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RESEARCH OF FATTY ACIDS, TOCOPHEROLS AND STEROLS OF SEED OILS EXTRACTED FROM *PACHYRHIZUS EROSUS* (L. URB.) IN VIET NAM

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Abstract. According to the research result, it can be seen that the seed oil of yam-bean (*Pachyrhizus erosus* (L.) Urb.) contains all basic substance layers of lipid: polar lipid (PL), sterol (ST), triacylglycerol (TG), and free fatty acid (FFA). Among them, triacylglycerol occupied the highest content (about 65.09 %) whereas the figures for polar lipid and free fatty acid were also relatively high. In the studied seed samples, the highest content of linoleic acid achieved around 32.37 %. There are no published results up to date about the content of fatty acid, tocopherol (with γ-T of 59.73 mg/kg), sterol (with β-Sitosterol of 973.36 mg/kg) of yam-bean seed oil in Viet Nam.

Keywords: lipid, saturated fatty acid, unsaturated fatty acid, seed oil, Fabaceae family.

Classification numbers: 1.1.3, 1.4.1.

1. INTRODUCTION

The scientific name of Yam-bean plant which is also called Mexican yam bean or jicama (according to south Viet Nam) is *Pachyrhizus erosus* (L.) Urb. It is one kind of the liana trees originated from Mexico and Mid-America. Yam-bean plant belongs to *Pachyrhizus* genus in the bean family (Fabaceae). This plant was imported into Viet Nam and grown popularly in several provinces such as Bac Giang, Phu Tho and western provinces. It adapted to the hot and wet tropical climate of Viet Nam. It usually blooms from June to August while the tuber root is harvested from September to November annually. The tuber root with sweet and fresh taste is used as food whereas its leaf and seed are applied by our ancestors to eliminate the pest, treat scabies and dermatology, etc. [1]. However, the jicama seed itself is completely "drug store" if

we develop research and understand about it. In this paper, we study about lipid, fatty acid, tocopherol, and phenolic of the yam-bean seed in Viet Nam.

2. MATERIALS AND METHOD

2.1. Materials

The seed samples were harvested in November 2019 in Phu Tho province and identified for the scientific name by Dr. Nguyen Quoc Binh,Vietnam National Museum of Nature. The voucher specimen was stored in Institute of Natural Products Chemistry. The seed samples were cleaned and dried at temperature of 40 - 50 ^{o}C.

2.2. Research method

2.2.1. Extraction method of total lipid

The fatty oil of seed samples was extracted and determined of the total lipid content according to the ISO/DIS 659:1988 method of Germany [2]. 10 gram of the seed sample was selected according to the standard which then was ground into flour by the mill before being extracted by n-hexane (the ratio of sample : solvent was 1:10 v/v) with the support of ultrasonic in 6 hours. Then, the solvent of collected extracts was evaporated completely by using the vacuum rotary at 40 ^{o}C, pressure of 25 tors before weighing the mass by Sartorius analysis scale. The collected fatty oil content was calculated following the percentage of gained oil mass comparing with the initial dried sample.

2.2.2. Determination of the composition and content of the substance layers of total lipid

The total lipid was covered on a thin plate (6 cm × 6 cm), 3 tracks corresponding to 3 volumes of 5 µg 10 µg; 15 µg at the same concentration, after that: they were subjected to chromatography using solvent system n-hexane/diethyl ether/acid acetic = 85/15/1 (v/v/v) until reached to the thin plate height of 95 %. Then the plates were dried before running the chromatography with the second solvent system $CHCl_3$/MeOH/C_6H_6/28%NH_4OH = 65/30/10/6 (v/v/v/v) to reach 1/6 heigh of the thin ones. Afterward, they were dried at room atmosphere and visualized using 10 % H_2SO_4/ MeOH as reagent at 240 ^{o}C in 10 minutes. The chromatograms were scanned by picture scanning (Epson Perfection 2400 PHOTO) with the grey standard. Determining the composition of the substance layers of lipid by comparing R_f of the lipid component layers of the samples with the standard ones at the same TLC chromatography condition. The content of lipid's component layers were calculated using the image analysis software (Sorbfil TLC Videodensitometer, Krasnodar, Russia).

