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Extraction and Application Eurycoma Longifolia Jack Medicine Extracts from Eurycoma Longifolia Root in Ia-Grai District, Gia Lai Province, Viet Nam

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Abstract: Eurycoma longifolia jack root is one of famous medicine, it has high medicinal value and can be applied to produce health food. This study was conducted to research some factors affecting on extraction of extracts from eurycoma longifolia jack root by distillation in water: Extraction temperature at 100^{o}C, in 120 minutes with the ratio between solvent and material is 20/1 (mL/g) for the highest 9,10-dimethoxy canthin-6-one concentration of 13.9 mg/kg. Establishing the eurycoma longifolia jack root medicine extracts and evaluating the quality with the results meet food hygiene and safety standards prescribed by the Ministry of Health in Viet Nam.

Keywords: *9, 10-dimethoxy canthin-6-one; extract; eurycoma longifolia jack root; health food; medicine..*

I. INTRODUCTION

Eurycoma longifolia, also known as stagnation, "tho nan" (Laos), "antongsar" (Cambodia), scientific name: *Eurycoma longifolia* jack, is a flowering plant in the *Simaroubaceae* family, native to Malaysia and Indonesia, Vietnam, they are less distributed in Thailand, Laos and India. In Indonesia, the natural *eurycoma longifolia* jack grows only in Sumatra and Kalimanta [1] [2].

Eurycoma longifolia jack is a common usable, the scientific name is *eurycoma longifolia* jack, also known as the panacea, nectarine or male post-branch. Extracts have been used by humans for antimalarial, sex hormone growth drugs, and antipyretics. *Eurycoma longifolia* jack is known as a valuable herbal medicine and is commonly used in the Central - Highland. Previous studies have proved that the root is the most valuable ingredient and is used to treat pain, indigestion, gas, bloating, persistent fever, malaria, and yang failure, dysentery, swollen glands and can be used as a health-promoting tonic. Extract from the roots are usable to restore energy and vitality, enhance blood circulation, and has a good role for women after childbirth. In addition, this extract contains compounds with anti-tumor, anti-parasitic, anti-ulcer activity... Among which, the best known is the effect of increasing the amount of endogenous hormone testosterone in the body male [3] [4].

The application of medicinal herbs derived from plants is a popular trend, even in advanced countries with a developed medical background, medicinal herbs both act as medicine and cause little harm to the bod and can also add many substances with health benefits to food production, especially food for health protection that improving the aim of diversifying food products, improving economic value and encouraging local conservation of these natural resources.

This study was conducted to research some factors affecting on extraction of extracts from *eurycoma longifolia* jack root by distillation in water and establishing for of *eurycoma longifolia* jack root medicine extracts.

II. MATERIAL AND METHODS

2.1 Material

- The *eurycoma longifolia* jack tree (about 13 to 15 years old) were collected in the hilly areas of Ia Grai district, Gia Lai province, Viet Nam. Plant samples were identified by MSc. Nguyen The Anh (Institute of Chemistry - Vietnam Academy of Science and Technology).
- Pure distilled water.
- Analytical balance (Marcus, Germany);

- Vacuum evaporator (Heidolph, Germany);
- HPLC high-performance liquid chromatography system (Model: Hitachi, Japan), DAD detector, wavelength: 254 nm, column C8-250 mm;
- Atomic Absorption Spectrometer (Model: Zeenit, Germany)

2.2. Methods

Investigate the factors affecting the extraction of extracts from the distillation process in water

Based on the research results of CK Foong et al (2015) [5], Truong Thi Minh Hanh, Tran Y Doan Trang (2015) [6] combined with preliminary research, conducted a study on the influence of 3 factors: extraction temperature (^oC), extraction time (minute) and solvent/material ratio (mL/g).

The experimental tests setup are detailed as below:

Test 1: Investigate the effect of temperature on the extraction process of *eurycoma longifolia* roots. These tests were carried out with the parameters are shown in table 1.

