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SUSTAINABLE DEVELOPMENT OF *AMOMUM VILLOSUM:* A SYSTEMATIC INVESTIGATION ON THREE DIFFERENT PRODUCTION MODES

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Abstract

Background: *Amomum Villosum* (A. *Villosum*), called Chunsharen in Chinese, is widely used in treating gastrointestinal disease. Its clinical benefits have been confirmed by both *in vitro* and *in vivo* studies. Facing the shortage of wild A. *Villosum*, artificial cultivating and natural fostering have been practiced in recent years. Therefore, it would be wondered whether the three different types of A. *Villosum* are comparable or not, particularly the herbal qualities, technological challenges, ecological impacts and economic benefits.
Material and methods: In this study, we combined quality research by using GC-MS, and field investigation to provide a systematic assessment about the three types of A. *Villosum* from these four aspects.
Results: It found that the wild type had low output and was in an endangered situation. The artificial cultivation had larger agriculturing area with higher productivity, but faced the ecological challenges. Lastly, the natural fostering type generated the highest economic benefit and relatively low ecological impact. In addition, the natural fostering type had relatively better quality than the other types.
Conclusion: Therefore, it suggests that natural fostering can be applied for long-term sustainable development of A. *Villosum*.

Key words: production mode; *Amomum Villosum*; natural fostering; artificial cultivating; quality evaluation

Introduction

Amomum Villosum (A. *Villosum*), belonging to the genus Zingiberaceae, is native to Guangdong Province of China, with more than 1,300 years of medical application history (Duang et al., 2009). Called as *Chunsharen* in Chinese, it is a very important Chinese herb distributed in southeast and east Asia (Li et al., 2011; Lee et al., 2012; Zhao et al., 2013; Zhao et al., 2011). The main chemical components in A. *Villosum* are camphor, borneol, borneol acetate, copaene, etc, and these main ingredients are very high in content (Chen et al., 2014; Yin et al., 2008; Wang & Situ, 2010; Zhang et al., 2011). Research found that the pharmacological activities of A. *Villosum* include direct protective effect on gastric mucosal barrier (Jafri et al., 2001; Chinese Pharmacopoeia Commission, 2010; Zhao et al., 2011), treatment on growth retardation during adolescence (Lee et al., 2012), anti-inflammation, analgesic and anti-diarrhea (Zhao et al., 2009), anti-platelet aggregation, and prolonging coagulation time (Zhang & Shen, 2013). It is not only an important herb included in many prescriptions of traditional Chinese medicine (TCM), but also used for health food (Zhou, 1993). Therefore, A. *Villosum* is widely regarded as a kind of herbal resource that has high medical and economic values (Zhang et al., 2013a; Feng et al., 2012).

With the development of TCM industry, the demand on Chinese herb rapidly increased. Up to 80% of Chinese herbs come from continuous wild collection without scientific planning (Chen & Xiao, 2006). However, the natural resource is limited and hard to meet the speedily growing demand. As one of the most commonly used Chinese herbs, A. *Villosum* is facing huge market demand and cannot be supplied by wild collecting (Tang et al., 2012). In fact, the market demand of A. *Villosum* has grown to more than 2.2×10^6 kg in 2008, but the total production in China was only about 1.6×10^6 kg (Tang et al., 2012). Therefore, natural resource of A. *Villosum* has already been in the endangered state (Li & Wu, 2012), so artificial cultivating and natural fostering have been practiced in recent years for A. *Villosum* production to make up the shortage of A. *Villosum*.

Artificial cultivating uses artificial environment and techniques to produce herbs in a large scale (Li et al., 2015). On the contrary, natural fostering named as wild nursery or semi-imitational cultivation, emphasizes planting herbs in their natural habitats with limited human intervention (Li & Chen, 2007; Li et al., 2015; Li et al., 2012). As three different production modes are used for A. *Villosum*, comparative study on these three production modes began to attract the attention of researchers. For example, Zhou (1993) and Liu et al. (2006) introduced the ecological characteristics of A. *Villosum* in natural forest and tree plantation. Ducourtieux et al.