2.2.3. Method to measure the composition and content of fatty acid

The composition and content of fatty acid were measured according to the method of ISO/DIS5509:1997 (International Organisation for Standardisation/Final draft) [3]: 100 mg fatty oil was dissolved in 1 ml n-hexane, then added with 50 µl $NaOCH_3$/CH_3OH, stirred carefully for 1 minute before adding with 100 µl H_2O and centrifuged at speed of 5000 rpm. After that, 50 µl HCl was added to the solution to collect the two-phase mixture. Eliminating the below filtrate to gain the rest and anhydrous using Na_2SO_4. The fatty oil samples that was in the form of methyl ester was moved to sample tube to analyze by Hewlett Packard instrument Model 5890 Series II,

CP-Sil 88 (specialized capillary column CP-Sil 88, 100 mm/0.25 mm/0.25 mm with the standard substance system C16:0,C18:0).

Temperature program: temperature: 155 - 220 ^{o}C (1.5 ^{o}C/min), speed: 10 ^{o}C/ min, 260 ^{o}C/5 minutes ; split:1:50; injector 250 ^{o}C, detector 250 ^{o}C, bearing gas of 36 cm/s hydrogen, detector gas of 30 ml/min hydrogen. 300 ml/min air and 30 ml/min nitrogen, pumping automatically sample with the volume of 0.9 µl. Using the library of spectrum - standard compounds to identify the fatty acids *via* specialized software and then calculating to convert them to the value of equivalent keeping time ELC (Equivalent Chain- lengths of methyl ester derivatives of fatty acids) for specialized capillary column CP-Sil 88 that using the standard compound of C16:0, C18:0 on C-R3A machine following the equation:

$$ECL = 16 + \frac{2(lgRT_x - lgRT_{16:0})}{lgRT_{18:0} - lgRT_{16:0}} \quad (1)$$

2.2.4. Determination of composition and content of tocopherol

The content and composition of tocopherol were determined according to the ISO/9936:2006 method [4].

Experiment: 70-100 mg of fatty oil was dissolved in 100 µl heptane before taking 20 µl of this solution to analyze on high performance liquid chromatography (HPLC) of Merck-Hitachi F-1000 Fluorescence Spectrophotometer, 295/330 nm, D-2500. The samples were automatically pumped in the sample pumping chamber of Merck 655-A40, column of 25 cm × 4.6 mm ID, speed: 1.3 ml/min, mobile phase system using heptane/tert-butyl : methyl ether (99/1,v/v), Chromato integrator, running solvent system heptane/tert-butyl: methyl ether (99/1,v/v). The mobile phase was water:methanol: 2-propanol (50:45:5) with the current speed of 0.6 ml/min.

2.2.5. Determination of sterol composition and content

The phytosterol composition of *Dalbergia tonkinensis Prain* seed was measured following to ISO/FIDS 12228:1999 [5].

Experiment: 150 mg of lipid was dissolved in 100 ml ethanol before saponified with the potassium hydroxide solution at 70^{o}C. The collected mixture was passed through an aluminum oxide column (Merck, Darmstadt, Germany) to separate the sterol and retain the fatty acids. The sterol part separated from column was purified by using thin layer chromatography (Merck, Darmstadt, Germany), then used betulin as internal standard compound to determine the composition and content of sterol by GLC. The compounds were isolated on SE 54 CB column (length of 50 m, 0.25 mm ID, the thickness of thin layer was 0.25 µm) (Macherey-Nagel, Düren, Germany).

3. RESULTS AND DISCUSSION

3.1. The total lipid content of seed sample

The content of lipid was measured by the percentage ratio of the lipid weight total and the mass of died seed. The fatty oil content of yam-bean seed achieved 20.91 %, similar to the results of other objects such as soy-bean oil (22.1 %), or *Vernicia montana* oil (25.1 %) [6]

which was higher than that of *Dalbergia tonkinensis Prain* seed oil and (13.64 %), or *Entada phaseoloides (L.) Merr*. seed oil (2.69 %) [1].

