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Optimized extraction of *gynostemma pentaphyllum*: advancing natural resource utilization for sustainable development

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Abstract

Gynostemma pentaphyllum, a renowned medicinal herb from Asia celebrated for its health benefits, has entered a new frontier of optimization and innovation. This groundbreaking study unveils a cutting-edge approach to ultrasonically-assisted liquid-solid extraction and encapsulation, designed to maximize bioavailability and therapeutic potential. Through meticulous experimentation, optimal extraction parameters were identified: 5 minutes of ultrasound at 37 kHz, 90 minutes of extraction, 70°C temperature, and a 1:15 g/ml material-to-solvent ratio. Antioxidant analysis revealed a remarkable IC50 value of 7.21 mg/ml (DPPH assay), alongside significant anti-cancer properties targeting liver and blood malignancies. Encapsulation further enhanced the extract's pharmaceutical application by integrating 45 ml of the extract with 75 g of glucose, followed by drying at 70°C for 75 minutes, yielding a powder with 1.45% saponin and 8.61% moisture content. The study's statistical rigor, verified through SPSS at a 95% confidence level, highlights the reliability and scalability of this novel process. These findings position Gynostemma pentaphyllum as a next-generation powerhouse in pharmaceutical innovation, unlocking unprecedented opportunities in natural medicine and therapeutic development.

Keyword: *Gynostemma pentaphyllum, Optimized extraction, Ultrasonically-assisted extraction, Pharmaceutical herb, Saponin extract*.

Introduction

Gynostemma pentaphyllum (G. pentaphyllum, Jiaogulan, Giao Co Lam*)* is an herb usable as a type of pharmaceutical food in many regions worldwide. Gynostemma pentaphyllum (commonly known as Jiaogulan or Giảo Cổ Lam) emerges as a botanical marvel with transformative potential. Known as a "miracle herb," it has long been celebrated for its anti-tumor, pain-relieving, and antibiotic properties (Zhao *et al.*, 2024). Beyond its therapeutic uses, G. pentaphyllum is revered for its ability to enhance overall health and longevity, a reputation supported by both historical records and modern scientific research (Linfu *et al.*, 2016). Furthermore, This herb has not only been a staple in Asian traditional medicine but has also garnered growing interest from researchers

across Vietnam, China, and Western nations like the United States, Italy, and Croatia (Lin-Na & Yong-Xiu 2014; Gelen *et al.,* 2017; Mastinu *et al.,* 2021). Despite its proven efficacy and global recognition, G. pentaphyllum remains underutilized. In many parts of the world, its use is confined to limited forms, such as tea bags or dried herbs, predominantly in Japan, Korea, China, and Vietnam. This restricted application underscores a critical gap in leveraging the herb's full potential. With rising consumer demand for innovative, bioactive health solutions, there is an urgent need to modernize the extraction, processing, and application of G. pentaphyllum to unlock its broader benefits.

There are many opportunities for research on *G. pentaphyllum.* The study of Espinosa-Leal et al., (2018) indicated the realistic potential of in-vitro cultivation for herbal plants to obtain bioactive compounds. This was supported by other researchers (Saldarriaga *et al.,* 2020, Chandran *et al.,* 2020). For *Gynostemma pentaphyllum*, consequently, the technique is an opportunity for standardize the raw material. Regarding bioactivity, saponins are the most prominent group of compounds offering health effects in the herb. In 2006, Utama-Ang et al. successfully demonstrated the presence of ginsenocide Rb1 and Rg1 among some other saponin compounds through the gas-chromatographic-mass spectrometric method. The two saponins Rb1 and Rg1 are also present in Panax Ginseng (Lee *et al.,* 2016; Mohanan *et al.,* 2018). In a comparison of total saponin content between the two herbs, Liang et al. (2019) indicated the amount of saponins in G. pentaphyllum that was 5 times higher than Panax ginseng. In addition, flavonoids and beneficial polysaccharides like GPMPP, GPA, GPP, CGPP, GPS-2, and GPS-3 were also recorded in research (Ji *et al.,* 2018, Zhao *et al.,* 2024). This study aims to address the existing limitations by introducing a next-generation approach to G. pentaphyllum. Through optimized ultrasonically-assisted extraction and innovative encapsulation techniques, this research not only enhances the herb's bioavailability but also broadens its applications, paving the way for G. pentaphyllum to become a global powerhouse in health and wellness. By bridging traditional knowledge with cutting-edge technology, this work sets the stage for G. pentaphyllum to enter mainstream pharmaceutical and functional food markets, unlocking its untapped potential for the benefit of millions.