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(2006) and Choocharoen et al. (2013) analyzed the different production features of A. *Villosum* in Laos. In order to identify the quality, Fu et al. (2010) and Wang et al. (2013) analyzed the volatile oil components of A. *Villosum* from different production regions by Gas Chromatography-Mass Spectrometry (GC-MS), and found that the quality of A. *Villosum* grown in Yangchun area of Guangdong Province is much better. However, the systematic evaluation about the different production modes of A. *Villosum* is still rare, which has been a key challenge for the sustainable development of A. *Villosum*.

Therefore, in this paper, we combined quality research by using GC-MS and field investigation to provide a systematic assessment about three production modes of A. *Villosum* by four aspects: herbal quality, production technology challenges, ecological impacts, and economic benefits. The findings are expected to provide references for sustainable development of A. *Villosum*.

Material and methods

The sample of A. *Villosum*

This study targeted A. *Villosum* in Yangchun area because of its reputation in China (Zhang et al., 2009). Specifically, Yangchun is geographically located in southwest Guangdong Province of south China (21°50’36" to 22°41’01" N, 111°16’27" to 112°09’22" E). Wild A. *Villosum* is rich in Yangchun area, especially in Panlong Village, which is the geo-authentic habitats of A. *Villosum*. A. *Villosum* of Panlong in Yangchun is well known as the highest quality (Liu et al., 2001). Artificial cultivating and natural fostering were also developed to plant A. *Villosum* in Yangchun area.

12 batches (S1–S12) of A. *Villosum* (the dried ripe fruit of *Amomum villosum* Lour.) were obtained from different places of Yangchun. 3 batches were wild collecting (S1, S2, S3); five batches were natural fostering (S4, S5, S6, S7, S8); and four batches were artificial cultivation (S9, S10, S12, S12). The sample information is summarized in Table 1, and voucher specimens were deposited at the Institute of Chinese Medical Sciences, University of Macau, Macao.

Camphor and borneol were purchased from the National Institutes for Food and Drug Control. Bornyl acetate was obtained from the Shanghai Research Center of Standardization of Chinese medicines. The purity of all compounds was more than 95%. Methanol for GC was purchased from Merck (Darmstadt, Germany). Ethyl acetate for GC was purchased from Sigma – Aldrich (St. Louis, MO). Reagents not mentioned here were from standard sources.

Table 1: Summary of the investigated samples

Code	Sample type	Source	Year
S1	Wild collecting	Panlong, Yangchun	2014
S2	Wild collecting	Panlong, Yangchun	2014
S3	Wild collecting	Panlong, Yangchun	2014
S4	Natural fostering	Panlong, Yangchun	2013
S5	Natural fostering	Heshui (A) , Yangchun	2014
S6	Natural fostering	Heshui (B) , Yangchun	2014
S7	Natural fostering	Chunwan (A) , Yangchun	2014
S8	Natural fostering	Chunwan (B) , Yangchun	2014
S9	Artificial cultivating	Chunwan, Yangchun	2014
S10	Artificial cultivating	Heshui, Yangchun	2014
S11	Artificial cultivating	Yongning (A) , Yangchun	2014
S12	Artificial cultivating	Yongning (B) , Yangchun	2014

Preparation of sample extract

The dried sample powder (0.1 g, 40 mesh) and 3 mL ethyl acetate were transfered into 5 mL extraction vessels made of borosilicate glass. The microwave-assisted extraction was carried out in Multiwave 3000 (Anton Paar GmbH, Graz, Austria), which was performed at 100 W and 80 °C for 3 min. Then, the extract was compensated the loss of weight with extraction solvent and subsequently centrifuged at 5000 × g for 5 min. After centrifugation, the supernatant was filtered through a 0.45 μm filter for further analysis.