Table 1. Test arrangement to investigate the temperature factor

Sample	1	2	3	4
Temperature (^oC)	70	80	90	100
Solvent/material ratio (mL/g)	20/1			
Extraction time (minute)	120			
Content EL4 (mg/kg)	Y_1	Y_2	Y_3	Y_4

Test 2: Investigate the effect of solvent/material ratio on the extraction of *eurycoma longifolia* roots. These tests were carried out with the parameters are shown in table 2.

Table 2. Test arrangement to investigate the solvent/material ratio factor

Sample	5	6	7	8
Solvent/material ratio (mL/g)	10/1	20/1	30/1	40/1
Extraction time (minute)	120			
Temperature (^oC)	Results from test 1			
Content EL4 (mg/kg)	Y_5	Y_6	Y_7	Y_8

Test 3: Investigate the effect of extraction time on the extraction process of *eurycoma longifolia* roots. These tests were carried out with the parameters are shown in table 3.

Table 3. Test arrangement to investigate the extraction time factor

Sample	9	10	11	12
Extraction time (minute)	60	90	120	150
Temperature (^oC)	Results from test 1			
Solvent/material ratio (mL/g)	Results from test 2			
Content EL4 (mg/kg)	Y_9	Y_{10}	Y_{11}	Y_{12}

The main substance selected for the investigation process is the active ingredient that accounts for a large proportion of the composition of the aqueous extract of the bile root and has valuable biological activity, substance 9,10-dimethoxy canthin-6-one (EL4) satisfies the above two conditions because it has a relatively high concentration in the root juice extract of the studied material and has antitoxic activity against HT-1080 blood cancer cells [7]. Therefore, the objective function of the survey process is the highest EL4 content.

Extraction method by distillation: *Eurycoma longifolia* jack root powder and water in appropriate proportions into a flask, install a condenser tube and simmer under different conditions, then filter out the residue with filter paper. Collect the filtrate and conduct vacuum evaporation to collect the extract.

Quantification of EL4 in a sample by HPLC is based on the principle: Determination for the peak area in the chromatogram at the time when the retention time of the sample coincides with the retention time of the EL4 standard. Then, from the standard curve, the relationship between concentration and peak area is deduced the content of EL4 substance [8].

Chromatographic conditions:

- Mobile phase: MeOH : H_2O = 70 % : 30 %
- Flow rate: 0.8 mL/min

- Injection sample volume: 5 micro
- Detector DAD, wavelength: 254 nm
- Column C8-250 mm
- Retention time: About 5 to 6 minutes

Product quality testing method

- Determination of heavy metal content according to AOAC 999.11;
- Determination of total aerobic microorganisms (TCVN 4884-1:2015);
- Determination of *Colifoms* (TCVN 6848:2007);
- Determination of *E.coli* (TCVN 7924-2:2008);
- Determination of total number of yeast and mold spores (TCVN) 8275-1:2010).

III. RESULTS AND DISCUSSION

3.1. Investigate some factors affecting the extraction process of *eurycoma longifolia* roots by distillation in water

3.1.1. Effect of temperature on *eurycoma longifolia* root extraction

The results of EL4 content are according to the extraction temperature are shown in figure 1.

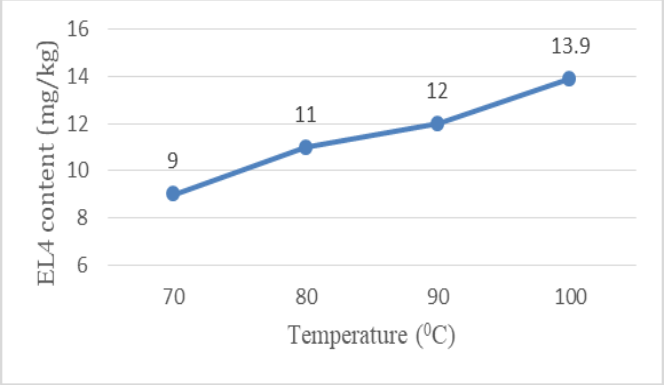

Fig 1. Effect of extraction temperature on EL4 content

The results in figure 1 are shown that, the extraction temperature were increased, the obtained EL4 content increased and reached the highest concentration at 100^{o}C. This is explained as follows: When the temperature has increased, the diffusion rate has increased, the viscosity of the solution has decreased, thus creating favorable conditions for the extraction process. At 100^{o}C is the boiling point of water, so the water molecules are more mobile, making the solvent's penetration into the material faster, combined with the effect of high temperature, it will help break the cell membrane of the raw materials thereby contributing to the easy diffusion of the components in the solvent. Result: Extraction temperature to obtain the highest EL4 content when extracting for 120 minutes, solvent/material ratio: 20/1 (mL/g) is 100^{o}C.

