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Gynostemma pentaphyllum cultivation in Sydney, Australia and its comparison with products from China

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Abstract

Traditional Chinese medicine has revealed an abundant source of plants containing compounds with the potential for pharmaceutical preparation. Due to favourable climatic and geographical conditions, many of these plants are grown in Asia. One such plant is *Gynostemma pentaphyllum*, a popular herb containing saponin components analogous to *Panax ginseng* and used for the treatment of hyperlipidaemia in China. This paper documents the cultivation of *G. pentaphyllum* in Sydney, Australia outside its native China. The dry weight yield of the Sydney-grown plant was in the range of the yield obtained in China. Compared to *G. pentaphyllum* products other than Sydney, the percentage of extracted material was approximately 2.5 times more for the locally-grown material and thin layer chromatography revealed a different saponin profile for all *Gynostemma* products. Initial results suggest that *G. pentaphyllum* could be easily grown and may be an alternative to *Panax ginseng* due to similar biological activities. Thus, Australia may be a new growing location for the herb.

Key words: Dammarane, *Gynostemma pentaphyllum* (Thunb.) Makino, gynosaponin, gypenoside, herbal medicine, sapcomparisononins.

Introduction

As the popularity of herbal medicine grows, so does the quest to uncover new and exciting natural materials that may effectively treat many debilitating diseases. One such plant with increasing clinical significance is *Gynostemma pentaphyllum* (Thunb.) Makino, a perennial climbing herb of the Cucurbitaceae family (China Pharmaceutical University, 1996). The plant is also known by other names including Jiaogulan, Qi Ye Dan, Gong Luo Guo Di, Pian Di Sheng Gen, Xiao Ku Yao, Amachazuru and Penta.

There are 21 species of *Gynostemma* growing abundantly in southern Shaanxi and areas south of the Yangtze River, China (Blumert and Liu, 1999, China Pharmaceutical University, 1996). *G. pentaphyllum* only occurs in the subtropics and tropics of Asia (China, India, Nepal, Bangladesh, Sri Lanka, Laos, Malaysia, Indonesia, Korea, Japan and Papua New Guinea) and is distributed naturally as a climber in mountain forests, mountain valleys, wood, scrub, stream banks, roadsides, bushes, shaded and humid places at an altitude of 300-3200 m (China Pharmaceutical University, 1996).

G. pentaphyllum is shown in Fig. 1. The morphology consists of slender, branched stems which are arris, glabrous or pubescent in nature. The palmately-compounded leaves are membranous and 3-9 foliate (usually 5-7 foliate). The leaflets are ovate-elliptic to lanceolate, rarely inverted and are acuminate or obtuse. They are scabrous on both sides, are deep green on the upper side and light green underneath. The complex tendrils are slender and two-branched. *G. pentaphyllum* is dioecious. The conical, inflorescent male flowers are 10-15 (up to 30) cm. The small, multi-branched tube consists of branches 3-4 (up to 15) cm in length. The thread-like stalk is 1–4 mm long. The short calyx consists of five triangular, apiculate lobes ~0.7 mm long. The 5-cleft corolla is pale green or white. The acuminate, single-veined petals (2.5-3.0 mm long, 1 mm wide) are ovate-lanceolate and slightly serrated. The stamens consist of five short, connate filaments, forming a column with anthers at the apex. The female flowers are similar to the male but are much smaller. The 2-3 loculated ovary is globose. The three styles are short and bifid; the stigma also bifid. Short remnant stamens may be present. The fruit consists of a glabrous, non-bifid, globose small berry about 5-6 mm in diameter and black when ripe. The two ovo-heart shaped seeds inside (~4 mm in diameter) are greyish brown or deep brown in colour and are compressed with irregular warty or papillary tubercles. The apex of the seed is obtuse and the base is heart-shaped. The flowering seasons in the northern hemisphere are March to November (September to May for the southern hemisphere); the fruiting seasons are April to December (October to June) (China Pharmaceutical University, 1996). Harvesting season in China is September to October.