GC-MS analysis

GC-MS was performed with an Agilent 6890 GC instrument coupled to an Agilent 5973 mass spectrometer and an Agilent ChemStation software (Agilent Technologies). A HP-5MS capillary column (30 m × 0.25 mm i.d.) coated with 0.25 μm film of 5% phenyl methyl siloxane was used for separation. The extracted ion chromatogram (EIC) (Chromatogram created by plotting the intensity of the signal observed at a chosen *m/z* value) of GC–MS was applied for accurate quantification of camphor, borneol and bornyl acetate. Characteristic fragment ions, *m/z* 95 was selected for the GC–MS quantification (Lv et al., 2015).

Field investigation

To collect data about technology challenges, ecological impacts and economic benefits, field investigation was designed and carried out in 2014. First, data was collected from exploratory interviews (n = 2) with government staff of Yangchun Municipal Finance Bureau and experts of Yangchun Agricultural Bureau. These interviews helped to collect data of the financial, commercial and regulatory situation affecting A. *Villosum* development. Second, the field interviews (n = 10) were also conducted with administrators, farmers and interns (students) of three A. *Villosum* cultivation bases in Yangchun area. These interviews provided a significant understanding of the current situation of different production modes of A. *Villosum*. Third, the information of natural environment of these three production modes was collected on site. The summary of field investigation was shown in Table 2.

Table 2: Summary of field investigation

Data sources	Data
• Government staff	• Financial, commercial and regulatory situation affecting A. *Villosum* development
• Farmers	• Technical requirements (planting, collecting), cost-effectiveness (cost structure, input, output), ecology impact (positive, negative)
• Interns (students)	• Technical requirements (planting, collecting), cost-effectiveness (cost structure, input, output), ecology impact (positive, negative)
• Base administrators	• Technical requirements, input and output
• Expert	• Opinions of three production modes

Results

Current situation of three production modes in Yangchun

For wild collecting, by the end of 2013, total area of wild A. *Villosum* was estimated to be 0.41 km^2, mainly distributed in intermountain valley of Panlong Village (Figure 1-a). For artificial cultivating, total area was about 42 km^2, mainly distributed in Kongdong Village, Jiuzikeng Village, Heshuinaruan Village, and Yongningshuangdi Village of Chunwan District (Figure 1-b). For natural fostering, total area was about 15.33 km^2, and mainly distributed in the middle of the valley and the two sides of mountain pit, where Panlong is a well-known location. Natural fostering was mostly operated by local farmers (see Figure 1-c).

Figure 1: a, Wild A. *Villosum*; b, A. *Villosum* in Artificial cultivating; c, A. *Villosum* in Natural fostering

Herbal quality analysis

The samples were prepared and subsequently determined by GC-MS. The contents of other compounds (Copaene, Caryophylly + α-Santalene, γ-Elemene, δ-Cadinol, δ-Cadinene, Torreyol, Methenolone) were estimated approximately by using calibration curve of borneol, which is one of the major components in A. *Villosum*. Specifically, the chromatogram of A. *Villosum* (sample S9) is shown in Figure 2. In addition, based on the GC-MS total ion chromatograms of 12 samples, the main components of all the samples were the same, including camphor, borneol, and bornyl acetate. Supplementary information of GC-MS total ion chromatograms of the other 11 samples is available in the attachment.