3.2. Composition and content of the lipid substance layers

According to the achieved result in Table 1 and Figure 1, the total lipid of bean seed samples contained the basic compound layers of lipid including polar lipid (PL), sterol (ST), triacylglycerol (TG), free fatty acid (FFA). The content of triacylglycerol was the highest with 65.09 % which was similar to the data for *Dalbergia mammosa* seed oil 69.51 %), *Afzelia xylocarpa* seed oil (63.94 %) [7], or *Madhuca pasquieri* seed oil (63.2 %) [8].

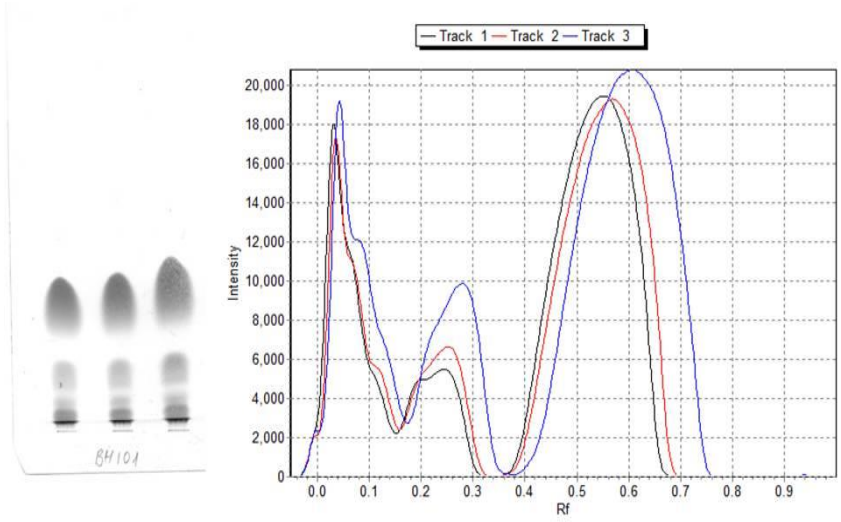

Figure 1. TLC graph and chromatogram result for calculation of lipid content of compound layers of yam-bean seed oil.

Table 1. Composition and content results of lipid compound layers of jimica seed oil.

No	Name of compound layer	Symbol of compound layer	Content %
1	Polar Lipid	PL	17.88
2	Sterol	ST	3.46
3	Free fatty acid	FFA	13.57
4	Triacylglycerol	TG	65.09
5	**Total**		**100**

3.3. Composition and content of fatty acids

According to Table 2, all investigated samples contained saturated fatty acids commonly presented in seed oil: Palmitic acid (C16:0), stearic acid (C18:0), arachidic acid (C20:0). The references showed that the content total of saturated fatty acid was high leading to the increase in the cholesterol content of blood which caused higher risk for cardiovascular disease and

arteriosclerosis [7]. The saturated fatty acid content for yam-bean seed oil achieved around 37.73 % which reached average result, corresponding to the results for lime bean oil (35.51 %) [9], or sunflower seed oil and soy-bean oil, etc.[10]. The unsaturated fatty acids found mainly in the oil of yam-bean seed were oleic acid (C18: 1n-9), linoleic acid (C18: 2n-6) or α-linolenic acid (C18: n-3), etc. Meanwhile, linoleic acid (ω-6) is an essential acid for health which can not be synthesized in body. This acid contents of yam-bean seed were relatively high (32.37 %) approximating to the content for soy-bean, sunflower, corn oil (average of 50 %) [11]. This acid helps to prevent the cardiovascular disease and decreases the pressure blood [12]. The content of oleic acid occupied around 25.74 % which was near the studied result for soybean oil (29.66 %) [9]. This acid is a necessary component in the human regimen which also has positive effects on the health of cardiovascular system, circulation system. Its impact was previously studied by scientists, especially the effect on the decrease in cholesterol in blood [13]. The content of such unsaturated fatty acid can be a potentially high nutrition source.

