EXTRACTION OF POLYSACCHARIDES AND TANNIN FROM SOME MEDICINAL PLANTS

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SUMMARY

Medicinal plants such as *Ganoderma lucidum* (Leyss. Ex Fr.) Karst, *Morinda officianalis* How and *Camellia tamdaoensis* Ninh et Hakoda have been identified and used as medicinies to support human health. They were reported contain many chemical substances (like polysaccharides, tannins, and saponins) that can perform many biological functions, for example, defence against fungis, insects, and herbivoros mamals. However, the extraction of these bioactive subtances from medicinal plants is poorly understood. Of siginificance is optimization for the extraction of them from the medicinal plants, we extracted polysaccharide and tannin from *Ganoderma lucidum* (Leyss. Ex Fr.) Karst, *Morinda officianalis* How and *Camellia tamdaoensis* Ninh et Hakoda with the temperature from 70 - 90^{0}C in 30 - 90 minutes, and ratio of materials and solvent is from 1:25 to 1:100. The results showed that the optimized extraction condition of polysaccharide and tannin from *Morinda officianalis* How (water: ethanol = 1:1, solid material: liquid solvent = 1:25, at 100°C in 30 minutes); *Camellia tamdaoensis* Ninh et Hakoda (water: ethanol = 1:1, solid material: liquid solvent = 1:100, at 90^{o}C in 30 minutes); *Ganoderma lucidum* (Leyss. Ex Fr.) Karst (water: ethanol = 1:2, solid material: liquid solvent = 1:75, at 100°C in 60 minutes). These results provide important insight regarding bioactive compounds from medicinal plants that may be useful for scientists in future.

Keywords: *Camellia tamdaoensis* Ninh et Hakoda, *Ganoderma lucidum* (Leyss. Ex Fr.) Karst, *Morinda officianalis* How, polysaccharide, tannin.

I. INTRODUCTION

Ganoderma lucidum (Leyss. Ex Fr.) Karst, *Morinda officianalis* How and *Camellia tamdaoensis* Ninh et Hakoda are valuable medicinal plants, which provide noticeable amount of bioactive compounds such as polysaccharide, tannin, triterpenoid, steroid, saponin, and so on. Among those, polysaccharide and tannin group are the most important because of their noticeable ability in anti-cancer and anti-oxidative, respectively (Sakai and Chihara, 1995).

Extraction of bioactive compounds from medicinal plants depends on many factors (like type of solvent, temperature, and time), which can affect to extraction yield as well as the functional stability of extracted compounds. Solvent type plays critical roles during the extraction. Together with the solvent, temperature also has significant role in the extraction. For example, using the low temperature used to extract the ionic compounds, whereas the non-ionic compounds can be extracted at high temperature (above 100^{0}C). However, extraction at temperature lower than 100^{0}C leads to solubility of short polysaccharide and soluble tannin, and conversely extraction at above 100^{0}C can trigger the solubility of hemicellulose (Sattler *et al*., 2008) and non-soluble tannin. According to Askin *et al.* (2007), increasing extraction temperature above 100^{0}C can slightly increase proportion of polysaccharide in the extract, however, the extraction temperature should not over 200^{0}C, which leads to destroy of organic compounds, specially is the polysaccharide. In addition, tannin also has non-stable extraction temperature that depends on types of tannin and environmental conditions. With the aims is the optimization of temperature, time and material/solvent ratio for the extraction of bioactive compounds from medicinal plants that can be applied for large scale production. In this study, we carried out the optimization of extraction condition of polysaccharide and tannin from three medicinal plants (*Ganoderma lucidum* (Leyss. Ex Fr.) Karst, *Morinda officianalis* How and *Camellia tamdaoensis* Ninh et Hakoda).

II. RESEARCH METHODOLOGY

2.1. Materials

Ganoderma lucidum (Leyss. Ex Fr.) Karst (Pileus), *Morinda officianalis* How (root) were collected at Hoanh Bo, Quang Ninh, Vietnam. *Camellia tamdaoensis* Ninh et Hakoda (leaves) was collected from Tam Dao national park, Tam Dao, Vinh Phuc, Vietnam. Ten samples for each medicinal plants.