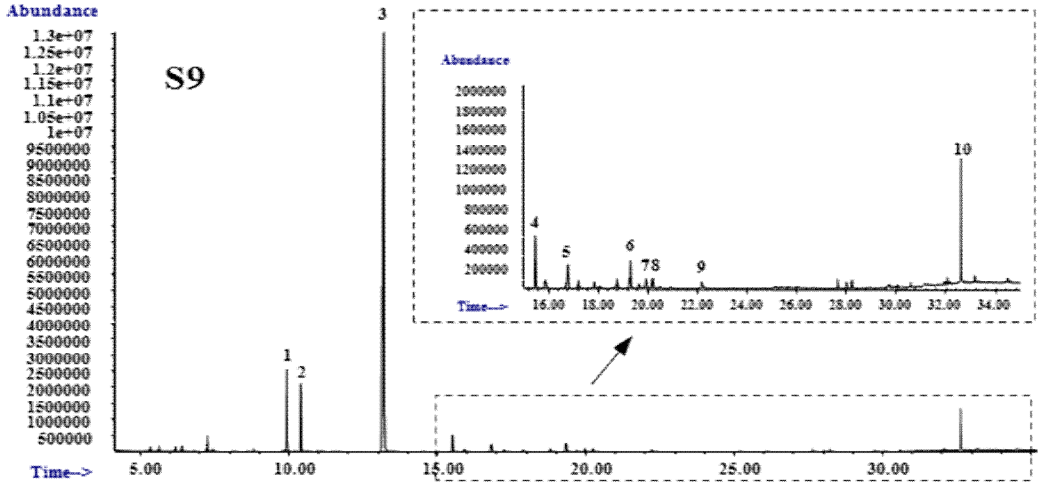

Figure 2: GC-MS total ion chromatograms of A. *Villosum* (Sample S9)
(1. Camphor, 2. Borneol, 3. Bornyl acetate, 4. Copaene, 5. Caryophylly + α-Santalene, 6. γ-Elemene , 7. δ-Cadinol, 8. δ-Cadinene, 9. Torreyol, 10. Methenolone)

For identifying the components in A. *Villosum* samples, the MS matching results of the main components in A. *Villosum* showed that camphor, borneol and bornyl acetate are the main components (Table 3).

Table 3: MS matching results of the main components in A. *Villosum* (total samples)

Retention Time (Peak no.)	Compound	MW	EIC[a]	Molecular Formula
9.885 (1)	Camphor	152.23	95	$C_{10}H_{16}O$
10.361 (2)	Borneol	154.25	95	$C_{10}H_{18}O$
13.099 (3)	Bornyl acetate	196.29	95	$C_{12}H_{20}O_2$
15.114 (4)	Copaene	204.35	-	$C_{15}H_{24}$
16.778 (5)	Caryophyllene+α-Santalene	204.35	-	$C_{15}H_{24}$
19.293 (6)	γ-Elemene	204.35	-	$C_{15}H_{24}$
19.940 (7)	δ-Cadinol	204.35	-	$C_{15}H_{24}$
20.204 (8)	δ-Cadinene	204.35	-	$C_{15}H_{24}$
22.170 (9)	Torreyol	222.37	-	$C_{15}H_{26}O$
32.607 (10)	Methenolone	302.45	-	$C_{22}H_{22}O_3$

Note: a, Ions for extracted ion chromatograms.; "—", not analyzed in EIC mode.

Table 4 further illustrated the comparison of the contents of main components in different samples. Natural fostering from Chunwan (S8) has the highest content in camphor (3.47 mg/g) and borneol (1.63 mg/g). For bornyl acetate, artificial cultivating from Yongning (S12) has the highest content (20.88 mg/g).

For the total contents of the three main components, among all the samples, S12 (23.5 mg/g) has the highest contents. However, the average contents of the three main components in wild collecting (S1, S2, S3) was 13.44 mg/g, natural fostering (S4, S5, S6, S7, S8) was 18.76 mg/g, and artificial cultivating (S9, S10, S11, S12) was 18.64 mg/g. Therefore, natural fostering had higher content of main components than wild collecting and artificial cultivating.

In addition, in Heshui, the samples of natural fostering have higher contents than artificial cultivating; in Panlong, the samples of natural fostering have higher contents than wild collecting; but in Chunwan, there are no obvious difference between natural fostering and artificial cultivating.

