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RESEARCH ARTICLE

Various Technical Parameters Influencing to Production of *Polyscias Fruticosa* Tea

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Abstract

Polysicas fruticosa is available throughout Vietnam. This plant species contains large quantity of saponin. We have identified some basic chemical components of the leaf *Polyscias fruticosa* as moisture, ash and saponin content. On that basis, we chose the initial drying of the raw material with a vacuum drying method, the drying temperature of 50°C, for 6 hours to achieve a humidity of < 13% as required for medicinal herbs; The method of hot extraction in solvents is 70% ethanol with a solid/liquid ratio of 1/8, at a temperature of 70°C and extraction for a period of 1 hour limit affecting product quality. Examining the heavy metals and microbiological indicators of dissolved powders from *Polyscias fruticosa*, the product has produced standards for food safety as prescribed by the Vietnamese Ministry of Health.

Keywords: *Polyscias fruticosa, saponin, Drying, Extraction, Ethanol, Powder.*

Introduction

Saponins are second metabolites which are widely distributed in the plant kingdom. It acts as a chemical barrier or shield in the plant defense system to counter pathogens and herbivores [1]. Saponins divided into two major classes which are triterpenoid and steroid glycosides which their structure characterization are varied by the numbers of sugar units attached at different positions [2]. Saponins have been discovered scientifically having pharmaceutical properties of antioxidant [3]. *Polyscias fruticosa* belongs to Araliaceae family and distributes widely in Vietnam. It is a shrub to small tree to 4 m tall.

The stems are hollow in the internodes and solid at the nodes. Flowers are umbellate inflorescence with epigynous flowers. There are five petals and the base is broad with petals and the base is broad with petals alternate. Fruits are compressed ovoid and about 4 cm in length. Seeds are also found compressed. Leaves alternate, petiolate, irregularly pinnately compound, the leaflets with conspicuous toothed margins, blades often yellowish in color and fragrant if crushed. Roots are cylindrical, lengthy, and slightly fasciculated in appearance. Roots are yellowish brown in colour, usually much branched and woody. The taste of the roots is slightly bitter followed by sweet and mucilaginous [4]. The roots smell and taste like parsley [5]. The leaves are used as atonic, anti-inflammatory, antitoxin, and antibacterial [6]. The root is used as a diuretic, febrifuge, antidysentery, and for treatment of neuralgia, rheumatic pain, asthma [5, 7, 8]. Amino acids, polysaccharides, steroids, sesquiterpenoids, triterpenoid saponins, and polyacetylenes are among the components of *P. fruticosa* [9, 13].

Tran Thi Hong Hanh et al [14]. Suggested the use of *P. fruticosa* and its major saponin for the prevention and treatment of diabetes .Nguyen Thi Luyen et al [15].Evaluated the inhibitory effect of 3-*O*-[ß-d-glucopyranosyl-(1→4)-ß-d-glucuronopyranosyl] oleanolic acid 28-*O*-ß-d-glucopyranosyl ester (PFS), a major saponin isolated from *Polyscias fruticosa* leaves, on α-amylase and α-glucosidase. Alex Boye et al [16].Assessed effects of P. fruticosa leaf extract (PFE) on male fertility and

toxicity in adult male Wistar rats. Nguyen Phuoc Minh et al [17].Focused on the effect of blanching time and temperature, $CaCl_2$ concentration in blanching; *Polyscias fruticosa* leaf size and temperature in drying; and storage condition to saponin (µg/g) content in the herbal tea. The present study focused on the examination of phytochemical substances in raw material of *Polyscias fruticosa* leaves; the drying and extracting process to the saponin (µg/g) content.

