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# Gas Chromatography-Mass Spectrometry Analysis of Natural Products in *Gypsophila paniculata*

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***Abstract. Gypsophila paniculata*** **is an ornamental crop with medicinal value. To date, limited information has been reported about the natural products in *G. paniculata* to explain its medicinal function. The current study reports the natural products found in *G. paniculata* stem for the first time. Thirty-three compounds were isolated from the extract of *G. paniculata* stem and identified by gas chromatography-mass spectrometry, 10 of which have contents >2%. These were 2-O-methyl-D-mannopyranose (37.4706%), glycerol (12.5669%), two tetratetracontane isomer (7.6523 + 3.5145%), tetrahygro-4-pyranol (5.3254%), 1,6-anhydro-beta-d-glucopyranos (4.7507%), palmitic acid (4.1848%), 4-hydroxy-3-methoxystyrene (3.7439%), methyl-octadeca-9,12-dienoate (2.7490%), and 2-deoxy-D-galactose (2.6193%). Another bioactive compound, condrillasterol, was identified with 1.3384% content. We also reported that *G. paniculata* possesses antioxidant activity possibly associated with the presence of a phenolic chemical 4-hydroxy-3-methoxystyrene. Our data collectively demonstrate that *G. paniculata* contains some bioactive compounds with high contents and antioxidants, consistent with its role as a medicinal herb.**

*Gypsophila paniculata*, a flowering plant of the Caryophyllaceae family, is the only species used as a cut flower in the genus *Gypsophila*, ranking as one of the top 10 best-selling cut flower species globally (Li et al., 2019). Fragrance is an attractive and prominent trait of an ornamental plant and is also important for attracting insects to finish its pollination. The scent of plants is complex and composed of various volatile organic compounds such as benzyl acetate, eugenolbenzyl alcohol, cinnamyl alcohol, cinnamyl acetate, and benzyl benzoate (Yuan et al., 2019; Zhang et al., 2020). There is an unpleasant smell exuded from the flower of *G. paniculata*, which reduces its ornamental value. Plants belonging to genus *Gypsophila* release an unpleasant odor during bud opening, associating with the presence of methylbutyric acid (Nimitkeatkai et al., 2005), which has also been isolated from *G. paniculata* (Furukawa, 2019). However, there is no reported chemical component related to the odor of the stem, the major part of *G. paniculata*, to date.

In addition to the ornamental purposes of cut or dried flowers, the root and stem of *G. paniculata* are also used as traditional Chinese herbal medicine (Lu and Nicholas, 2001). It displays spermicidal activity due to the presence of saponins (Primorac et al., 1985). Moreover, it can be used as an adjuvant for veterinary vaccines (Turmagambetova et al., 2017). Medicinal herbs have historically been applied to various diseases, particularly in Asian countries (Tyler, 2000). The practice of traditional Chinese herbal medicine has been successful in immunomodulatory and antimicrobial activities (Tan and Vanitha, 2004). In addition, Chinese medicinal herbs such as *Anemarrhena asphodeloides*, *Artemisia argyi*, and *Commiphora myrrha* play a role in anticancer activity because their stem aqueous extracts can inhibit the growth of cancer cell lines in vitro (Shoemaker et al., 2005). However, herbal medicine involves a combination of chemicals, and thus the evaluation of its safety is then complex (Chang, 2000).

Mass spectrometry (MS) plays an important role in determining the bioactive compounds associated with the pharmacological activity in medicinal herbs. Such information has been obtained for many Chinese medicinal herbs used to treat diseases such as neurological disorders, cardiovascular diseases, inflammatory diseases, and cancer (Wang et al., 2003). The application of gas chromatography-MS (GC-MS) facilitates the research of chemical constituents and metabolic profiling in medicinal herbs because of its high resolution, selectivity, and sensitivity. Benefiting from this, effective constituents such as nonprotein amino acids, steroid, alkaloid, fatty acid, terpene, and organic acid, have been identified in various medicinal herbs (Ye, 2009).