Table 4: Contents (mg/g) of main components in different A. *Villosum* (total samples)

Compounds	**Samples**											
	S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**	**S11**	**S12**
Camphor	2.20	0.71	0.74	2.05	3.43	2.82	1.93	**3.47**	1.12	1.42	2.31	1.33
Borneol	0.44	0.26	0.32	1.47	0.56	0.41	1.59	**1.63**	1.05	0.70	0.40	1.29
Bornyl acetate	12.05	10.40	13.19	12.20	14.94	14.08	15.91	17.30	17.79	13.32	12.93	**20.88**
Copaene	0.10	0.06	0.23	0.24	0.19	0.15	0.27	0.35	0.33	0.28	0.24	0.42
Caryophyllene+ α-Santalene	0.13	0.06	0.15	0.17	0.13	0.13	0.19	0.22	0.22	0.17	0.15	0.29
γ-Elemene	0.25	0.19	0.41	0.21	0.33	0.31	0.19	0.15	0.19	0.13	0.26	0.28
δ-Cadinol	0.09	0.04	0.06	0.06	0.05	0.19	0.06	0.07	0.08	0.05	0.04	0.09
δ-Cadinene	0.05	0.03	0.06	0.05	0.06	0.06	0.07	0.08	0.08	0.06	0.06	0.09
Torreyol	-	0.05	0.02	0.02	0.04	0.03	0.06	0.08	0.07	-	0.06	0.07
Methenolone	0.46	-	-	0.04	0.17	0.13	0.05	0.07	0.36	0.29	0.36	0.38

Note: "—", Undetected

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By comparing the average contents of all the components of natural fostering, artificial cultivating and wild collecting samples, it showed that the difference between natural fostering and artificial cultivation A. *Villosum* is not obvious, but both of them are better than the wild collecting (Figure 3).

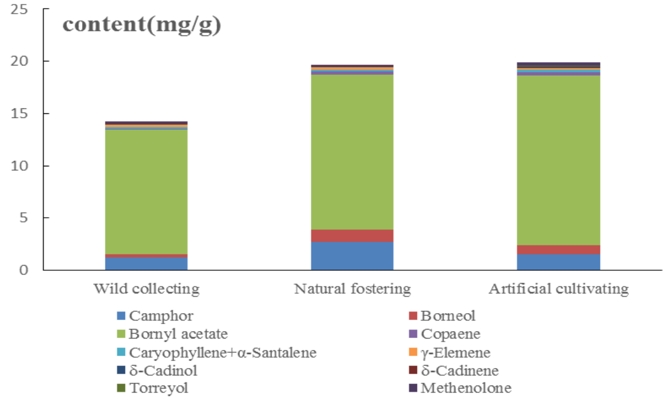

Figure 3: Comparison of the average contents of the main components of A. *Villosum* in wild collecting, natural fostering and artificial cultivating

Production technology challenges

Table 5 provides a comparative summary of the technological challenges of the three production modes. For the investigation of technology challenges, there are no technical requirements at all in the production mode of wild collecting. It is very different in the natural fostering because many planting technologies are required, including seed selection, seeding, artificial pollination, fertilization and pest control. However, in the artificial cultivating, most attention should be paid on location selecting, artificial pollination, and scientific harvesting.

Table 5: Technology challenges comparison of three production modes

Wild collecting	Artificial cultivating	Natural fostering
• None	• location selecting • artificial pollination • scientific harvesting	• seed selection • seeding • artificial pollination, fertilization • pest control

Ecological impacts

Regarding ecological impact analysis of wild collecting and natural fostering of A. *Villosum*, both production modes were good for protecting ecological environment. Particularly, wild collecting was very helpful for protection of biodiversity and germplasm resources. Artificial cultivating can improve water and soil loss, enhance plant population and improve the orchard environment, but pesticide residues and heavy metal pollution cannot be ignored (Table 6).