2.2. Methods

2.2.1. Extraction and quantitation of polysaccharide

Method to extract polysaccharide was adapted from Jin-Gao (2015). In briefly, the material was washed briefly with ethanol 95%, dried, and ground into powder. The powder was used to extract polysaccharide with different conditions: (1) water/ethanol ratio from 1:0 to 1:3; (2) solid material/liquid solvent ratio from 1:25 to 1:100; (3) extraction temperature from 70 to 90^0C; and (4) extraction time from 30 to 90 minutes. The extraction solution was filtered by Whatman filter paper No.1 and vacuum centrifuge concentrated at 3,000 rpm in 15 minutes. The second solution (25% n-butanol:choloform (v/v)) was added into the extract solution to remove protein. The mixture was centrifuged at 6,000 rpm in 15 minutes. The supernatant was collected. Added three times volume of Ethanol 95% into the supernatant and incubate overnight. The pellet was collected by centrifuge at 10,000 rpm in 5 minutes.

Quantitation of polysaccharide by using method adapted from Foster *et al*. (1961) and Harshal and Priscilla (2011). The percent of polysaccharide in the sample is the ratio of polysaccharide amount per dried weight of sample.

2.2.2. Extraction and quantitation of tannin

Material was washed briefly with ethanol 95%, dried, and ground into powder. The powder was used to extract tannin with different conditions: (1) water/ethanol ratio from 1:0 to 1:3; (2) solid material/liquid solvent ratio from 1:25 to 1:100; (3) extraction temperature from 70 to 90^0C; and (4) extraction time from 30 to 90 minutes. The extraction solution was filtered by Whatman filter paper No.1 and vacuum centrifuge concentrated. Add $(NH_4)_2SO_4$ solution into the extraction solution to pellet the tannin. Collected the pellet by centrifuge and resuspended the pellet in acetone solution. Tannin solution was put into the vacuum centrifuge concentrator until dry.

Tannin amount was identified by BSA method (Magdalena *et al*., 2007). The percent of tannin in the sample is the ratio of polysaccharide amount per dried weight of sample.

III. RESULTS AND DISCUSSIONS

3.1. Effect of water/ethanol ratio to extraction yield of polysaccharide and tannin

Medicinal plants were collected and ground into powder as shown in Fig.1 below. Identifying effect of water/ethanol ratio to extraction yield was investigated with a range from 1:0 to 1:4. The results were presented in Table 1. The data shown that the increasing ethanol proportion leads to higher percent of collected polysaccharide from all three samples. However, when the ethanol was too high (over 70%) caused the decrease of collected polysaccharide. This result might be the soluble polysaccharide dissolves easily in low-ionic solvent, hence when proportion of ethanol increased leading to the ionic increase of the solvent and subsequently reducing the extraction yield of polysaccharide. The data also indicated that the optimized ratio of water/ethanol for polysaccharide extraction from *Ganoderma lucidum* (Leyss. Ex Fr.) Karst and *Morinda officianalis* How is 1:1, with respective percent of collected polysaccharide was 19.03% and 7.39%. The optimized ratio for *Camellia tamdaoensis* Ninh et Hakoda was 1:2 and the proportion of collected polysaccharide is 8.27%.