Medicinal plants are also frequently used as preservatives in the food cosmetics industries. In addition, they are valuable resources for antiaging ingredients supplied in cosmetics as well (Škrovánková et al., 2012). Substances capable of scavenging free radicals, referred to as antioxidants, function in preventing oxidative processes in mammalian cells (Škrovánková et al., 2012). A variety of phytochemicals are involved in the antioxidative process, including phenolics, flavonoids, anthocyanins, polyphenols, carotenoids, ascorbic acid, terpenoids, tannins, and tocopherols (Bhatt et al., 2013).

Many previous studies have reported the isolation of triterpenoid saponins from the extract of *G. paniculata* root and demonstrated the inhibition ability against yeast α-glucosidase of some identified triterpenoid saponins (Yao et al., 2010, 2011); however, the chemical composition of *G. paniculata* stem has never been reported. In the present study, we analyzed the natural products of *G. paniculata* stem extract and screened phytochemicals with potential bioactivities by GC-MS. The antioxidative capacity of the medicinal part, the stem, was also evaluated for further application of this ornamental plant in the food or medicine industry.

## Materials and Methods

*Plant materials and extraction.* The commercial cultivar 'Cloudstar 4' of *G. paniculata* was used in this study. The plant materials were obtained from Yuxi Yunxing Biological Technology Co., Ltd. (Yunnan, China). *G. paniculata* stem extraction was performed as described by Mayer et al. (2006). Raw material (150 g) with 1000 g of trichloromethane as solvent were added to a 2000-mL extraction flask. With heating mantle, oily water separating installation and steam distiller were used for extraction. The extraction temperature was set at 100 °C and the time was set for 10 h.

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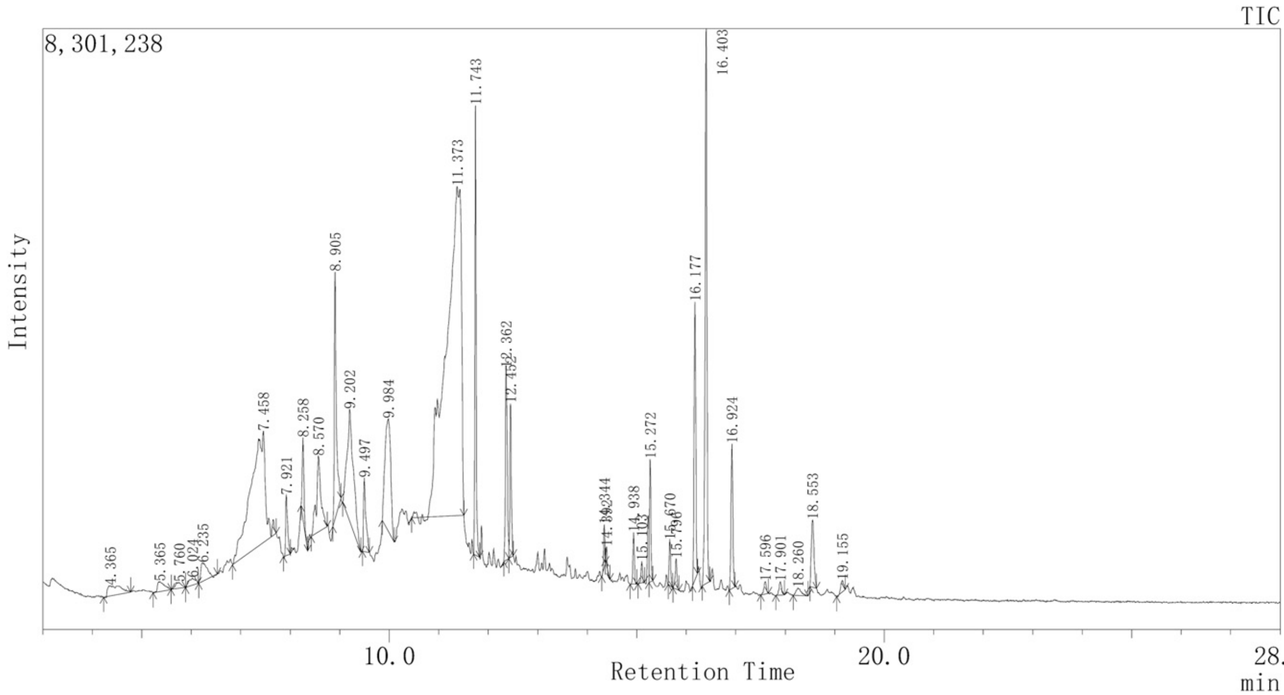