3.1.2. Effect of solvent/material ratio on the process of *eurycoma longifolia* roots extraction

The result of EL4 content are according to the solvent/material ratio are shown in figure 2.

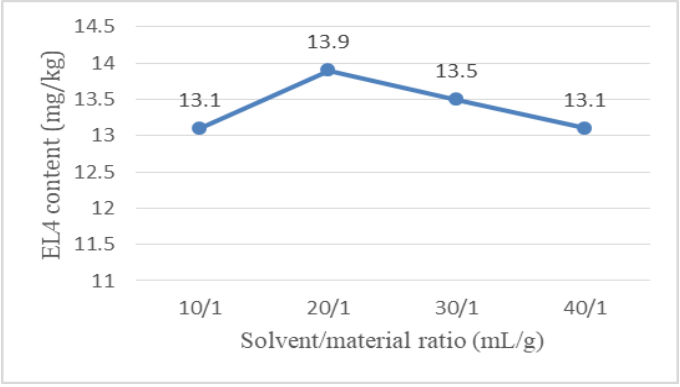

Fig 2. Effect of solvent/material ratio on EL4 content

The results in figure 2 are shown that, when increase the solvent/material ratio from 10/1 to 20/1 (mL/g), the EL4 content increases and reaches the highest concentration at the ratio 20/1 (mL/g). After that, when we continue to increase the solvent/material ratio, the EL4 content decreased. This is explained as follows: With the same amount of material, when the amount of solvent is different, the solubility of substances is different. The solvent used is water, so substances with higher polarity and better solubility in water will be extracted first. Therefore, at the ratio of 10/1 (mL/g), the amount of solvent is not enough to dissolve all the EL4 present in the material. When the ratio of solvent/ raw materials are increased, they will facilitate the dissolution of all components to be extracted and at the ratio of 20/1 (mL/g), the amount of solvent is just enough to dissolve all the components, so here the value is reached maximum value. Since then, when continuing to increase this ratio of lips/materials, the EL4 content does not increase. On the other hand, when the amount of solvent is high, the solvent evaporation time in the HPLC pre-concentration process is long, leading to the loss of EL4 to the solvent. Therefore, at the ratio of 30/1 and 40/1 (mL/g) the EL4 content decreased. Result: The appropriate solvent/material ratio to obtain the highest EL4 content at 100^{o}C for 120 minutes is 20/1 (mL/g).

3.1.3. Effect of time on process of *eurycoma longifolia* root extraction

The results of EL4 content with extraction time are shown in figure 3.