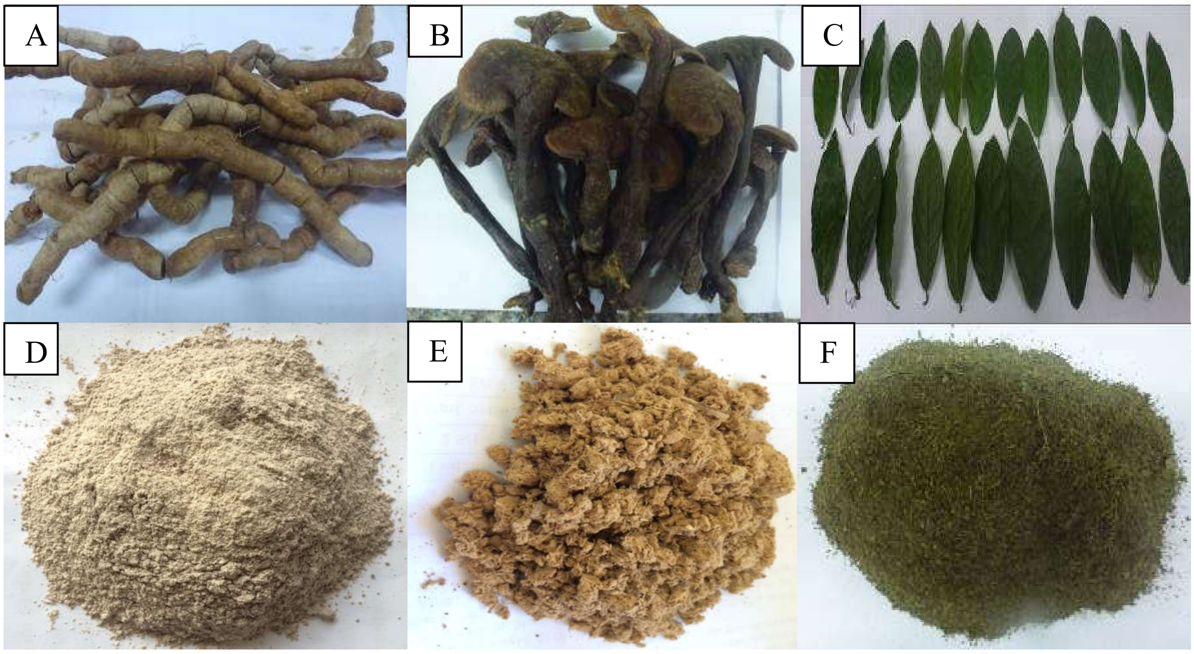

Figure 1. Experimental materials in fresh phenotype (up panel) and in powder (down panel)
A and D: *Ganoderma lucidum* (Leyss. Ex Fr.) Karst, B and E: *Morinda officianalis* How,
C and F: *Camellia tamdaoensis* Ninh et Hakoda

Table 1. Effect of water/ethanol ratio to the polysaccharide and tannin extraction

Samples	Water: ethanol (v/v)	Collected polysaccharide (%)	Collected tannin (%)
Ganoderma lucidum (Leyss. Ex Fr.) Karst	1:0	17.76 ± 0.53	1.21 ± 0.04
	1:1	**19.03 ± 0.38**	**1.65 ± 0.03**
	1:2	15.39 ± 0.46	1.49 ± 0.04
	1:3	12.18 ± 0.24	1.38 ± 0.03
Morinda officianalis How	1:0	7.33 ± 0.22	2.28 ± 0.07
	1:1	7.77 ± 0.16	2.43 ± 0.05
	1:2	**8.27 ± 0.25**	**2.69 ± 0.08**
	1:3	6.81 ± 0.14	2.01 ± 0.04
Camellia tamdaoensis Ninh et Hakoda	1:0	6.65 ± 0.22	**25.8 ± 0.77**
	1:1	**7.39 ± 0.11**	24.3 ± 0.47
	1:2	5.38 ± 0.12	23.7 ± 0.61
	1:3	4.01 ± 0.13	20.2 ± 0.49

Similar results were observered during the tannin extraction from *Ganoderma lucidum* (Leyss. Ex Fr.) Karst and *Morinda officianalis* How with the optimized ratio is 1:1 and 1:2, and the highest proportion of collected tannin is 1.65% and 2.69%, respectively. However, the collected tannin from *Camellia tamdaoensis* Ninh et Hakoda was reduced from 25.8 to 20.2% when the proportion of ethanol increased from 0 to 75%. Tannin is a polyphenol compound, which is easily soluble in ethanol, hence the 1:1 and 1:2 ratio generated the suitable ionic condition for tannin be dissolved. Moreover, increased ethanol percent might caused the solubility of contaminated compounds, which were dissolved in ethanol and be extracted together with tannin. These contaminated compounds were increase together with the ethanol increase leading to low yield extraction of tannin.