Methods

Material

Fresh *Gynostemma pentaphyllum* leaves and stems were collected from the ecological garden located at the Faculty of Biotechnology – Food (Thai Nguyen University of Agriculture and Forestry, Vietnam) after 18 months planting. Subsequently, the leaves were dried at 60^0C to reach moisture of 10% before being stored in PE bags under ambient conditions.

The material was characterized in terms of moisture content, ash content, and the initial saponin content. Moisture and ash were determined using high temperature-based methods, which either dried out all the moisture and used the difference to identified the moisture or applied extreme heat to remove all organic compounds. For the initial saponin content of *G. pentaphyllum*, the method for this category was ethanol extraction and then quantifying the total saponin content with High Performance Liquid Chromatography (HPLC).

Batch experiment for extraction optimization

The method of extraction was ultrasonically-assisted liquid-solid extraction in the description of Chemat *et al* (2017) .In these experiments, 3 grams of dried G. pentaphyllum was installed in an Erlenmeyer flask (100 ml) and the solvent was added subsequently. The flask was then ultrasonically processed in a certain amount of time before settled in the water-bath, which managed the temperature. Therefore, it was required to determine the optimal conditions for extraction. Specifically, the experiments were as follows:

- Ultrasound time: Ultrasound was employed to support the extraction process. Particularly, the instrument generated 37 kHz soundwaves in the degas mode. The range of experimenting time was 10 minutes with records after 1, 3, 5, 7, and 10 minutes, corresponding to 5 formulas. Each formula consisted of 3 g of the sample and ethanol solvent with a ratio of 1:15 g/ml in an Erlenmeyer flask. After finishing, the records were analyzed in total saponin extract

- Solvent concentration: The study examined 4 different concentrations, including 50%, 60%, 70%, and 80%. The solvent was the one concluded from the previous experiment while other operational conditions remained with 5 minutes ultrasonic processing, 60^0C, 60 minutes, and 1:15 (g/ml) material-solvent ratio. The optimal concentration from this experiment was subsequently used in later contents.

- Extraction time: The study investigated a range of 120 minutes. The solvent and concentration used for this experiments were derived from previous contents while the extraction temperature and material-solvent ratio remained at 60^0C and 1 : 15 (g/ml). Extraction samples were recorded in terms of the total saponin extract each 15 minutes from the 45th minute. Consequently, there were 6 records.

- Extraction temperature: The study focused on a range from 60^0C – 80^0C (Ji *et al.*, 2018). Other conditions including the solvent, solvent concentration, and extraction time were the optimal parameters selected following previous experiments. The material-solvent ratio remained at 1:15 (g/ml). Extraction efficiency was recorded at 60^0C, 65^0C, 70^0C, 75^0C, and 80^0C. The optimal temperature was subsequently used in next experiments.

- Solvent – material ratio: There were 3 ratios in examination including 1:10, 1:15, and 1:20(g/ml). Other conditions including the solvent, solvent concentration, extraction time, and temperature were the optimal factors

from previous experiments. The extract of G. pentaphyllum then was analyzed in terms of total saponin extract to determine the best material-solvent ratio for extraction.

After each experiment, the samples were analyzed in terms of total saponin (%). The quantification of saponin extract was based on the Herbal Extraction Techniques (2008) book of the National Institute of Medicinal Materials (NIMM).