Usage of *G. pentaphyllum* dates back 500 years as a food alternative, especially in times of famine. Traditional use of the herb includes treatment of chronic tracheitis, bronchitis, infectious hepatitis, pyelitis and gastroenteritis (China Pharmaceutical University, 1996). Biological activity of *G. pentaphyllum* has been attributed to its saponins of a dammarane nature called gypenosides or gynosaponins (Cui *et al.*, 1999). Gypenosides have also been discovered in *Gymnema sylvestra* (Asclepiadaceae) (Yoshikawa *et al.*, 1991) and *Panax* species such as *P. notoginseng* (Araliaceae) (Yoshikawa *et al.*, 1997) and *P. quinquefolium* (Araliaceae) (Wang and Li, 1997). Gypenosides have evoked much research interest due to their similarity in structure to ginsenoside saponins from *Panax ginseng* (Arialaceae), an immensely popular herbal medicine. Latest literature searches have uncovered over 100 saponins for *G.*

pentaphyllum, compared to around 28 for *P. ginseng* (Bergner, 1996). Approximately 25% of the saponin content in *G. pentaphyllum* is ginsenosides of the panaxadiol type such as Rb_1, Rb_3, Rc, Rd, F_2, Rg_3, malonyl-Rb_1 and malonyl-Rd. Rest of the saponin content is distinct to *G. pentaphyllum* (Qin *et al.*, 1992). The similar pharmacological activities of *G. pentaphyllum* and *P. ginseng* include effects on the cardiovascular, endocrine, immune and central nervous systems. Both plants exhibit anti-inflammatory, hypolipidaemic, anti-oxidant, anti-bacterial, hypoglycaemic and anti-cancer activities (Jang *et al.*, 2001; Gillis, 1997; China Pharmaceutical University, 1996). Numerous and diverse medicinal properties consequently increase the appeal of *G. pentaphyllum* as a main stream herbal remedy. *G. pentaphyllum* is also known as 'Southern Ginseng' and is sometimes praised as 'the herb of immortality' (Blumert and Liu, 1999). The plant has adopted many other uses including oral fluid (Kadota, 1986; Gao and Yu, 1993), health foods (Osaka Yakuhin Kenkyusho K. K., 1985a, b and c) and cosmetic products (Takemoto, 1983; Takemoto *et al.*, 1986).

Thus, *G. pentaphyllum* could provide a variety of saponin types, an inexpensive source of saponins for pharmaceutical use and possibly, a substitute for *P. ginseng*.

G. pentaphyllum is not native to Australia and has never been cultivated locally. This study attempts to cultivate and harvest *G. pentaphyllum* outside its native Asia, in Sydney, Australia for further chemical and biological studies of the local material. Growing conditions in China and Sydney were also compared. It has also been reported that different growing localities produce different saponins (Ding and Zhu, 1992). Saponin profiles of the locally produced material and commercially available products were compared and percentage of extracted material calculated.

Materials and methods

Cultivation: Greenhouse and outside garden at the University of Sydney and a house yard at Randwick, N.S.W., Australia (both areas approximately 10 m^2) were used to grow *G. pentaphyllum*. The seeds were obtained from the Hubei University, Wuhan, China and handled in accordance with the Australian Quarantine Inspection Service (AQIS) permit conditions. "Hortico Aquasol" fertiliser (containing nitrogen (23%), phosphorus (4%), potassium (18%), plus trace elements such as iron), "Hortico Kelthane Red Spider Miticide" (active constituent: 75 g l^{-1} Dicofol) and "Dipel HG Bio-insecticide" (active constituents: 4320 International units of potency mg^{-1} of *Bacillus thuringiensis* var. Kurstaki mg^{-1}) were from Arthur Yates and Co. Ltd. (Homebush, N. S. W., Australia). "Ant-rid" (active ingredient 9.24 g kg^{-1} boron as disodium tetraborate decahydrate) was from LS Russell Manufacturing Pty (Moorabin, VIC., Australia). "Baysol Snail and Slug Bait" (active ingredient: 20 g kg^{-1} methiocarb) was from Bayer Cropscience (East Hawthorn, VIC., Australia).

