin receptor signalling (Goldstein, 2001). The study of Chen et al. showed that 3,5-dicaffeoylquinic acid and 3,4-dicaffeoylquinic acid isolated from *G. divaricata* are of an obvious inhibitory effect on PTP1B, compared with the positive control in a parallel assay (Chen et al., 2014). They also found 4,5-dicaffeoylquinic acid, 3,4-dicaffeoylquinic acid, methyl 4,5-dicaffeoylquinic acid and methyl 3,4-dicaffeoylquinic acid isolated from this plant showed significant inhibitory effects against yeast  $\alpha$ -glucosidase, with  $IC_{50}$  values much lower than that of acarbose (Chen et al., 2014). The results suggested that phenolic acids in *G. divaricata* are responsible for its hypoglycaemic activity. The number of caffeoyl groups attached to a quinic acid core is important for the  $\alpha$ -glucosidase inhibitory activity. Moreover, the methyl esters of the isolates always exhibit more significantly inhibitory effect on  $\alpha$ -glucosidase. Flavonoids and caffeic acid were also proven to show strong inhibitory activity on PTP1B (Zhang et al., 2010). Importantly, our latest studies also demonstrated the good hypoglycaemic activity of lyophilised powder of *G. divaricata* in type 2 diabetic mice (Xu et al., 2015). The lyophilised powder of *G. divaricata* aerial parts retained the plant's physicochemical properties and physiological activity to maintain as much of the plant's active chemicals as possible. In addition, the powder induced a significant decrease in fasting blood glucose by more than 55% in *G. divaricata*-treated diabetic groups, relative to diabetic model mice, which successfully demonstrated that *G. divaricata* has potential to be an effective medicine for the treatment of diabetes.

### **Antihypertension**

Huang et al. reported that the water extract of *G. divaricata* had significant effects on reducing blood pressure in spontaneously hypertensive rats. Meanwhile, it can also increase the content of serum nitric oxide and superoxide dismutase activity significantly in spontaneously hypertensive rats (Huang et al., 2009). Wu et al. also reported antihypertensive effects of water and solvent fractions of *G. divaricata* (Wu et al., 2011).

### **Hypolipidaemic Effect**

The flavonoids from *G. divaricata* have obvious glucose-lowering effect and antiatherosclerotic activities (Jie et al., 2011). Tong et al. reported that both water and ethanol extracts of *G. divaricata* had obvious effects on reducing the contents of triglycerides on experimental hyperlipidaemia in rats, which indicated that *G. divaricata* may have therapeutic effects on hyperlipidaemia (Tong et al., 2012).

### **Antiproliferation**

It has been reported that many constituents (kaempferol and quercetin) in *G. divaricata* are of antiproliferation activity (Chen et al., 2009b; Chen et al., 2003; Wan et al., 2011b).

### **Antioxidation**

Reactive free radicals, including superoxide, hydroxyl radical, and peroxy radical, generally result in degradation of protein, lipid peroxidation and oxidation of DNA, which have been considered to be linked with many chronic diseases, such as diabetes, cancer and atherosclerosis (Riley, 1994; Willcox et al., 2004). Polyphenols and flavonoids are the major antioxidant constituents isolated from many medicinal and edible plants (Moon and Shibamoto, 2009), so is *G. divaricata*. It is well known that flavonoids function as natural antioxidants by virtue of the number and arrangement of their phenolic hydroxyl groups attached to aromatic ring structures (Rice-Evans, 2001). The protective effects of flavonoids in diabetes as antioxidants are often owed to their capacity to activate antioxidant enzymes to

scavenge free radicals in vitro (Hirano et al., 2001; Yokozawa et al., 2002; Esmaeili et al., 2009).

In addition, phenolic acids, which are found in abundance in *G. divaricata*, are characterised as natural antioxidants. Our recent research analysed *G. divaricata* methanol extract and abundant phenolic acids were found. They are chlorogenic acid, 3,4-dicaffeoylquinic acid, 3,5-dicaffeoylquinic acid and 4,5-dicaffeoylquinic acid. The results of quantitative determination are shown in Table 4. The contents of these four bioactive components in the *G. divaricata* were about 2.337%. Chlorogenic acid, a phenolic compound found widespread in plants, is widely recognised as an antioxidant (Sotillo and Hadley, 2002). The three dicaffeoylquinic acids also have radical-scavenging activity (Chen et al., 2014). Regarding to structure-function relation, the four bioactive components have vicinal hydroxyl groups on an aromatic residue (Sato et al., 2011), and the C = C linked to the phenyl ring may play a important role in stabilizing the radical by resonance which ensures their antioxidant capacity (Rice-Evans et al., 1996; Cuvelier et al., 1992). It is generally known that the diabetogenic action of streptozocin can be illustrated as free radical caused toxicity particularly to pancreatic  $\beta$ -cells. And antioxidants, the free radical scavenger, are considered to be the potential cure for diabetes (Matsushige et al., 1996). Thus, with this background, we could conclude that *G. divaricata* is of prominent antioxidant capacity.

**Table 4:** Quantification of four bioactive components in methanol extract of *G. divaricata*.

Bioactive components	Contents (mg/g <i>G. divaricata</i> powder)
Chlorogenic acid	5.57±0.35
3, 4-dicaffeoylquinic acid	0.67±0.08
3, 5-dicaffeoylquinic acid	10.29±0.74
4, 5-dicaffeoylquinic acid	6.84±0.14

### Antitumor

Flavonoid compounds, a large group of secondary metabolites in higher plants (Gattuso et al., 2007), are known to be responsible for antioxidant (Gould et al., 2006; Heim et al., 2002), anticancer (Cibin et al., 2010; Fang et al., 2009) and antibacterial activity (Kouam et al., 2007; Naeem et al., 2010). Currently, epidemiological studies have strongly demonstrated that consumption of plant flavonoids is helpful to reduce the risk or incidence of some cancers (Wang et al., 2009).

### Prospective

*G. divaricata* has been gradually developed into a high-grade vegetable produced by artificial cultivation from a wild vegetable since the middle of the 1990s. It is easy to grow, maintain and harvest. The whole plant can be applied, which has good effect on many diseases. In addition, it is a kind of medicinal and edible plant with rich nutrients and special healthcare functions so that many Chinese people like using it to make dishes. The manuscript systematically summarises the folk application, the main chemical components, the extraction and separation methods of main chemical components and pharmacological effects of *G. divaricata* for the first time, which provided a foundation for the further research of *G. divaricata*.

Currently, diabetes mellitus has become the third major world public health problem after cardiovascular disease and tumours. Folk medication experience and modern research all suggest that *G. divaricata* has obvious hypoglycaemic and hypolipidaemic effects.

At present, great progress has been made in the research of extraction, identification, and pharmacological action of the *G. divaricata* chemical components in China. However, the pharmacological action mechanism of this medicinal plant has not been deeply studied. Therefore, strengthening the basic research of the *G. divaricata* in these areas is of great significance to the development of new medicinal resources and medical care.

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