Among three samples, the highest proportion of collected polysaccharide was from *Ganoderma lucidum* (Leyss. Ex Fr.) Karst (19.03%) and of collected tannin was from *Camellia tamdaoensis* Ninh et Hakoda (25.8%).

3.2. Effect of solid materials/liquid solvent ratio to extraction yield of polysaccharide and tannin

Polysaccharide was extracted from fine powder according to Jin Gao method (2015) with the change in the ratio for the solid material and liquid solvent to get the highest extracts from samples.

Optimized ratios of water/ethanol from previous experiments were applied in these experiments. The results were shown in the Figure 2 following.

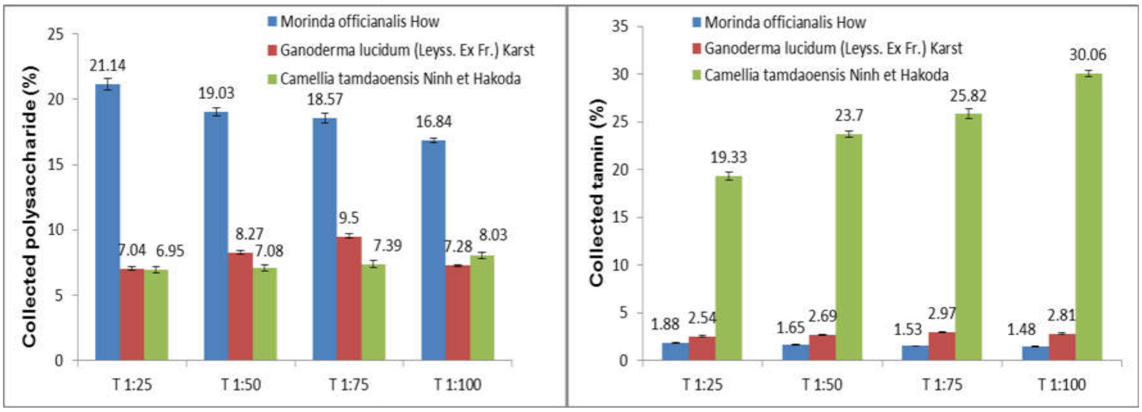

Figure 2. Effect solid material/ liquid solvent ratio to extraction yield of polysaccharide (left panel) and tannin (right panel)

The figure presented the different ratio of solid material and liquid solvent slightly affected to yield extraction of polysaccharide and tannin among three samples. The yield extraction depended highly on the plant species and type of bioactive compounds. For example, the polysaccharide extraction with the ratio is 1:25, the highest yield was observed in *Ganoderma lucidum* (Leyss. Ex Fr.) Karst (21.14%) while the polysaccharide yield extraction for *Morinda officianalis* How and *Camellia tamdaoensis* Ninh et Hakoda was rapidly dropped to 7.04% and 6.95%, respectively. However, with the same ratio for tannin extraction, the highest yield was observed for *Camellia tamdaoensis* Ninh et Hakoda (19.33%) and dammatical yield decrease was seen at *Ganoderma lucidum* (Leyss. Ex Fr.) Karst (1.58%) and *Morinda officianalis* How (2.54%). Hence, based on the observation, we chose the optimimum ratio for solid material and liquid solvent is 1:25 for *Ganoderma lucidum* (Leyss. Ex Fr.) Karst, 1:75 for *Morinda officianalis* How, and 1:100 for *Camellia tamdaoensis* Ninh et Hakoda. With these ratio, the collected percent for polysaccharide were 21.14%, 9.50%, and 8.03%, and for tannin were 1.88%, 2.97% and 30.06%, respectively for *Ganoderma lucidum* (Leyss. Ex Fr.) Karst, *Morinda officianalis* How and *Camellia tamdaoensis* Ninh et Hakoda.

3.3. Effect of temperature to extraction yield of polysaccharide and tannin

Extraction at above 100^0C caused the contamination of hemicellulose, which have branch structures that might be broken down generating smaller molecules at high temperature (Sattler *et al*., 2008; Yu *et al*., 2008). Hence, the extraction of polysaccharide and tannin were carried out with the above optimized ratio and in the range of temperature from 70 - 100^0C. The results were presented in Table 2 below.