The methods and optimal conditions for growing *G. pentaphyllum* are outlined in China Pharmaceutical University (1996), Wang *et al.* (1996) and Guo and Wang (1993). The cultivation conditions for China and Sydney are summarised in Table 1. Guidelines on agricultural practices for medicinal plants by the World Health Organisation (WHO, 2003) were also followed. The Sydney harvest of *G. pentaphyllum* was grown from both stem and root sections (rhizomes). For stem propagation, the aerial part was cut into a section comprising of two nodes consistent with the common growing practice of China (China Pharmaceutical University, 1996). The two major soil types in the Sydney Region are sandy soils (derived from Hawkesbury sandstone) and clay soils (derived from shales or volcanic rocks). Some soils may be a combination of the two (Department of Environment and Conservation (N.S.W.)).

The fertile sandy loam as in the garden at the University and the house yard was well aerated and retained water. In the University yard, the plant was protected from strong sunlight and wind using a steel frame and mesh (Fig. 2). Wooden sticks were placed in the soil outside to allow the plant to grow upwards by intertwining itself. *G. pentaphyllum* was grown as pot plants in the greenhouse (Fig. 3). Cultivation was in full sunlight in the house yard (Fig. 1). The plants formed a thick horizontal intertwined mat that was resistant to sun burning where no soil was exposed to strong sunlight. In the house yard, the plants were sheltered from strong wind by fences and buildings. The climate range in N.S.W. is entirely temperate (Australian Government Bureau of Meteorology). The air temperature in N.S.W. was suitable for growing *G. pentaphyllum* with outside temperature ranging from 10-35°C. The glasshouse temperature was set at 22°C. The viable outside air humidity was 50% and water content of the soil was 13-16%.

Optimal glasshouse soil water content was 25-40% and optimal air humidity was at 75%. In the glasshouse, watering was done three times a day. Watering in the University yard was initially six times a day but later reduced to three times as the plants showed a yellow discolouration, possibly due to over-watering. Watering in the house yard was when required (daily in hot weather) to keep the soil moist and prevent burning of the delicate growing shoots.

Nitrogen-phosphate-potassium compound chemical fertiliser (2-3 times) was administered during the active growth period. In the house yard, "Hortico Aquasol" fertiliser was dissolved as 8 g in 10 l of water as directed by the manufacturers and applied with a large watering can. The ants could be exterminated by "Ant-rid" and the red spider exterminated by "Hortico Kelthane Red Spider Miticide" (20 ml in 3 l water). Cabbage white moth could be exterminated by "Dipel HG Bio-insecticide" and snails and slugs exterminated by "Baysol".

Harvesting, processing and storage: In Sydney, the herb was collected twice a year, after each growing period (March and September). Weeding was also performed at the time of collection. The soil was dug up and overturned. Four banks were formed with channels on either side. The roots were covered again with the soil and watered extensively.

After collection, the aerial part of the plant (which included mainly stems and leaves) was cleaned by removing impurities such as earth or sand, rock and non-medicinal parts. The whole plant was dried in direct sunlight or in an oven at a temperature of 50°C over three days. The plant was then cut into smaller pieces and subsequently milled using the High Moisture-High Fat Cyclone Mill (UDY) (Sietronics, Belconnen, A.C.T., Australia) with a particle mesh size of 1 µm. The herb was stored in sealable plastic bags in cardboard boxes in a dry, well ventilated room or

refrigerated to prevent damage due to dampness, mildew, rot and insect pests.