Materials and Method

Material

Polyscias fruticosa samples were naturally collected from Dak Lak province, Vietnam. These samples were graded to remove the damaged or decay leaves as well as foreign matters. They were then washed under tap water for cleaning. These samples were then dripped before experiments. This research was conducted in the Central Highland Hygiene Institute (Vietnam). Lab utensils were used including electric oven, rotary evaporator, circulating dryer, vaccum dryer, burning oven, water bath, fume hood, ultrasonic, incubator, digital balance, and glassware. Chemical substances were also used such as ethanol, N-butanol, diethyl-ether.

Figure 1: ***Polyscias fruticosa***

Researching Procedure

Phytochemical Analysis in Raw Material of Polyscias Fruticosa Leaves

Raw material of *Polyscias fruticosa* leaves was primarily analyzed to measure moisture (%), ash ((%) and saponin (µg/g). The experiment was repeated 3 times, taking the average result.

Effect of Drying Process to saponin (µg/g) Content in the Dried Polyscias Fruticosa Leaves

Samples of fresh materials, with the same volume, after being washed, drained to be carried away drying with the storage drying, the vacuum at 40°C, 50°C, 60°C, 70°C and exposed naturally for 6 hours.

Dry materials after being finely crushed, taking 5g of raw materials to analyze the content of saponins. Based on the results of the analysis, choose the appropriate method *Polyscias fruticosa*.

Effect of Extraction (Solvent, Concentration, temperature) to saponin (µg/g) Content in the Extract

Take 10g powdered raw materials to be extracted by two different extraction methods, hot and cold extraction, in the following conditions: Duration (4 hours); material/Solvent ratio (1/8); solvent concentration (Ethanol 40%); hot extraction temperature (70°C); cooling temperature (ambient temperature). Based on the measured result, select the appropriate extraction method.

Physico-chemical and Biological Analysis

The moisture content (%) was measured by weighing samples before and after drying to constant weight. Ash content (%) was analyzed by burning sample at 550°C to white ash. Saponin (µg/g) was quantified by spectrophotometer [18].

Statistical Analysis

The experiments were run in triplicate with three different lots of samples. Data were subjected to analysis of variance (ANOVA) and mean comparison was carried out using Duncan's multiple range test (DMRT). Statistical analysis was performed by the Stat graphics Centurion XVI.

Result & Discussion

Phytochemical composition in *Polyscias fruticosa*

Polyscias fruticosa contains many chemical components of high nutritional value, which contain highly bioactive compounds such as saponins, alkaloids, tanin. Phytochemical composition in *Polyscias fruticosa* was primarily analyzed. Results were depicted in Table 1.

Table 1: Phytochemical composition in *Polyscias fruticosa*

Segment	Moisture (%)	Ash (%)	Saponin (µg/g)
Leaf	89.47±0.05^{a}	4.66±0.05^{ab}	4.18 ±0.02^{a}
Body	61.34±0.02^{c}	4.13±0.05^{b}	0.52±0.03^{c}
Root	81.16±0.05^{b}	5.12±0.02^{a}	1.18±0.03^{b}

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%).

Figure 2: Extract of Polyscias *fruticosa* collected from its leaf, body and root

Effect of Drying Process to Saponin (µg/g) Content in the Dried Polyscias Fruticosa Leaves

Since the result stated above, *Polyscias fruticosa* has a high moisture content of about 89%. In order to facilitate the preservation process as well as improve the extraction capacity, we conduct a drying survey in vacuum and drying modes and expose at different temperatures.

Table 2: Effect of drying method and drying temperature to saponin content (µg/g) in dried *Polyscias fruticosa*

Drying	Temperature (°C)	Moisture (%)	Saponin (µg/g)
Circulation	40±1°C	11.03±0.18^{b}	3.27±0.10^{c}
	50±1°C	9.67±0.15^{de}	3.39±0.10^{b}
	60±1°C	9.88±0.15^{d}	3.08±0.12^{de}
	70±1°C	9.02±0.19^{e}	3.11±0.11^{d}
Vaccum	40±1°C	11.76±0.18^{a}	3.62±0.15^{ab}
	50±1°C	10.12±0.17^{c}	3.72±0.17^{a}
	60±1°C	10.04±0.14^{cd}	3.31±0.13^{bc}
	70±1°C	9.89±0.16^{d}	3.21±0.11^{cd}
Sunlight	30-36	11.12±0.11^{ab}	2.94±0.16^{e}