Table 2. Effect of temperature to the polysaccharide and tannin extraction

Samples	Temperature (^{o}C)	Collected polysaccharide (%)	Collected tannin (%)
Ganoderma lucidum (Leyss. Ex Fr.) Karst	70	18.46 ± 0.37	1.26 ± 0.03
	80	20.11 ± 0.30	1.42 ± 0.02
	90	21.14 ± 0.42	1.88 ± 0.04
	100	**23.69 ± 0.24**	**2.57 ± 0.03**
Morinda officianalis How	70	7.43 ± 0.15	2.07 ± 0.04
	80	8.01 ± 0.12	2.45 ± 0.04
	90	9.50 ± 0.19	2.97 ± 0.06
	100	**10.43 ± 0.10**	**3.73 ± 0.04**
Camellia tamdaoensis Ninh et Hakoda	70	6.24 ± 0.12	25.67 ± 0.51
	80	7.08 ± 0.12	27.21 ± 0.41
	90	**8.03 ± 0.14**	**30.06 ± 0.60**
	100	7.15 ± 0.07	28.32 ± 0.28

The results showed that increasing extraction temperature lead to the increase of polysaccharide extract (Table 2). The biggest proportion of polysaccharide was observed at 100^0C for *Ganoderma lucidum* (Leyss. Ex Fr.) Karst (23.69%) and *Morinda officianalis* How (10.43%) and at 90^0C for *Camellia tamdaoensis* Ninh et Hakoda (8.03%).

At optimized temperature, the soluble polysaccharides, mostly are high molecular (about $5x10^5$ Da), were extracted. Polysaccharide compounds consist of polysaccharide molecules and amio acids, in which polysaccharide colecules including glucose, galactose, arabinose, xylose and mannose that connect to each other by the β-glucoside linking. There are approximately 17 acid-amin molecules that linked the polysaccharide molecules (Chan *et al*., 2006). These acid amin molecules have high ionic strength that helps them dissolve more easily during the increase of temperature.

The results also indicated that when the temperatures increase from 70 to 100^0C, the collected tannin parallelly increase (Table 2). The collected tannin rapidly increased in the range of temperature from 90 to 100^0C. The optimum range of temperature for tannin extraction were from 90 to 100^0C for all three samples with the percent of collected tannin were 2.57%, 3.73% and 30.06%, respectively for *Ganoderma lucidum* (Leyss. Ex Fr.) Karst, *Morinda officianalis* How, and *Camellia tamdaoensis* Ninh et Hakoda.

The parallell increase of extraction temperature and collected tannin amount means tannin was not be oxidative during the extraction. Tannin can be oxidative in present of oxygen at quite high temperature under the activity of polyphenol oxydase. However, the extraction was carried out in water solvent, which can prevent the interaction between tannin and oxygen reducing steadily the oxidation. In addition, the increasing temperature also had role in deactivating the polyphenol oxydase. Therefore, extraction at high temperature can reduce the oxidation of tannin.

3.4. Effect of time to the extraction yield of polysaccharide and tannin

In these experiments, we applied the optimum conditions from above experiments in different time range from 30 to 90 minutes. The results were presented in Fig 3 and 4 and Table 3 following.

The data showed a parallel increase for extraction time and yield extraction for polysaccharide from all three samples (Table 3). The optimum time for polysaccharide extraction were 30, 60 and 30 minute for *Camellia tamdaoensis* Ninh et Hakoda (collected polysaccharide was 9.15%), *Morinda officianalis* How (15.02%), and *Ganoderma lucidum* (Leyss. Ex Fr.) Karst (25.17%), respectively.

This process used to extract the soluble molecules, hence, extraction in long time may lead to an increase of soluble polysaccharide to maximum level. However, extraction at high temperature in a long time might cause the degradation of organic compounds including polysaccharides (Askin *et al*., 2007).