Fig. 1. Gas chromatography-mass spectrometry chromatogram of extract of *Gypsophila paniculata* stem. The x-axis represents the retention time, and the y-axis represents the intensity of each peak.

*GC–MS analysis.* GC–MS analysis of constituents in the stem extract was carried as described by Bouchra et al. (2003). Compounds were identified by matching the retention time peaks of known compounds and then compared the mass spectra with those present in the computer data bank (US National Institute of Standards and Technology) and published spectra.

*Antioxidant assay.* The antioxidative capacity of *G. paniculata* stem extract was tested by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assay as described by Eleazu (2016). Samples were extracted by trichloromethane and diluted to eight concentrations (0.08, 0.10, 0.20, 0.30, 0.40, 0.50, 1.00, and 2.00 mg/mL). Of 0.3 mM DPPH, 0.1 mL was added to each sample, and they were kept in the dark. The absorbance of incubated samples was detected after 30 min by spectrophotometer stabilized by DPPH control at 517 nm. The scavenging activity was calculated using the equation:

$$\textit{Scavenging activity}\ (\%) = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100\%$$

## Results

*2-O-methyl-D-mannopyranose is abundant in G. paniculata.* Thirty-three compounds were identified in the stem extract of *G. paniculata* by GC-MS, corresponding to the peaks at different retention time (Fig. 1), whereas the contents of 10 compounds, 2-O-methyl-D-mannopyranose, glycerol, two tetratetracontane isomer, tetrahygro-4-pyranol, 1,6-anhydro-beta-d-glucopyranos, palmitic acid, 4-hydroxy-3-methoxystyrene, methyl-octadeca-9,12-dienoate, and 2-deoxy-D-galactose were >2% (Table 1). Notably, 2-O-methyl-D-mannopyranose, a methylated mannose, made up 37.4706% of the extract. Glycerol, a hyperosmolar agent, ranked second, comprising 12.5669% in the extract. Another chemical with bioactivity, 4-hydroxy-3-methoxystyrene, which has been reported to be a natural germination inhibitor exerting potent antiinflammatory effects (Sudhagar et al., 2018), made up 3.7493% of the extract. Additionally, condrillasterol a chemical with antibacterial properties, made up 1.3384% of the extract. The structures of compounds having a potential medicinal function or antioxidative capacity is shown in Fig. 2.

*G. paniculata possesses antioxidative capacity.* To investigate the potential antioxidant activity of *G. paniculata*, a DPPH scavenging assay was performed using the trichloromethane extracts of its stem. The scavenging effect was concentration dependent (0.08–2.0 mg/mL) as the scavenging rate rose with the increase of extract concentration (Fig. 3). Nevertheless, the rising ratio was retarded when the concentration of extract reached 1.0 mg/mL. The $IC_{50}$ value of *G. paniculata* stem predicted by trend was 11.45 mg/mL.

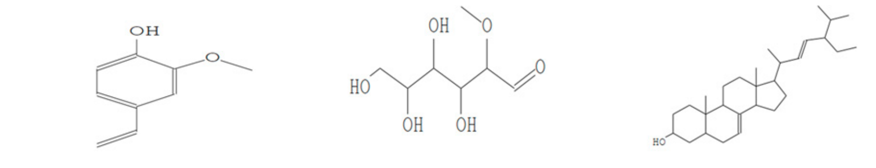
*Compounds behind G. paniculata scent.* To understand what causes the unpleasant smell of *G. paniculata*, we pursued the odor chemicals in the stem extract by GC-MS. Four significant peaks were shown in the chromatogram at 1.4, 7.16, 8.31, and 9.39 min (retention time) respectively, corresponding to oxygen (68.62%), dimethyl succinate (8.51%), dimethyl glutarate (19.82%), and dimethyl adipate (2.83%) (Fig. 4). The identified compounds are either without odor or with agreeable odor that cannot explain the smell of *G. paniculata*.