Table 6: Ecological impact comparison of three production modes

Wild collecting	Artificial cultivating	Natural fostering
• good for protecting the biodiversity and germplasm resources	• improve water and soil loss • enhance plant population • improve the orchard environment • but cause pesticide residues and heavy metal pollution	• good for protecting ecological environment

Economic benefits

Economic input and output analysis was showed as Table 7. The economic input and output analysis is calculated in one-year period of one km^2 area. It is obvious that A. *Villosum* in wild collecting had the highest price while A. *Villosum* in artificial cultivating had the lowest price. However, the highest turnover was natural fostering in Panlong with 14,080 RMB per km^2. It should be noticed that the price of natural fostering in Panlong was much higher than other areas because of brand effect. In terms of cost, the cost structure of different patterns was different, but labor cost was the main cost of all the patterns. The total cost of artificial

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cultivating is the highest. Finally, the profit margin of wild collecting was 95.5%, 87.2% to natural fostering in Panlong, 73.5% to natural fostering in other areas in Yangchun except Panlong, and 50.6% to artificial cultivating.

Table 7: Economic input and output comparison of three production modes [a]

	Wild collecting	Artificial cultivating	Natural fostering (Panlong)	Natural fostering (other areas except Panlong)
Fresh fruit yield (Kg)	2	40	22	22
Price (RMB per Kg)	1,000	180	640	240
Turnover (RMB)	**2,000**	**7,200**	**14,080**	**5,280**
Labor cost (RMB)	90	1,800	1,600	1,200
Land rent (RMB)	0	200	0	0
Seedling (RMB)	0	960	0	0
Fertilizer (RMB)	0	500	200	200
Pesticide (RMB)	0	100	0	0
Total cost (RMB)	**90**	**3,560 (2,600)[b]**	**1,800**	**1,400**
Gross profit (RMB)	**1,910**	**3,640 (4,600)[c]**	**12,280**	**3,880**
Profit margin (%)	**95.5**	**50.6 (63.9)[d]**	**87.2**	**73.5**

[a] the comparison in per km^2 per year; [b] the total cost per year without seedling; [c] the gross profit per year without seedling; [d] the profit margin per year without seedling

Discussion

Comparison of the main components of A. *Villosum* of three production modes

The chemical components of essential oil among A. *Villosum* are important quality indicators (Wang et al., 2013). Researchers found that the principal components of volatile oils were similar (Wang et al., 2013; Zhang et al., 2011; Yan et al., 2014). However, the percentages of each component in these volatile oils were different (Fu et al., 2010). Our study found that A. *Villosum* from natural fostering has higher average contents of the main components.

Natural fostering is a production mode that combines wild and artificial cultivation, which brings several advantages for planting A. *Villosum*. Firstly, in the process of seed selection, only the high-class germplasm resource is selected for natural fostering. Secondly, natural fostering is mainly operated by local farmers who have much experience of planting A. *Villosum*. Particularly, the artificial pollination can improve the efficiency of fructification. Finally, the location selection is very important for planting A. *Villosum*. The ideal location should meet these conditions: the altitude should be lower than 500 meters; the slope should be less than 30 degrees; the shade should be among 50% and 60%; and it must be planted in loam with yellow soil as subsoil and loose topsoil. In addition, the most important condition is that it must grow under trees. All these conditions specify the growing environment for A. *Villosum*. Therefore, natural fostering with the optimized combination of these environmental requirements can produce A. *Villosum* with high content.

Comparison of technology challenges, enological impact and economic benefits of three production modes

As shown in this study, different production modes have different development advantages. It is obvious that they are very different in production scale. The scale of wild A. *Villosum* is very small, while the emerging production mode of artificial cultivating is rapidly increasing. However, the scale only reflects the current situation of different production modes. The factors of technological challenges, ecological impact and economic benefits need to be considered.