Assessment of G. pentaphyllum extract's bioactivity

The anti-oxidation capability of *Gynostemma pentaphyllum* extract was assessed using the Relative DPPH Radical Scavenging method (RDSC) of Blois (1958). The cell proliferation inhibitory effect of *G. pentaphyllum* extract was evaluated using the MTT assay, which stimulates cellular metabolic activities and indicates the cytotoxicity of the test subject.

Encapsulation

After obtaining the extract glue from extraction under optimal conditions, it was diluted with dewater (10% w/v). Afterward, the extract was mixed with glucose in different ratios to obtain the pharmaceutical flour with greenish color. There were 5 ratios examined, which included 45 ml/65 g, 45 ml/70 g, 45 ml/ 75 g, and 45 ml/ 80 g of glucose. Subsequently, the mixtures were dried and evaluated in terms of total saponin extract. The range of experiment was from 50 – 90^0C with corresponding time. The experiment was commenced in triplicate. In addition, the pharmaceutical flour was evaluated in terms of moisture content and total saponins according to the Vietnamese Standard TCVN I-4:2017

Data analysis

The data collected from experiments were synthesized through Microsoft Excel and processed according to the ANOVA method through PASW 18 (SPSS). Optimization experiment utilized software Design – Expert to commence surface response. Numerical and graphic exhibition was conducted via Microsoft Word 2017 and Origin 2019.

Results

Material characterization

Moisture content and ash content are the most fundamental indexes in evaluations of botanical materials. For this research, the total saponin content was also the primary criterion. The moisture content, ash content, and total saponin content of *G. pentaphyllum* are displayed in Table 1. The average moisture content and ash content were 89.56% and 1.16%, respectively.

Table 1 Initial indexes of the raw material

Moisture content (% raw material)	Ash content (% raw material)	Total saponin (% dried material)
89.56	1.16	15.67

The effects of extraction parameters on optimization

Ultrasonic processing is a supporting factor in botanical extraction as it breaks down fibrous cell walls using vibration. Nevertheless, the extreme soundwaves can also damage molecular levels including also include the saponin group. That had been well demonstrated through the findings of this experiment (Fig. 1a). With the effect of 37 kHz soundwaves, the resulting total saponin extract promptly accelerated from 6.42 g/100g to 8.13 g/100g in the first 5 minutes. After 7 minutes, the total saponin extract reduced slightly to 8.05 g/100g, statistically similar to 5 minutes. Subsequently, the total saponin extract declined to only 7.62 g/100g.

For the type of solvent, Fig. 4 clearly demonstrated the superiority of alcoholic solvents compared to water. The maximum saponin extract was 10.97 g/100g, corresponding to 69.99% efficiency, produced by *G. pentaphyllum* extraction by ethanol. This result was statistically equivalent to that of methanol and about 3% higher than water.

With ethanol as the primary solvent, the experiment subsequently tested 4 different concentrations including 50%, 60%, 70%, and 80%. The increasing solvent concentration led to an incline in terms of total saponin extract (Fig. 1c). Nevertheless, the acceleration rate was different among every 2 concentrations. When the increase of total saponin extract between 50% ethanol and 60% ethanol was sharp (9.91 to 10.73) corresponding to 5.26% efficiency higher, the gap between 60% and 70% was lower with 3.07% efficiency difference. More significantly, the acceleration rate was reduced to only 0.6% efficiency difference when it came from 70% to 80%. The extraction process with 80% ethanol resulted in 11.31 g/100g (72.17%). Statistically, this result was no different from that of 70% ethanol (11.21 g/100g corresponding to 71.57% efficiency).

Extraction time refers to how long the mixture of material and solvent stays in the water bath. In this experiment, the investigated range was 120 minutes. According to Fig. 1d, he acceleration rate from 45th min to 90th min was relatively consistent, rising from 9.98 g/100g to 13.51 g/100g (63.70% - 86.20%). Each 15 minutes passing resulted in an average increase of 1.18 g/100g (7.5% efficiency). In the beginning, the osmotic pressure between the solvent and the inner matters was high as the solvent did not have much matter. As a result, mass transfer occurred at a fast rate and saponins were quickly extracted. However, after 90 minutes, the acceleration rate suddenly stopped.