Comparison of locally-grown *G. pentaphyllum* to other sources: The commercially-supplied extract (ANK) (containing 99.5% total saponin fraction), ANK capsules and tablets (which contained 60 mg of the total saponin fraction) and raw plant material (researched by Ankang Medical Plants Development Institute, Shaanxi, China) were a kind gift from Ankang Pharmaceutical Institute of the Beijing Medical University (Beijing Medical University (Ankang) Pharmaceutical Holdings Co. Ltd). Lanwang tea of "*Gynostemium pentaphylla*" was from Ankang "*Gynostemium pentaphylla*" Technology Development Corporation, China (researched by Ankang Medical Plants Development Institute, Shaanxi, China). *Gynostemma* plant was obtained from Sheng Tang Shan, Guangxi Province, China. Jiagulan Tea (Jin Xiu Sheng Tang Shan, Jin Xiu Country, Guangxi, China), Five leaves Ginseng (Guangzhou Natural Products Company LTD, Huaxian, Guangzhou, China) and Tsai's Amachazuru (Zhuhai Sez, Agricultural Biotechnology Development and Research Centre, Xiangzhou District, Zhuhai Guangdong, China) were purchased from 'Chinese Ginsengs and Herbs' (Haymarket, N.S.W., Australia).

The solvent fractions were evaporated under reduced pressure to dryness using a Rotovapour R-114 rotary evaporator with a water bath (B-480) temperature ranging between 40-60°C (Büchi, Flawil, Switzerland). Camag Planar Chromatography System (Linomat 4 machine), 20 cm x 10 cm Camag dipping chamber tank, Camag hotplate and Camag Reprostar 3 with Video Store/ VideoScan were purchased from MEP Instruments Pty. Ltd. (North Ryde, N.S.W., Australia). Horizontal tanks (20 x 20 cm and 10 x 10 cm) were used for TLC (Desaga, Heidelberg, Germany).

In order to investigate the different *Gynostemma* products available and their respective TLC profiles, *Gynostemma* product (4 g) was extracted three successive times with stirring in boiling water or cold 90% ethanol in water (100 ml). The Sydney-grown plant material was extracted with cold methanol, ethanol, 90% ethanol or boiling water. The capsule and tablet form were extracted in cold 90% ethanol. Each extract was filtered in a Buchner funnel and concentrated to dryness on a rotary evaporator. Extract solutions (10 mg ml^{-1} in dissolving solvent) were made up for TLC. The percentage of *Gynostemma* plant extracted from dried material using 90% ethanol is listed in Table 3.

The solutions were applied onto the silica gel 60 F_{254} thin layer chromatography sheets using the Camag Planar Chromatography System (Linomat instrument). The conditions set on the Linomat instrument were 15 mm from the left hand edge of the TLC plate, 4 µL on 4 mm band, with bands 5 mm apart. The plate was dried and then placed in a developing tank flat bottom chamber with the mobile phase consisting of a miscible solution of chloroform:ethyl: acetate:methanol:water (15:40:24:7 modified from 15:40:22:10 (Xie and Yan, 1987) as it showed better resolution and separation of the bands). The plate was left to elute in the tank until the solvent front was 1 cm from the top (approximately 30 min.). After the plate was air dried, UV absorbance at 254 nm was also checked under the UV lamp and any absorbing spots were noted. The plate was then dipped in a solution of sulfuric acid:methanol:water (20:10:175) (Jork *et al.*, 1990) which developed the profile. This allowed visualisation of polar and non-polar components (predominantly plant saponins, sugars and sterols) when heated to 120°C on the Camag hot plate. The plate was then photographed using the Camag image-capturing apparatus (Video Store) under visible light (Fig. 4). The TLC was repeated to ascertain reproducibility.