## Discussion

The demand for natural antimicrobial and antioxidant agents from herbs replacing synthesized ones in the food and medicine industry is growing due to the consumption custom (Ortega-Ramirez et al., 2014).

Table 1. Chemical compounds in *Gypsophila paniculata* stem with contents over 2% revealed by gas chromatography-mass spectrometry.

| Compound | Molecular formula | Retention time (min) | % content |
|---|---|---|---|
| 2-O-methyl-D-mannopyranose | $C_7H_{14}O_6$ | 11.373 | 37.4706 |
| Glycerol | $C_3H_8O_3$ | 7.458 | 12.5669 |
| Tetratetracontane | $C_{44}H_{90}$ | 16.403 | 7.6523 |
| Tetrahydro-4-pyranol | $C_5H_{10}O_2$ | 9.202 | 5.3254 |
| 1,6-anhydro-beta-d-glucopyranos | $C_6H_{10}O_5$ | 9.984 | 4.7507 |
| Palmitic acid | $C_{16}H_{32}O_2$ | 11.743 | 4.1848 |
| 4-hydroxy-3-methoxystyrene | $C_9H_{10}O_2$ | 8.905 | 3.7439 |
| Tetratetracontane | $C_{44}H_{90}$ | 16.177 | 3.5145 |
| Methyl-octadeca-9,12-dienoate | $C_{19}H_{34}O_2$ | 12.362 | 2.7490 |
| 2-deoxy-D-galactose | $C_6H_{12}O_5$ | 8.570 | 2.6193 |




4-Hydroxy-3-methoxystyrene 2-O-Methyl-D-mannopyranose Condrillasterol

Fig. 2. Structures of compounds possess potential medicinal value or antioxidative capacity. 4-hydroxy-3-methoxystyrene is considered to possesses antioxidant activity, and 2-O-methyl-D-mannopyranose and condrillasterol are considered to have medicinal value.

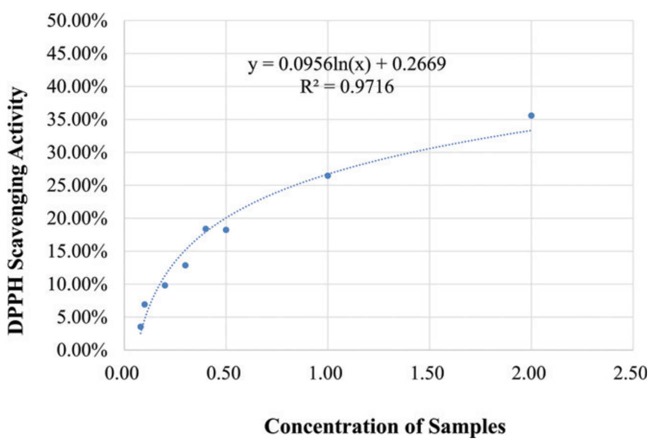



Fig. 3. Free radical scavenging capacity of *Gypsophila paniculata* stem extract. The experiment was performed based on 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging activity. The x-axis represents the concentrations of the extract used in the DPPH scavenging experiment, and the y-axis represents the DPPH scavenging activity according to the calculation of the percentage of free radicals being scavenged. Data were analyzed by Excel.

Benefiting from the developed techniques, the bioactive components against certain diseases have been isolated and investigated. For example, artemisinin and its derivatives (ARTs), isolated from *Artemisia annua* L., display anticancer activity associated with the expression of genes such as *c-MYC*, *cdc25A*, and *EGFR* (Tan et al., 2011).