In terms of extraction temperature, this experiment examined the range of 60^0C to 80^0C in *G. pentaphyllum* extraction. When extraction was conducted at 60^0C, the total saponin extract was 13.51 g/100g, corresponding to an efficiency of 86.24% (Fig. 1e). It grew to 13.84 g/100g (88.33%) at 65^0C before reaching the maximum point of 14.29 g/100g (91.20%) at 70^0C. However, when using 75^0C and 80^0C, the extraction efficiency decreased substantially to only 13.65 g saponin extract/100g (87.10%) and 12.56 g/100g (80.15%), respectively.

Finally, 3 different ratios including 1:10 g/ml, 1:15 g/ml, and 1:20 g/ml were investigated (Fig. 1f). The ratio 1:10 resulted in much lesser total saponin extract compared to the other two ratios with only 11.21 g/100g, corresponding to 71.57% efficiency. The 1:15 ratio resulted in 14.29 g saponin extract/100g (91.16% efficiency) and 1:20 yielded 14.49 g/100g (92.47%).

In conclusion, the optimal parameters included 5 minutes of ultrasound, a solvent of 70% ethanol, 90 minutes of extraction at 70^0C, and a material – solvent ratio of 1:15 g/ml. This condition produced a maximum extraction efficiency of 14.29 g saponins/100 g dried *G. pentaphyllum*, corresponding to 91,16% of efficiency.