Results and discussion

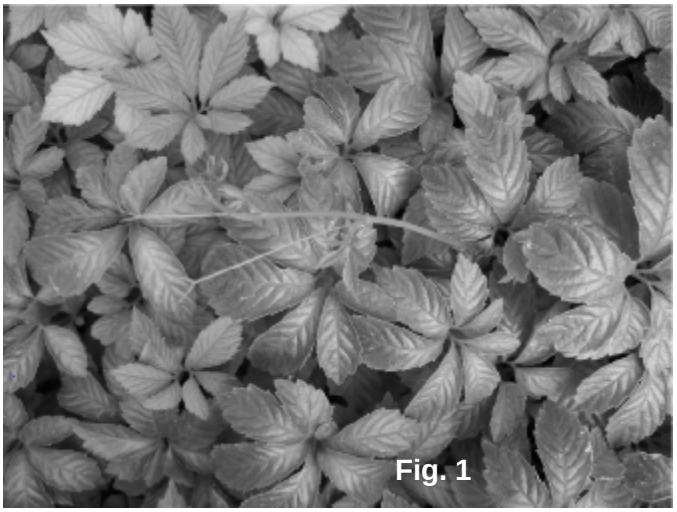
The plant grew very well in the glasshouse and field plot. The plant bore healthy flower and fruit indicating successful adaptation to the new environment. In a 1 m^2 plot, the plant bore around twenty fruits. Any shade was very beneficial to the propagation of the plant.

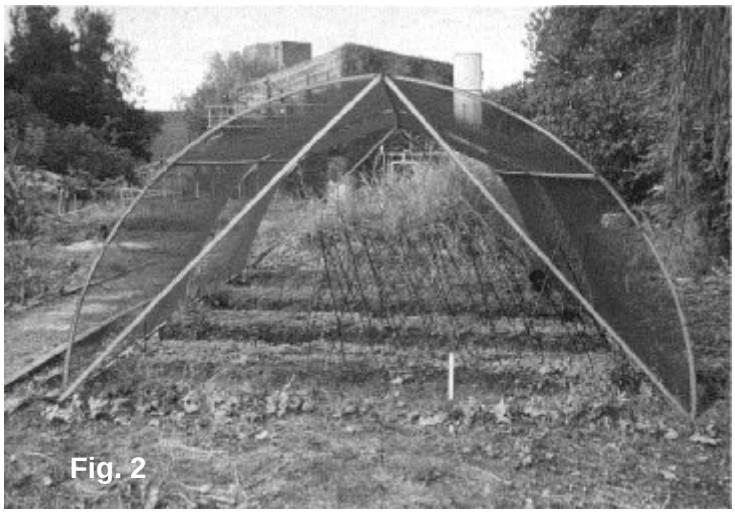
A comparison of Sydney and China growing conditions is shown in Table 1. The locality of Sydney incorporated the optimal growing conditions of *G. pentaphyllum* such as temperature, relative humidity and soil. Although not fastidious in soil preference, *G. pentaphyllum* is best grown on rich, loose, sandy soil. The herb prefers a warm, humid environment, with adequate water (China Pharmaceutical University, 1996). Throughout the study, *G. pentaphyllum* tolerated the environmental conditions in Sydney, Australia. The roots and stems of the herb were replanted after each cultivation period and continually produced more shoots. It grew abundantly in the house yard due to frequent attention and a natural resistance to attack by snails. 1 kg of fresh material produced approximately 140 g of dried material which could be extracted further and chemically assessed.

The cultivation of *G. pentaphyllum* was cheap, easy and required no extraordinary treatment or attention. *G. pentaphyllum* may be collected when the vine reaches 2-3 m in length. In the subtropics and tropics, the herb is collected 4-5 times a year. With optimal growing conditions and luxuriance, collection may be every 20-30 days. In a high-yield plot, 4000-5000 kg of dried herb may be collected every hectare (Guo and Wang, 1993). This compares favourably to the dry weight yield of the local material at 0.5 kg m^{-2}

Table 1. Summary of China, Sydney and optimal growing conditions (China Pharmaceutical University, 1996; Wang *et al.*, 1996; Australian Government Bureau of Meteorology; BBC Weather Centre; University of Idaho)