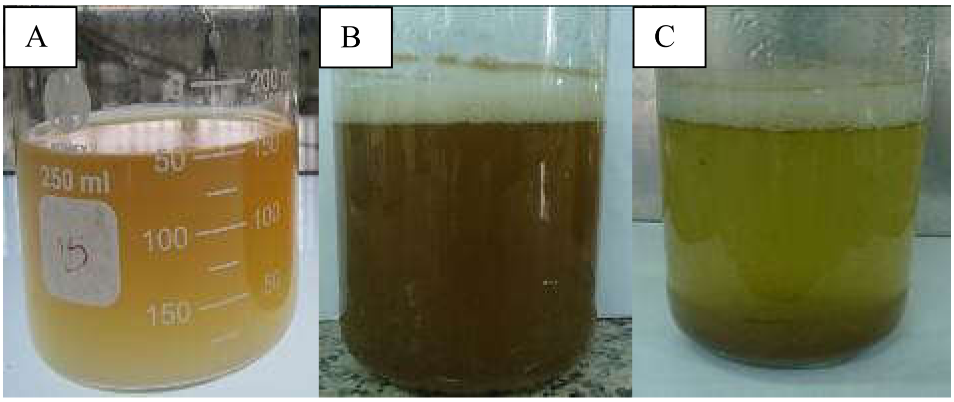

Figure 3. Extract solution from *Ganoderma lucidum* (Leyss. Ex Fr.) Karst (A), *Morinda officianalis* How (B) and *Camellia tamdaoensis* Ninh et Hakoda (C)

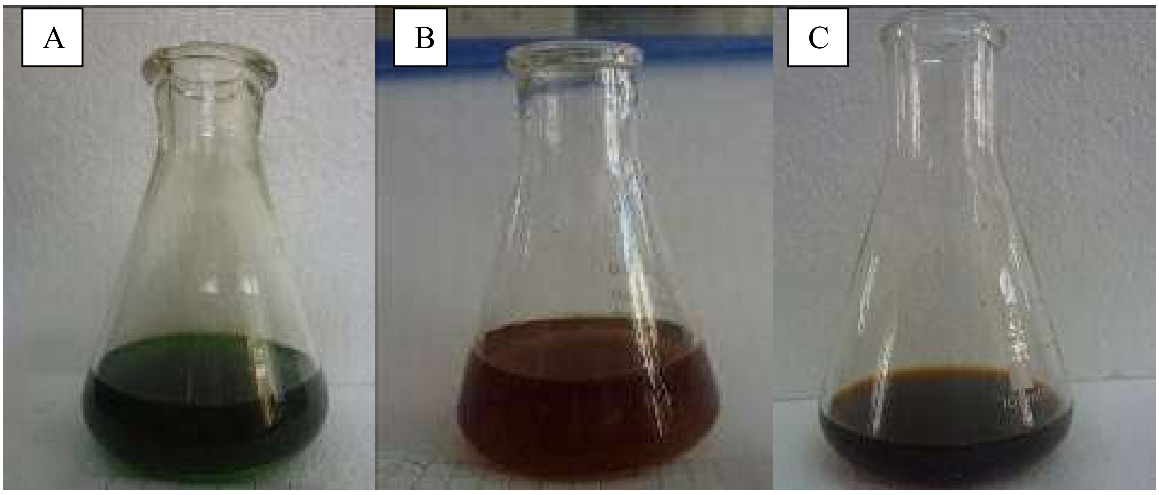

Figure 4. Tannin extract solution from leaf of *Ganoderma lucidum* (Leyss. Ex Fr.) Karst (A), *Morinda officianalis* How (B) and *Camellia tamdaoensis* Ninh et Hakoda (C)

Table 3. Relationship between time and yield for polysaccharide and tannin extraction