Table 2. Fatty acid composition and content of yam-bean seed oil.

No.	Fatty acid	Content %
1	14:0	0.64 ± 0.05
2	16:0	28.11 ± 0.05
3	16:1(n-9)	0.13 ± 0.04
4	17:0	0.19 ±0.04
5	18:0	5.78 ± 0.05
6	18:1(n-7)	0.06 ± 0.04
7	18:1(n-9)	25.74 ± 0.03
8	18:2(n-6)	32.37 ± 0.01
9	18:3(n-3)	0.79 ± 0.04
10	20:0	0.99 ± 0.04
11	20:1(n-11)	0.4 ± 0.02
12	20:2(n-6)	0.03 ± 0.03
13	22:0	2.02 ± 0.04
14	22:2(n-6)	0.09 ± 0.05
15	Squalen	0
16	Others	2.66 ± 0.05
Saturated		**37.73 ± 0.03**
Unsaturated fatty acid		**59.61 ± 0.02**

(Average values ± of standard deviation of repeated times)

3.4. Composition and content of tocopherol

From Table 3, it can be seen that the content of tocopherol of yam-bean seed oil is 62.87 mg/kg, which exists in 5 types: α-T, β-T, γ-T, P8, δ-T. Although the content of total Vitamin E is not high, the composition contains mainly γ-T with 59.73 mg/kg that is much higher than that of some other bean seed oil like Glycine soya (3.94 mg/kg), lima bean (19.96 mg/kg), *Vigna unguiculata* (12.74 mg/kg), etc. [9].

Recent studies also showed that γ-tocopherol can prevent cancer as well as heart attack better than α-tocopherol [14], so this is a basis to continue further research on the impact of yam-bean seed oil on medicine and life.

Table 3. Composition and content of tocopherol (Vitamin E).

No	Tocopherol composition	Content (mg/kg)
1	α-T	1.13 ± 0.05
2	β-T	0.36 ± 0.05
3	γ-T	59.73 ± 0.01
4	P8	1.28 ± 0.04
5	δ-T	0.38 ± 0.05
Total		**62.87 ± 0.01**

3.5. Composition and content of sterol of yam-bean seed oil

Table 4. Composition and content of sterol.

No	Composition of sterol	Content %
1	Cholesterol	5.25 ± 0.01
2	Brassicasterol	11.76±0.01
3	2,4-methylenecholesterol	1.57 ±0.02
4	Campesterol	133.73 ± 0.02
5	Campestanol	5.94 ±0.05
6	Stigmasterol	418,22 ± 0,01
7	Δ7-camersterol	11.02 ± 0.04
8	$\Delta^{5,23}$-stigmastadienol	30.16 ±0.05
9	Chlerosterol	7.41 ±0.02
10	β-sitosterol	973.36 ± 0.05
11	Sitostanol	40.66 ± 0.02
12	Δ^{5}-Avenasterol	38.83± 0.04
13	$\Delta^{5,24}$-Stigmastadienol	8.09 ± 0.02
14	Δ^{7}-Stigmastenol	10.95 ± 0.05
15	Δ^{7}-Avenastenol	5.30 ± 0.04
Total		**1702.24 ± 0.03**

According to Table 4, the yam-bean seed oil contains some types of typical sterol of plant oil such as campesterol, stigmasterol, β-sitosterol and sitostanol. Therein, the highest content belongs to β-sitosterol with 973.36 mg/kg. Several researches indicated the impact of β-

Sitosterol to: decline the content of blood cholesterol, antifungus, prevent hyperlipidemia, inhibit carcinogenic process [15, 16] and decrease the glucose concentration of hemoglobin and blood glucose while the insulin content increases.

4. CONCLUSION

For the first time, this research provides data about the composition and content of the compound layers of total lipid, fatty acid components, tocopherol, and sterol of oil from yam-bean seed oil which is grown in Viet Nam. In these studied seed oil samples, linoleic acid, γ-tocopherol, and β-sitosterol are the main components which occupy high content. Thus, this is an important basis for further research on lipid of yam-bean seed oil.

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