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%)

The *Polyscias fruticosa leaves* were washed, shade-dried (26- 30°C) and pulverized into fine powder using a mechanical blender (George Asumeng Koffuor et al., 2014). Data from the above table, vaccum drying and circulate drying are obtained higher levels of saponins than natural exposures. When drying with the method of saving and vaccum drying at 40°C, 50°C, the material has the highest content of saponins and has a statistical aberration in terms of the values at 60°C, 70°C. However, vaccum drying is the method that has more advantages than circulate drying because it restricts the oxidation potential of some bioactive compounds available in plants.

So we chose the vachow drying method. Of the 40°C and 50°C temperature levels of the vacuum drying method, the material obtained does not have a statistically false difference. But, with the drying mode at 50°C, a shorter drying time so the application capabilities to the higher reality. On that basis, we propose the initial material drying by the vactemethod drying, drying temperature 50°C, for 6 hours to reach the drug < 13% as required for medicinal herbs.

Effect of Extraction (Solvent, Concentration, Temperature) to saponin (µg/g) Content in the Extract

Leaf of *Polyscias fruticosa* after drying proceeds finely crushed, preserved. Then to build the process of saponin extraction, we conduct research on the influence of extraction methods, solvent, solvent concentration, extraction time. From there select the most effective extraction mode.

Figure 3: Powder of *Polyscias fruticosa*

In order to select the highly effective extraction method we conduct the extraction by two different methods of hot extraction and cold extraction has been described above. The determination of the extraction content is also known as determining the content of dissolved substances in order to assess the possibility of extraction of raw materials in a certain solvent, resulting in the following:

Table 3: The results of the extraction method

Number	Extraction method	Extract content (%)
1	Hot extraction	10.15±0.01[a]
2	Cold extraction	9.03±0.01[b]

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%)

The results showed that the content of the extraction in both methods of extraction fluctuates around 10% powdered grain of raw material. In the hot extraction method higher cold extraction, but not significant. We choose the method of hot extraction with the reason when this method is made to save more time than cold extraction method, limiting the quality of the product. Solvents are an important factor in the process of extraction, the appropriate solvent selection has the essential role to obtain extraction fluid containing the highest necessary separation of the compounds. To assess the effect of the solvent extraction process to extract saponins compounds, we conduct extraction of steroidal saponins in these solvents in turn: water and tincture of different concentrations are: 40%, 50%, 60% , 70%. The results of the study are shown in the following table:

Table 4: The results of the solvent survey and the concentration affecting extraction process

Number	Solvent	Saponin (µg/g)
1	Water	2.53±0.21[c]
2	Ethanol 40%	3.05±0.05[bc]
3	Ethanol 50%	3.61±0.15[ab]
4	Ethanol 60%	3.14±0.14[b]
5	Ethanol 70%	4.15±0.31[a]

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%)

From the above results shows the extraction of ethanol solvent for higher content of saponins than distilled water, when changing the concentration of solvent extraction, the content of Saponins has a change according to. Ethanol extraction 70% for the saponins content is the highest and there are other discrepancies in terms of statistical significance, extraction of ethanol at a concentration of 60%, 50% and 40% obtained low saponins, due to the chemical nature of saponins compared with water solvent due to uncapable Soluble in alcoholic compounds. At the same time the extraction of cups by ethanol 70% keeps the fragrance characteristic of Polyscias fruticosa better so we choose ethanol 70% as solvent for subsequent studies. To assess the effect of the extraction time to the process of extraction of saponins compounds, we

conduct a separation of saponins compounds by means of hot extraction by ethanol solvents at the time of 1.0h, 1.5h, 2.0h, 2.5h, 3.0h. The results of the study are shown in the following table:

Table 5: The results of the survey time extraction to the extraction process

Number	Time of extraction (h)	Saponin (µg/g)
1	1.0	4.15 ± 0.11^{a}
2	1.5	4.12 ± 0.07^{ab}
3	2.0	4.05 ± 0.12^{b}
4	2.5	3.95 ± 0.15^{bc}
5	3.0	3.91 ± 0.21^{c}

Note: the values were expressed as the mean of three repetitions; the same characters (denoted above), the difference between them was not significant (α = 5%)

As the result shows that the longer the hot extraction time, the content of saponins tends to be reduced although insignificant, due to loss during evaporation or less durable decomposition, at the same time when to calculate the economic efficiency we choose the best extraction time is 1h. The extract is carried out spray-making powder using the bearing substance is maltodextrin with a content of 11% (w/V). Spray drying process at the parameters: drying temperature of 140-150°C, the speed of the spray disk 15000-16000rpm, injection pressure on the plate 2atm.After drying the spray into dissolved powder, analysis of heavy metal composition and micro organisms' targets. In the framework of the topic, has not studied the effect of drying method of spraying up the quality of tea products. We see the content of saponins before drying is 4.18% and after choosing the method of the influence of solvent and solvent concentration ethanol 70% time 1 hour saponins levels decreased to 4.15%. Thus through the effects, the content of the lost Saponins is negligible and the above condition will limit the quality effect of the product.

Quality of Polyscias Fruticosa Soluble Dried Powder

The instant soluble powder of Polyscias fruticosa dried powder was analyzed the heavy metal and microorganism to demonstrate the safety of its herb.

Table 6: Results of the analysis of heavy metals composition

Number	Parameter	Unit	Testing method	Result
1	Mercury (Hg)	mg/kg	TCVN 7604: 2007	0
2	Cadmi (Cd)	mg/kg	TCVN 7603: 2007	0.1
3	Lead (Pb)	mg/kg	TCVN 7602: 2007	0
4	Arsen (As)	mg/kg	TCVN 7601: 2007	0
5	Tin (Sn)	mg/kg	TCVN 7788: 2007	0
6	Methyl mercury	mg/kg	AOAC 988.11	0

Results of the analysis of heavy metals norms show the production of products in accordance with the proposed procedure of quality of food safety standards according to TCVN 8-1:2011/BYT of the Ministry of Health.

Table 7: Microbiological analysis results in dissolved powder

Number	Parameter	Unit	Testing method	Result
1	TPC	cfu/g	TCVN 4884: 2005	10^2
2	*Coliforms*	cfu/g	TCVN 6846: 2007	3
3	*E. coli*	cfu/g	TCVN 6848: 2007	0
4	*Salmonella*	cfu/g	TCVN 8342: 2010	0
5	*S. aureus*	cfu/g	TCVN 4830-1: 2005	2
6	*C. perfringens*	cfu/g	TCVN 4991: 2005	1

Microbial indicators analysis results show that product powders have produced the food safety standards specified in Decision No.: 46/2007/QD-BYT on Dec 19, 2007 of the Ministry of Health.

Conclusion

Polyscias fruticosa is used widely as food, remedy for diseases, and as an ornamental. *P. fruticosa* leaf powder is dark green in colour with a characteristic aromatic odour and having a slightly bitter taste. We have successfully investigated the soluble dried herbal tea production from *P. fruticosa* leaf. We recommend some other experiments to verify some affect the extraction process of saponin: as the drying method; constructing saponins quantitative process in *P. fruticosa* by optical measuring method, HPLC; perform extraction by more extraction methods.

Furthermore, we also suggest the study of antibacterial, antioxidant, antifungal, resistant to saponins extracts and its pharmacological effect; diversification of products from *P. fruticosa*: such as high *P. Fruticosa*, herbal drink from *P. fruticosa*, herbal tea bags from *P. fruticosa.*

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