Location Conditions	China	Sydney	Optimal
Climate type	Continental Subarctic in the north Tropical in the south	Temperate	Sub-tropics and tropics
Soil type	Aridisol, Mollisol (north) Ultisol (south) Inceptisol (east) Entisol	Ultisol Alfisol	Aerated and retains water, such as a fertile sandy loam.pH in the range 6.5-7.5
Temperature(°C)	-15-41.5	10-35	15-30
Relative Humidity(%)	50(30-95)	50(30-95)	75-85
Soil (water content %)	13-16	13-16	25-40
Altitude(m)	300-3200	40	Sheltered from wind
Illumination (%)	Under shade	Under shade and full sunlight	65-75
Harvest period	September -October	March -September	End of growing season





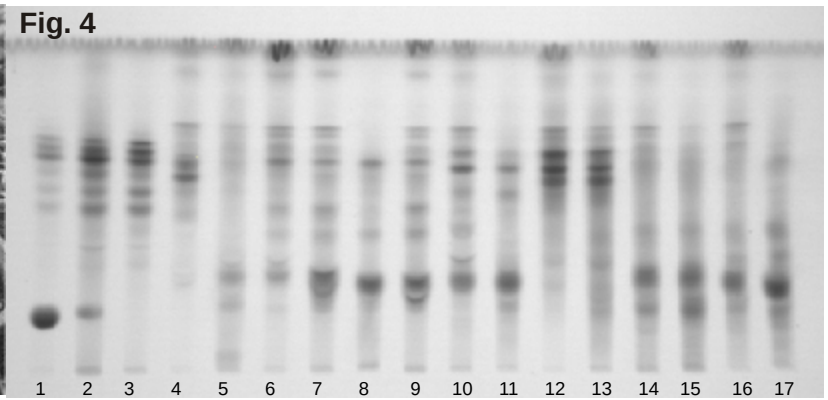


Fig. 1. *G. pentaphyllum* grown in the houseyard, Randwick, N.S.W., Australia (Closeup); Fig. 2: *G. pentaphyllum* grown at the University of Sydney (Outside yard). Fig. 3. *G. pentaphyllum* grown at the University of Sydney in glasshouse. Fig. 4. TLC of *G. pentaphyllum* products photographed under visible light. 4 g of *G. pentaphyllum* product was extracted with solvent. TLC condition of chloroform:ethyl: acetate:methanol:water (15:40:24:7) loaded at 10 mg m^{-1} in dissolving solvent. Plate dipped in sulfuric acid:methanol:water (20:10:175) to develop profile. Key: 1) Ankang (ANK) tablet, 2) ANK capsule, 3) ANK purified fraction (99.5% saponin), 4) *Gynostemma* (ANK- 90% ethanol), 5) Lanwang tea of "*G. pentaphylla*" (90% ethanol), 6) *Gynostemma* (University of Sydney (USyd)-ethanol), 7) *Gynostemma* (USyd-90% ethanol), 8) *Gynostemma* (USyd-hot water), 9) *Gynostemma* (USyd-methanol), 10) *Gynostemma* (Sheng Tang Shen- 90% ethanol), 11) *Gynostemma* (Sheng Tang Shen-hot water), 12) Jiagulan tea (90% ethanol), 13) Jiagulan tea (hot water), 14) Five leaf ginseng (90% ethanol), 15) Five leaf ginseng (hot water), 16) Tsai' s Amachazuru (90% ethanol), 17) Tsai' s Amachazuru (hot water).

(Table 2)**.** However, the yield obtained in Beijing was 70% lower (Chen *et al.*, 1991), probably related to an overall colder climate. In contrast, the cultivation of ginseng is quite different from other crops and requires special care from planting to harvesting. To maximise the ginseng yield, careful selection of the field (soil and topography) is required. In order to imitate the growing conditions of wild ginseng, the proper soil is located and fertilised with decayed leaves for one or two years. In this time, seedlings are nurtured in specially prepared nursery beds. A balance of sunlight and shade, as well as good air circulation, is essential for successful ginseng cultivation. To achieve optimal medicinal properties, *Panax ginseng* requires a long maturation period of up to seven years (Lee, 1992).