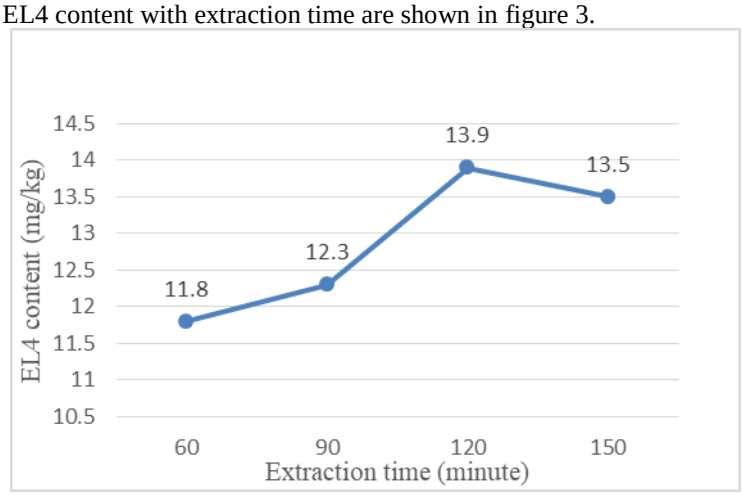

Fig 3. Effect of extraction time on EL4 content

The results in figure 3 are shown that, when increasing the extraction time, the EL4 content increases and reaches the highest concentration at 120 minutes. Then, when we continue to increase the extraction time to 150 minutes, the EL4 content tended to decrease. This is explained as follows: At the beginning of extraction, the substances with small molecular weight will be dissolved and diffused into the solvent first, then the substances with large molecular weight. Because EL4 compound is a large molecule alkaloid, during the first (60 to 90) minutes, mainly compounds with small molecules were extracted, so EL4 was not obtained much and the content increased slowly. In the next 30 minutes, when the small-molecule compounds were extracted, the diffusion rate of EL4 increased, leading to a rapid increase in the obtained concentration and reaches the highest concentration at 120 minutes. Thus, 120 minutes is enough time to extract all EL4 so when continue to prolong the time, there is no component to dissolve further. On the other hand, the prolongation of extraction time under the effect of high temperature makes the components likely to decompose, leading to a decrease in the content of components when increasing the extraction time to 150 minutes. Thus, the extraction for too long not only reduces the extraction efficiency for EL4 but also is not economically beneficial. The result shown that, the appropriate extraction time to obtain the highest EL4 content at 100^{o}C, solvent/material ratio: 20/1 (mL/g) in water is 120 minutes.

Extraction time to obtain the highest EL4 content at 100^{o}C, solvent/material ratio: 20/1 (mL/g) in water was 120 minutes to obtain EL4 content. The maximum of 9,10-dimethoxy canthin-6-one concentration was 13.9 mg/kg. According of CK Foong et al (2015), they showed that the ratio medium/material 20/1 (mL/g), at 90^{o}C for 90 minutes, the conditions are optimal, but at 120 minutes, it continues to increase [5]. Similarly, the study of Truong Thi Minh Hanh and Tran Y Doan Trang (2015), with the same experimental setup, presented that the highest extraction efficiency was achieved at 100^{o}C [6]. Compared with the survey results obtained in this study, we confirmed that at a temperature of 100^{o}C, the solvent/material ratio of 20/1 (mL/g) for 120 minutes not only extracted the EL4 content but also many other compounds.

3.2. Manufacture the *eurycoma longifolia* jack root medicine extracts

Currently, there are many herbal extracts that are sold quite popularly, their uses have also been studied and proven to be of high value to human health. The technological process of extracting extracts is generally the same in principle, however, for each type of material, each region has its own characteristics, so there are differences in the processing process.

In this study, we have researched and proposed the production process of *eurycoma longifolia* roots extract from selected material areas and evaluated the high quality of nectarine extract according to the technical requirements of the Vietnamese pharmacopoeia and in accordance with the requirements of the Vietnamese Pharmacopoeia. compliance with current regulations on food safety.

With the investigated conditions, propose the production process of *eurycoma longifolia* extracts according to as diagram below:

- Extraction: *Eurycoma longifolia* roots was put into a flask, fitted with a condenser tube system and extracted at a temperature of 100^{o}C for 120 minutes with a solvent/material ratio of 20/1 (mL/g).
  Filtration: The extract obtained after extraction is filtered by a vacuum filter to remove residue.
- Concentrate: The clear extract obtained after filtration is evaporated under vacuum at a temperature of 50^{o}C, carried out to Bx is about 20%. Then heat on a water bath to further concentrate to Bx is about 50 %.
- Heating: Gall bladder is heated on an electric stove to a temperature of 80^{o}C to eliminate gas [9], limiting the oxidation process and the growth of pathogenic microorganisms.
- Filling and capping: After heating, pour hot product and then close the cap immediately, to avoid the invasion of microorganisms.
- Pasteurization: Pasteurized at 80^{o}C for 10 minutes [9] to kill microorganisms harmful to human health, prolong product preservation time.