A previous study revealed that the root of *G. paniculata* produced gypsogenin, which can inhibit the catalyzation of α-glucosidase, indicating its potential role in suppressing postprandial hyperglycemia (Yao et al., 2010). Here, by analyzing the extract of *G. paniculata* stem, we found a methylated sugar, 2-O-methyl-D-mannopyranose, with high content in the extract. The methylation of hexose frequently exists in plants, but the 2-O-methylated of mannose has only been reported in fungi (Staudacher, 2012). Although there is no direct association between the medicinal function and methylated hexose, it has been reported that immune systems and glycans decorated with 2-O-methyl-D-mannose residues react with rabbit immunoglobulin G antibodies (Braaten et al., 1994; Wohlschlager et al., 2014). Condrillasterol displays antibacterial properties probably by inhibiting or disrupting the activity of biofilm of pathogens (Mozirandi et al., 2019). Thus, the presence of 2-O-methyl-D-mannopyranose and condrillasterol in *G. paniculata* might be involved in its role as a medicinal herb.

A medicinal plant that possesses antioxidant activity could be valuable. Here, we showed that the extract of *G. paniculata* displayed antioxidant activity. Phenolic compounds isolated from herbs possess antioxidant capacity (Balasundram et al., 2006; Cai et al., 2004; Fukumoto and Mazza, 2000; Wojdyło et al., 2007). They also possess antimicrobial effects against various bacterial, such as *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli* (Rauha et al., 2000). Here we isolated a phenolic compound, 4-hydroxy-3-methoxystyrene, that made up 3.7439% of the extract. A previous study on the liquid products of hazelnut shells demonstrated that all compounds containing phenolic hydroxyl structure, including 4-hydroxy-3-methoxystyrene, act as antioxidants (Zhao et al., 2021), implying that the antioxidant capacity of *G. paniculata* is probably attributable to 4-hydroxy-3-methoxystyrene.

As previously reported, *G. paniculata* possesses methylbutyric acid, which releases a bothersome smell (Furukawa, 2019). However, we did not isolate this chemical from the extract of its stem. In contrast, dimethyl glutarate, a chemical with a light, sweet smell, was identified. Thus, one may conclude that the unpleasant odor is mainly released from the flower buds of *G. paniculata.*

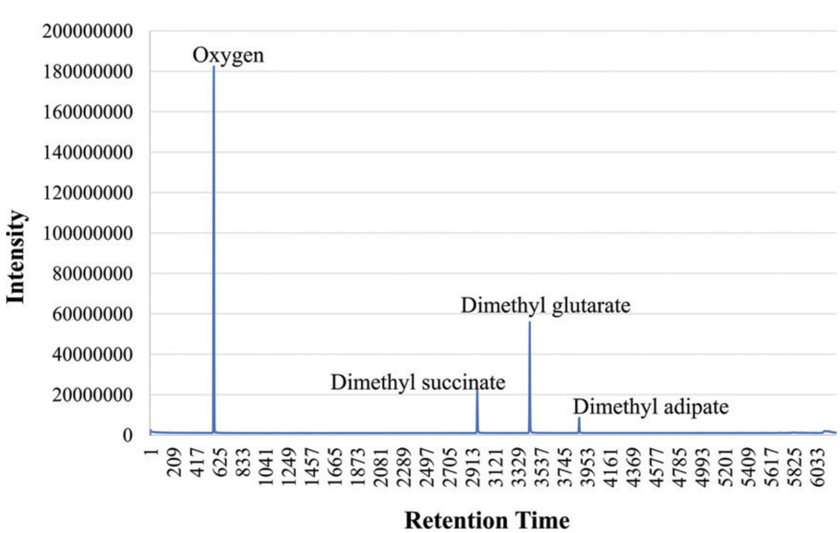



Fig. 4. The main odor chemicals in *Gypsophila paniculata* revealed by gas chromatography-mass spectrometry (GC-MS). Four chemicals were identified by GC-MS and are shown in the figure. The x-axis represents the retention time, and the y-axis represents the intensity of each peak.

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