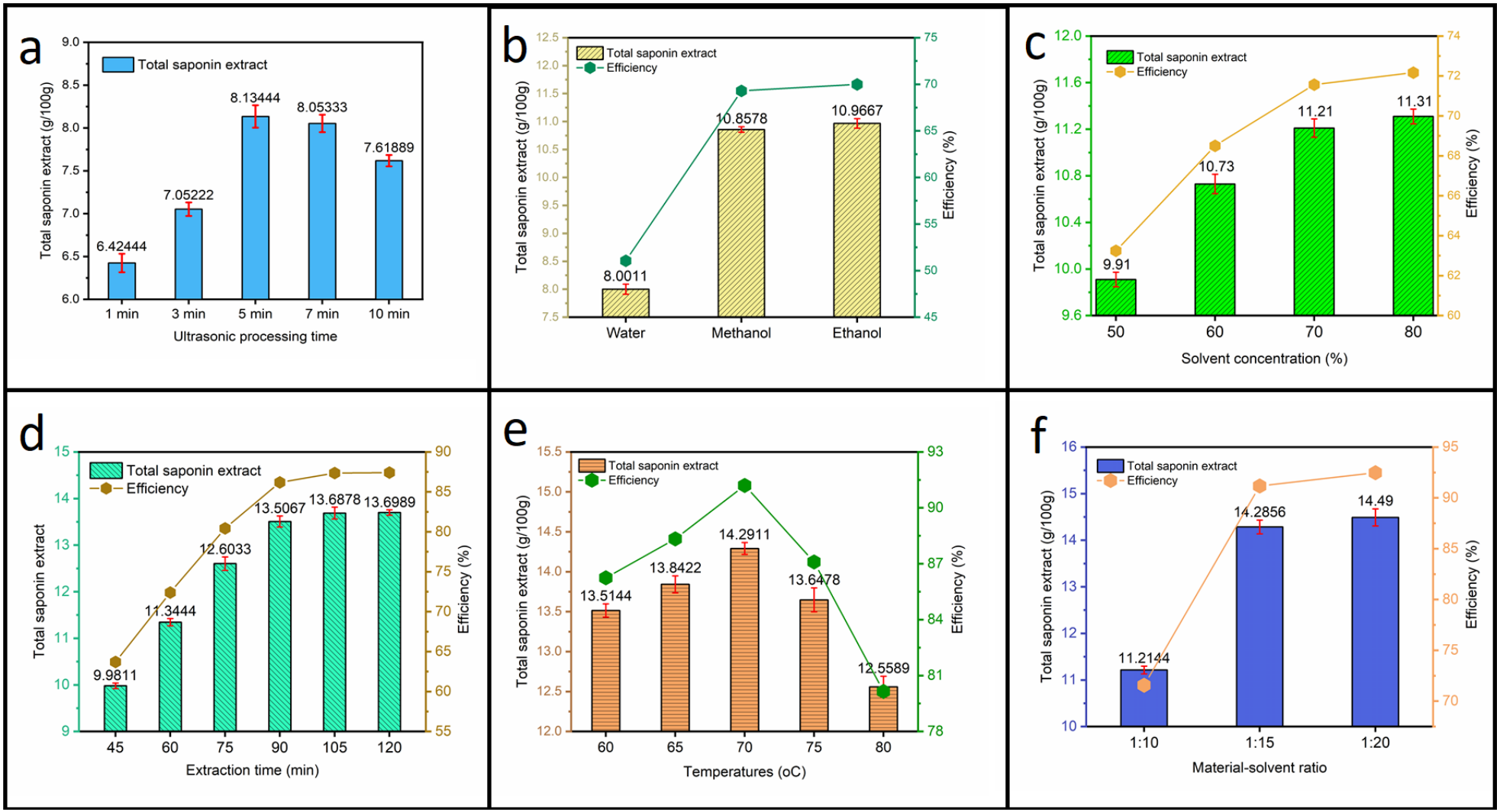

Fig. 1 The effects of (a) ultrasonic processing time; (b) types of solvent; (c) solvent concentration; (d) time; (e) temperatures; (f) material-solvent ratio on extracting G. pentaphyllum

Bioactivity assessment

Free radicals with oxidation capability are important constituents for a human body. Nevertheless, they excite aging and unwanted reaction within the organs, leading to inflammation and damages in molecular levels. The extract can exhibit a relatively high efficiency as an antioxidant with 48.23% of the free radical removed with only 0.5 mg extract equivalent/ml. As the concentration of the extract increased, the radical scavenging activity also rose. The IC50 value was 0.71 mg/ml based on y = 0.45745 + 0.0603x (Fig. 2).