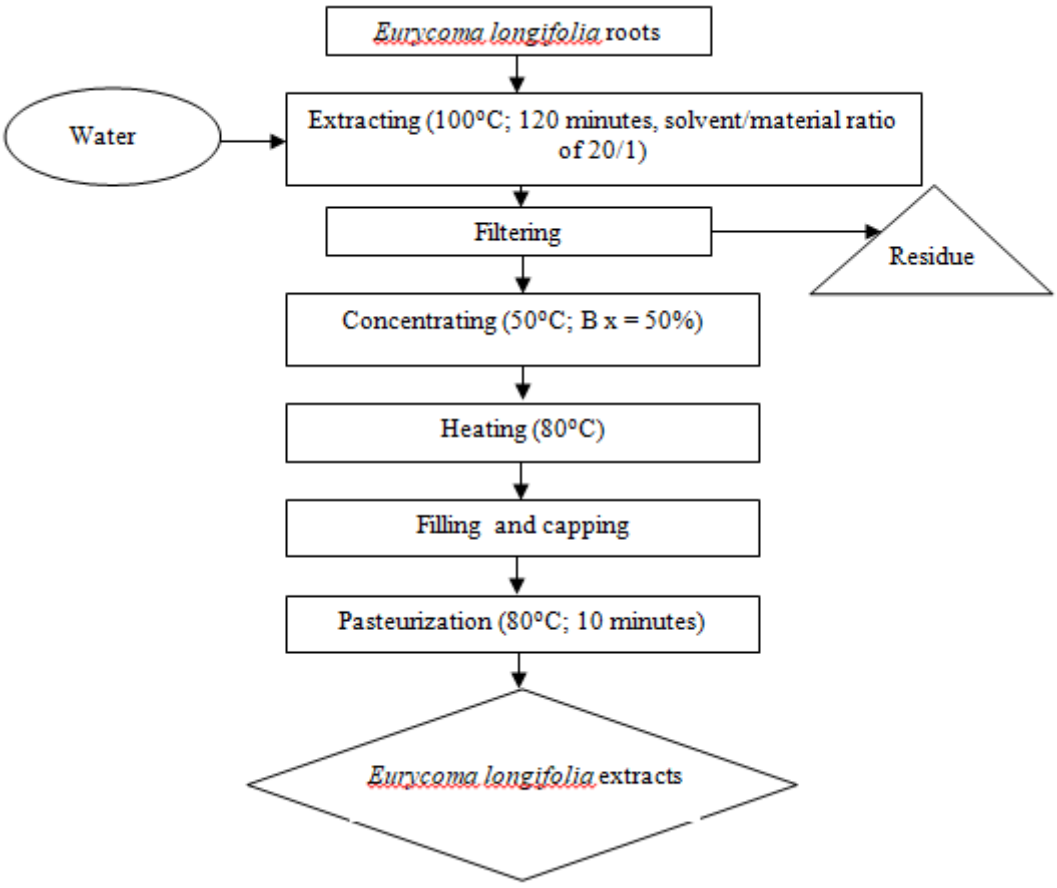

Fig 4. Diagram of *eurycoma longifolia* extracts process

3.3. *Eurycoma longifolia* extracts quality control

Quality control of *eurycoma longifolia* roots extracts are met the requirements of Viet Nam pharmacopoeia volume V.

Table 4. *Eurycoma longifolia* extracts quality control

Description	Liquid, viscous, homogeneous, dark brown, strong smell, characteristic of medicinal herbs, bitterness.
Insoluble in water residue	1.22 ± 0.05 %
Moiture	60.31 ± 0.04 %
Ash	4.56 ± 0.03 %
pH	5.68
Density d_{20}^{20}	1.2141
EL4 content	390 ± 7.07 mg/kg

The results of analysis of some natural compounds in the extract of *eurycoma longifolia* roots are shown in the table 5.