Samples	Time (minutes)	Collected polysaccharide (%)	Collected tannin (%)
Ganoderma lucidum (Leyss. Ex Fr.) Karst	30	**25.17 ± 0.5**	**2.68 ± 0.05**
	45	23.69 ± 0.36	2.57 ± 0.04
	60	20.43 ± 0.41	2.46 ± 0.05
	75	17.51 ± 0.18	2.3 ± 0.02
	90	10.68 ± 0.11	2.02 ± 0.02
Morinda officianalis How	30	8.90 ± 0.18	3.05 ± 0.06
	45	10.43 ± 0.16	3.73 ± 0.06
	60	**15.02 ± 0.30**	**4.08 ± 0.08**
	75	13.56 ± 0.14	3.87 ± 0.04
	90	8.03 ± 0.08	3.59 ± 0.04
Camellia tamdaoensis Ninh et Hakoda	30	**9.15 ± 0.18**	**33.04 ± 0.66**
	45	8.03 ± 0.09	30.06 ± 0.45
	60	6.22 ± 0.10	28.32 ± 0.57
	75	5.07 ± 0.08	24.83 ± 0.25
	90	4.16 ± 0.04	19.12 ± 0.19

The data from Table 3 also indicated that the amount of collected tannin depended strongly to extraction time. At the same temperature, an increase of extraction time caused increased amount of collected tannin in 30 - 60 minutes. However, the proportion of collected tannin was stable and tended to decrease after 60 minutes. The optimization of extraction time for tannin from *Ganoderma lucidum* (Leyss. Ex Fr.) Karst, *Morinda officianalis* How and *Camellia tamdaoensis* Ninh et Hakoda were 30, 60, and 30 minutes respectively.

IV. CONCLUSIONS

The optimized conditions have been identified for the extraction of polysaccharide and tannin from *Ganoderma lucidum* (Leyss. Ex Fr.) Karst, *Morinda officianalis* How and *Camellia tamdaoensis* Ninh et Hakoda.

For extraction from *Ganoderma lucidum* (Leyss. Ex Fr.) Karst, we identified optimum conditions were Water/Ethanol = 1:1, solid material/liquid solvent = 1:25, temperature = 100^0C and extrraction time is 30 minutes.

For extraction from *Morinda officianalis* How, we identified optimum conditions were Water/Ethanol = 1:2, solid material/liquid solvent = 1:75, temperature = 100^0C and extrraction time is 60 minutes.

For extraction from *Camellia tamdaoensis* Ninh et Hakoda, we identified optimum conditions were Water/Ethanol = 1:1, solid material/liquid solvent = 1:100, temperature = 90^0C and extrraction time is 30 minutes.

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NGHIÊN CỨU CHIẾT XUẤT POLYSACCHARIDE VÀ TANNIN TỪ MỘT SỐ LOẠI DƯỢC LIỆU

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TÓM TẮT

Nấm lim xanh, ba kích, trà hoa vàng là những dược liệu quý hiếm và chứa nhiều chất có hoạt tính sinh học như polysaccharide, tannin, saponin… Nghiên cứu chiết xuất polysaccharide và tannin từ các nguồn dược liệu nấm lim xanh, ba kích, trà hoa vàng bằng dung môi ethanol với tỷ lệ nguyên liệu rắn:dung môi lỏng 1:25 - 1:100, nhiệt độ 70 - 90^{o}C, thời gian 30 - 90 phút. Quy trình chiết xuất polysaccharide và tannin thích hợp với củ ba kích (tỷ lệ nước:ethanol = 1:1, tỷ lệ nguyên liệu rắn:dung môi lỏng = 1:25, nhiệt độ 100^{o}C, thời gian trích ly 30 phút); lá trà hoa vàng (tỷ lệ nước: ethanol = 1:1, tỷ lệ nguyên liệu rắn: dung môi lỏng = 1:100, nhiệt độ 90^{o}C, thời gian trích ly 30 phút); nấm lim xanh (tỷ lệ nước:ethanol = 1:2, tỷ lệ nguyên liệu rắn:dung môi lỏng = 1:75, nhiệt độ 100^{o}C, thời gian trích ly 60 phút). Tỷ lệ polysaccharide và tannin trong các mẫu lần lượt là: ba kích (25,17% và 2,68%), trà hoa vàng (9,15% và 33,04%) và nấm lim xanh (15,02% và 4,08%).

Từ khóa: Ba kích, nấm lim xanh, polysaccharide, tannin, trà hoa vàng.

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