Initial chemical evaluation of the Sydney plant material revealed a different saponin profile when compared with commercially available *G. pentaphyllum* products, with the possibility of previously unreported saponins. Recently we have reported a new compound from the Sydney-grown material, Gynosaponin TR1 (Huang *et al.*, 2005). TLC was useful in providing a 'fingerprint' of the extracts and the mobile-phase solution of chloroform:ethyl: acetate:methanol:water (15:40:24:7) was ideal for the separation of the gypenosides. The dipping solution of sulfuric acid:methanol:water (20:10:175) is known to be specific for saponins and gave a characteristic purple colour for *Gynostemma* saponins. The TLC saponin profile of Sydney *Gynostemma* was compared with other samples from various provinces of China: Shaanxi, Guangxi and Guangdong (Fig. 4). The samples showed common spots, indicative of either identical saponins or components of the same polarity, as well as different saponins and components. Some spots on the TLC were single bands, possibly indicating one gypenoside compound, while diffuse spots may denote a higher concentration of one saponin or many saponins showing similar polarity. This is the first time visual saponin patterns of *Gynostemma* from various sources have been documented and indicates TLC as a powerful tool for quality control of *Gynostemma*.

Table 2. Comparison of Sydney-grown *G. pentaphyllum* dry weight yield to China (China Pharmaceutical University, 1996; Chen *et al.*, 1991)

Location		Amount (kg m^{-2})
Sydney		0.50
Beijing	Open area:	0.035
	Under shade:	0.15
China (generally)		0.4-0.5

Depending on the polarity of the solvent, the extracted plant material also exhibited a unique saponin profile. Extraction with polar solvents such as methanol and ethanol gave a broader profile and thus a more diverse range of saponins, but ethanol extraction seemed to lead to inferior separation of the very polar saponins closer to the bottom of the TLC plate. Water extraction gave the lower half of the profile and thus the more polar saponins. Extracting with 90% ethanol gave a similar saponin profile to the methanol extraction and comparable percentage (28.5 versus 28.6% for methanol; Table 3). Thus, the preferred solvent for extraction of the saponins was 90% ethanol in water as it provided an alternative to extracting with the more toxic methanol. The percentage of extracted material obtained for the locally-grown material was around 28.5%. This value is ~2.5 times more than the other *Gynostemma* products. These high values could be due to extracting the recently dried harvested material or the harvest material having a greater range of gypenoside components compared to other sources such as Jiaogulan Tea from Guangxi, China (10.3%) (Table 3; Fig. 4).

Table 3. Percentage of extract from *G. pentaphyllum* products

Product name	Source	Extract (%)
Jiaogulan Tea	Guangxi, China	10.3
Five leaves Ginseng	Guangzhou, China	13.4
Tsai's Amachazuru	Guangdong, China	18.1
Lanwang tea of *Gynostemium pentaphylla*[a]	Shaanxi, China	11.6
Gynostemma plant[a]	Shaanxi, China	11.1
Gynostemma plant	Guangxi, China	11.1
Gynostemma plant	Sydney, Australia	28.5

4 g of *G. pentaphyllum* product was extracted three times with 90% ethanol (100 ml) solvent. The solvent was evaporated to dryness. The amount of extract was weighed and calculated as a percentage of the total plant material (4 g).

[a]Researched by Ankang Medical Plants Development Institute, Shaanxi, China.

Therefore, the Australian grown *G. pentaphyllum* could potentially be used as a source of commercial supply of the medicinal plant. *G. pentaphyllum* could provide an alternative source of the medicinal compounds such as dammarane saponins, which are also found in *P. ginseng*. Further study on the chemistry and pharmacological activities is being carried out and will be published in respective biochemistry and pharmacology journals.

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