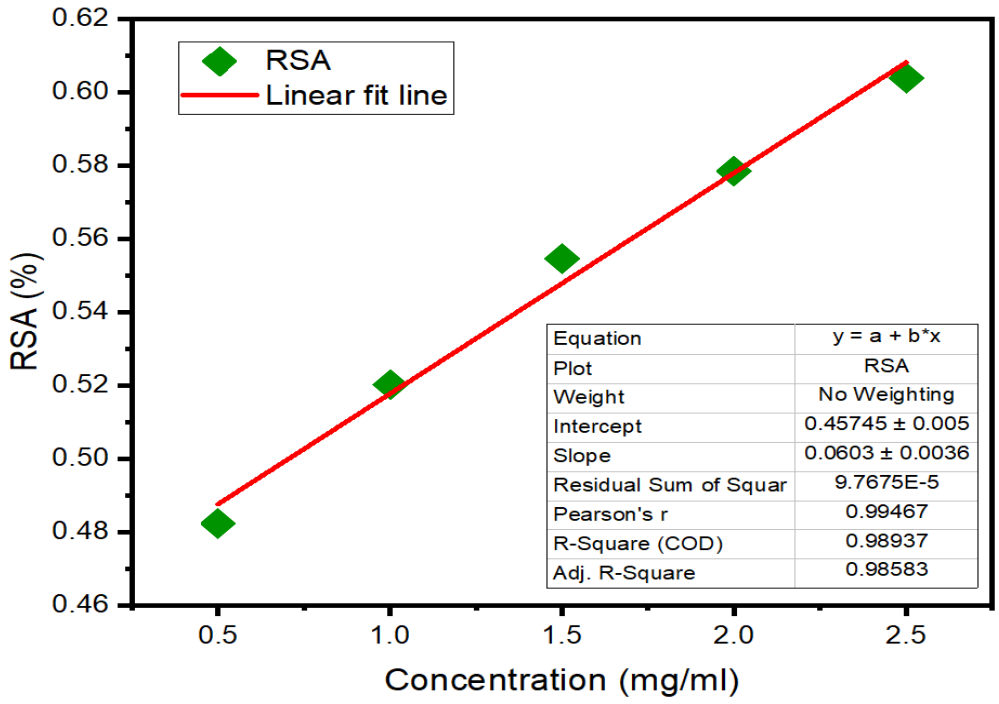

Fig. 2 Linear fitting for the radical scavenging activity performed by *G. pentaphyllum* extract

The MTT assays was recorded in Table 2 below. Gynostemma pentaphyllum extract was tested on the most common cell lines representing liver cancer (Hep-G2), lung tumor (LU-1), and blood cancer (HL-60). It showed anti-cancer effects against some types of common tumor cell line. Specifically, with a concentration of 49.57 µg/ml, the extract glue had inhibited 50% of the Hep-G2 cells, which represents common primary liver cancer. It also shows anti-blood cancer effects but it is relatively insignificant. When tested against the metabolisms of lung cancer cell line SK-LU-1, the inhibitory effect did not display.

Table 2 *Cell proliferation inhibitory effect of G. pentaphyllum extract*

	Cell proliferation inhibitory effect (IC_{50})		
	Hep-G2	Lu-1	HL-60
G. pentaphyllum extract	49.57 µg/ml	---	> 100 µg/ml

Encapsulation of G. pentaphyllum extract

After evaporating ethanol and obtaining the extract, it was mixed with deionized water before adding glucose to create the pharmaceutical flour. The tested ratios and descriptions were summarized as follows.

Table 3 *Mixing ratios and descriptions*

Formula	Ingredient	Ratio	Description
1	Glucose	65 g	The mixture had a dark green color with strong herbal odor of bitterness. The phrase was semi-solid and viscous
	G. pentaphyllum extract	45 ml	
2	Glucose	70 g	The mixture still had an unusual green color. The phrase remained viscous
	G. pentaphyllum extract	45 ml	
3	Glucose	75 g	The mixture had a light green color with no apparent odor. The phrase of the mixture was virtually solid pieces.
	G. pentaphyllum extract	45 ml	
4	Glucose	80 g	The green color was insignificant. The phrase was solid pieces with white glucose flour covering the surface.
	G. pentaphyllum extract	45 ml	

As a result, the ratio between the extract and the glucose was 45 ml/75 g. The mixture was also dried out at various temperature to find out the most appropriate level and results are in Table 4 below:

Table 4 *Total saponin extract of G. pentaphyllum extract flour after drying*

Temp (^{0}C)	Time (min)	Saponin extract (g)	Saponin extract (%)
50	105	0.098^{d}	1.40 %
60	90	0.096^{cd}	1.43 %
70	75	0.095^{c}	1.46 %
80	60	0.091^{b}	1.43 %
90	45	0.087^{a}	1.37 %

Note: In the same column, mean values with different lowercase letters indicate significant differences at $p < 0.05$.

In general, as the temperature ranged from 50^{0}C to 90^{0}C, the saponin extract decreased. Nevertheless, when accounting the efficiency of drying, the rate of saponin extraction rose when the temperature increased from 50^{0}C to 70^{0}C. The temperature of 70^{0}C resulted in the highest rate of extraction with 1.46% saponins corresponding to 0.095 grams out of 10 grams. Statistically, the saponin extract of 60^{0}C resembled both 50^{0}C and 70^{0}C. However,

its efficiency is much higher than 50^0C while slightly lower than 70^0C. The point 90^0C remarked the lowest numbers with only 0.087 g out of 10 g of flour, corresponding to 1.37%.