Table 5. Qualitative results of some natural compounds in *eurycoma longifolia* roots extracts

No.	Compound	Reagents	Description	Results	Conclusion
1.	Alkaloid	Wagner	Red-brown precipitate	+	appear
2.	Phenolic	$FeCl_3$ 1 % solution	Color solution became to dark blue	+	appear
3.	Steroid	Salkowski reaction	Color solution became to dark red	+	appear
		Lieberman-Bourchard reaction	Color solution became to orange	+	

The results from table 5 are shown, the presence of alkaloids, polyphenols, and steroids was found. These are all have been shown to have many biological activities. According to the study of Phan Quoc Kinh, alkaloids are organic substances found in plants that inhibit or stimulate the central nervous system, treat heart disease, high blood pressure, fight malaria, cancer,... [10]. Phenolics are aromatic secondary metabolites that give color and taste and have health effects such as a reduced risk of cardiovascular disease [11]. Furthermore, according to the study of Alyu et al (2009), phenolic compounds account for the majority of antioxidants in plants [12]. Steroids are fat-soluble organic compounds of natural or synthetic origin. Steroids are included in many important drugs and dietary supplements used in the treatment of musculoskeletal disorders and hypogonadism and infertility [10].

Previous studies, when isolated the chemical composition of bile, also identified the presence of these three compounds and demonstrated many of their biological activities. The study of Ping-Chung Kuo et al. reported that alkaloids present in *eurycoma longifolia* roots have significant cytotoxic activity against human lung cancer cell lines (A-549) and human lung cancer cell lines human breast (MCF-7) [13]. Anti-inflammatory activity and antimalarial ability were also reported by Pham Bich Ngoc et al [14] and Leonadus B. S. Kardono et al [15]. The study of Oei–Koch et al have determined the presence of plant sterols campesterol, stigmasterol and sitosterol in *eurycoma longifolia* roots [16] and the study of Ping-Chung Kuo (2004) has demonstrated the possibility anti-lung cancer of stigmasterol [13].

Product quality testing results

Table 6. Results of food hygiene and safety testing

No.	Test properties	Unit	Specification	Test results	Conclusion
1.	Total aerobic microorganisms	CFU/mL	$<10^4$ (*)	20	Pass
2.	*Coliforms*	CFU/mL	<10 (*)	Not detectable	Pass
3.	*E. coli*	CFU/mL	0 (*)	Not detectable	Pass
4.	Total number of yeast and mold	CFU/mL	10^2 (*)	Not detectable	Pass
5.	*Salmonella*	CFU/mL	0 (*)	Negative/25 mL	Pass
6.	Pb	mg/L	<10 (**)	< 0.05 (MQL)	Pass

7.	Cd	mg/L	<0,3 (**)	< 0.05 (MQL)	Pass

Note: MQL: Quantitative limit of the method; (*): QCVN 8-3:2011/BYT – National technical regulation on microbial contamination limits in food of Viet Nam; (**): QCVN 8-2:2011/BYT – National technical regulation on limits of heavy metal contamination in food of Viet Nam.

The results from table 6 are shown, *eurycoma longifolia* roots extracts are met the standards of food safety and hygiene according to QCVN 8-3:2011/BYT – National technical regulation on microbial contamination limits in food of Viet Nam; QCVN 8-2:2011/BYT – National technical regulation on limits of heavy metal contamination in food of Viet Nam.

Product quality publication

Submit Product quality publication to specialized State management agencies for approval, specifically, submit it to the Food Safety Department - Ministry of Health in Viet Nam.

IV. CONCLUSION

The best condition of *Eurycoma longifolia* root by distillation in water: Temperature at 100^{o}C, in 120 minutes with the ratio between solvent and material is 20/1 (mL/g) for the highest 9,10-dimethoxy canthin-6-one concentration of 13.9 mg/kg.

Quality of *Eurycoma longifolia* roots extracts are met the requirements of Vietnam Pharmacopoeia with the results: Liquid, viscous, homogenous, dark brown in color, strong in smell, and dense. characteristic of medicinal herbs, bitter taste; bite insoluble in water 1.22 ± 0.05%; moisture content 60.31 ± 0.04%; total ash 4.56 ± 0.03%; 5.68; the relative density at 20^{o}C is 1.2141; content of 9,10-dimethoxycathin-6-one 390 ± 7.07 mg/kg. The qualitative results of biologically active compounds showed the presence of alkaloids, steroids, polyphenols, the product ensures food safety in terms of microbiological criteria and heavy metal content according to the current regulations.

Acknowledgements

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