For technological challenges, artificial cultivating of A. *Villosum* needs to consider location selecting, artificial pollination and scientific harvesting. In particular, the artificial pollination was especially the key of high yield, which still has many technological problems unsolved. In addition, how to identify and protect the high-class germplasm resources is another technological challenge. Researchers have tried to compare and identify the different cultivated varieties of A. *Villosum*. For example, long-fruit and round-fruit types were suggested (Zhang et al., 2013b; Zhang et al., 2005). However, more research work is still needed. Comparatively, natural fostering meets fewer technological challenges because it plants A. *Villosum* in a more natural way that is similar with wild A. *Villosum*.

For ecological impacts, both wild collecting and natural fostering are good for protecting ecological environment. On the contrary, while artificial cultivating can improve water and soil loss, enhance plant population and improve the orchard environment, it leads to pesticide residues and heavy metal pollution. The cultivation of A. *Villosum* in Xishuangbanna area of Yunnan Province had significantly reduced plant diversity, tree biomass, litter production and soil nutrients, which would affect the structure and function of the seasonal rain forest (Liu et al., 2006).

Moreover, economic benefits play a fundamental role for planting A. *Villosum*. As shown in this study, while the price of wild collection is the highest, it is constrained by quantity. Considering the final profit, natural fostering is better than the other two modes. Hence, based on comprehensive consideration of technological challenges, ecological impact and economic benefits, natural fostering shows its advantages over wild collecting and artificial cultivating.

The medical efficacies of A. *Villosum* of different production modes

As different production modes and ecological habitats can result in varied plant morphology and medical traits (Feng et al., 2004; Gao & Liu, 2009; Zhang et al., 2005; Zhang, 2008), it is more important to consider pharmacological effects of A. *Villosum*. There

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have been studies that focused on medical application of A. *Villosum* (Jafri et al., 2001; Mathew et al., 2003; Zhao et al., 2009; Zhao et al., 2011; Lee et al., 2012; Zhang & Shen, 2013). However, there are fewer studies on medical efficacies of A. *Villosum* from different production modes (Ding et al., 2004; Liu et al., 2013; Peng et al., 2006).

For chemical components of A. *Villosum*, previous studies identified the main components of A. *Villosum* as camphor, borneol and bornyl acetate (Chen et al., 2014; Yin et al., 2008; Wang & Situ, 2010; Zhang et al., 2011), which is similar with the results of our GC-MS analysis. However, our study found that natural fostering generates higher content of these main components. Therefore, it is expected that A.*Villosum* of natural fostering can have more significant efficacies, which is worth deeper clinical exploration.

Implications and future study

Through comprehensive comparison of three production modes, this study has several implications for sustainable development of A. *Villosum*. Firstly, among the three production modes, natural fostering is more recommended while the other two production modes can play complementary roles in some aspects. Secondly, location selecting should be emphasized as natural fostering of A. *Villosum* is very sensitive to location environment. Thirdly, organic fertilizer is most suitable for natural fostering. Fourthly, scientific artificial pollination is crucial for both natural fostering and artificial cultivating.

In addition, some research limitations can be addressed through future studies. Firstly, in this study, we only chose Yangchun A. *Villosum* as the analysis sample. Future study could extend to test samples from other areas like Hainan Province, Yunnan Province, Guangxi Province, and Laos. greatly helpful to the development of sustainable production modes of A. *Villosum*. Secondly, future study can apply more technical methods to measure environmental factors and test their impacts on A. *Villosum* in different production mode. It will contribute to establish a systematic model of understanding environmental factors on sustainable production modes of A. *Villosum.*

Conclusion

This study found that the wild A. *Villosum* had low output and was in an endangered situation. The A. *Villosum* of artificial cultivation had larger agriculturing area with higher productivity, but faced ecological challenges. Lastly, the A. *Villosum* from natural fostering generated the highest economic benefit and relatively low ecological impact. In addition, the natural fostering type had relatively better quality than the other types. Therefore, natural fostering is suggested for sustainable development of A. *Villosum*.

Conflict of interest: The authors declare that there is no conflict of interests regarding the publication of this paper.

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