Discussion

Regarding the material characterization, the total saponin content of this study's raw material and those of other studies vary drastically. The average record of this study was 15.67% of total G. pentaphyllum's dried matters. The study of Z. Wang et al. (2007) reached 11.29%. Xie et al. (2010) while investigating 5 different commercial G. pentaphyllum in China recorded a maximum yield of 22.26%. It is noticeable that the yield of this study more closely resembles those of in-vitro G. pentaphyllum in China. This proves the variation attributed to the source of material as well as the quantification technique.

In the operation of *G. pentaphyllum* extraction, the tendency of ultrasound depleting the extraction efficiency was recorded before. In 2019, Lakka et al. examined the effect of ultrasound on extracting hops (Humulus lupulus) and found the identical tendency of results. Particularly, the total polyphenols extracted from hops increased sharply by approximately 17% in 20 minutes but subsequently reduced by 19% after 30 minutes. The extraction of Panax ginseng in the study of Liang et al. (2019) also recorded a decrease in Gypenoside III content after a certain amount of ultrasonic processing time. The solvent of ethanol at 70% was shown to be the most balanced solvent for G. pentaphyllum extraction for its polarity and non-toxic nature [20]. The extraction efficiency also declined over time due to mass transfer, which was not supported as equilibrium was reached and the rate of increase stopped (Raghav & Kumar, 2018; Q.-W. Zhang *et al.,* 2018). For temperature and material – solvent ratio, the arrangement in this study made those parameters heavily influenced by the results from previous experiments so the results varied significantly compared to other studies. For example, with an abundant amount of ethanol (ratio 1:67), Z. Wang *et al.*, (2007) reached an efficiency over 90% with 95^0C and only 10 minutes. On the other hand, (Xie *et al.,* 2010) and Wang *et al.,* 2020) needed 30 – 50 minutes and 60 – 70^0C to extract G. pentaphyllum using the material-solvent ratio of 1:30.

In the study of Xie et al. (2010), it was shown that their GP4 samples had approximately 49% anti-oxidation capacity with the concentration of 0.4 mg *G. pentaphyllum* extract/ml, which resembles the result of this study. The radical scavenging activity of 1.5 mg/ml was 57%. On the other hand, Xie et al. (2012) showed that it often ranged from less than 20% to approximately 70% depending on the origin. In a more detailed view, the capacity of 30% - 50% occurred most often with 10/16 cases showing the result in such a range. Therefore, the record of DPPH scavenging activities of *G. pentaphyllum* in this study was comprehensible.

Conclusion

This study has examined ultrasonically-assisted solid-liquid extraction for the retrieval of *Gynostemma pentaphyllum* saponins and bioactivity. The raw *G. pentaphyllum* leaves were determined with 89.50% moisture, 1.16 % ash, and 15.67 % saponins. The parameters and single-factor optimization were 5 min of 37 kHz ultrasonically processing time, 70% ethanol as the solvent, 90 min of extraction time, 70^0C of temperature, and a 1 – 15 g/ml material-solvent ratio. These conditions produced a maximum saponin extract of 14.49 g/100 g of dried material, corresponding to an efficiency of 92.47%. The extract in this study was also proved potent as a DPPH scavenging agent with an IC_{50} value of 7.21 mg/g. It also exhibited remarkable ability against liver cancer and potential against blood cancer after MTT assays. Therefore, the extract promises good use in the future

To produce the pharmaceutical flour, the extract glue was mixed with water and glucose. The mixing ratio was 45 ml of extract (10%) and 75 grams of glucose. The drying conditions were at 70^0C for 75 minutes. This stage resulted in a greenish flour with 1.45% of total saponin extract and 8.61% of moisture. After encapsulation, the production process was complete. This is an appropriate point of moisture content according to the Vietnamese Standard TCVN I-4:2017 for herbal pharmacy. This study has been successful in creating a procedure to store the optimized extract of *Gynostemma pentaphyllum*, initially laying a foundation for future research on the subject and realistic applications.

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