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GC-MS Analysis the Fatty Acid Components of *Tamarindus indica S*eeds in Vietnam

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ABSTRACT

Tamarindus indica is an edible plant, especially its fruits were wildly used as food or herbs in Vietnam. The seed of *Tamarindus indica* has been already studied for its phytochemistry and some bioactivities, but there still have limited publications about the fatty acid part of this material. The dried seed (8 kg) of *T. indica* was extracted with methanol to yield a methanol crude extract (1 kg) and partitioned successively with *n*-hexane, chloroform, ethyl acetate to obtain *n*-hexane (0.25 g), chloroform (15 g), ethyl acetate (70 g) and water (700 g) extracts, respectively**.** Then, seven sub-fractions were prepared from ethyl acetate extract and labelled as TI-A to TI-G. The fatty acids in the TI-A fraction were hydrolyzed to make derivatives and identified by GC-MS equipment. There are seven fatty acids, both saturated and unsaturated acids, myristic acid, palmitic acid, stearic acid, isoarachidic acid, docosanoic acid, linoleic acid (54.17%), and oleic acid (20.32%) were analyzed from the high polarity fractions of ethyl acetate extracts. This is the first public about the fatty acids from *T. indica* seed collected in Vietnam and analyzed by gas chromatography – molecular spectrometric method.

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1. Introduction

Fabaceae is a big botanical family with approximately 500 genus and nearly 1200 species. *Tamarindus indica* is a tropical plant which origins in Africa, it is planted popular around the world [1]. It is distributed wildly in some countries in Asia such as India, Thailand, Bangladesh, Sri Lanka and Indonesia. In Vietnam, *T. indica* is found in provinces such as Ha Giang, Tuyen Quang, Vinh Phuc, Quang Ninh, Hoa Binh, Ninh Binh and southern provinces.

There are many benefits to using this plant for medical purposes. *T. indica* fruit has been employed to treat different diseases such as digestion disorders, rheumatism, allergy, constipation, and fever [2]. There are many studies about *T. indica* bioactivities in other parts, the antioxidant activities of 2-hydroxy-3',4'- dihydroxyacetophenone; methyl 3,4-dihydroxybenzoate; 3,4- dihydroxyphenylacetate, epicatechin, and acid linoleic were isolated from this plant [3], the polysaccharide reduces cholesterol *in vivo* model [2], the extraction of tamarind seed polysaccharide also increases the inflammation effect of NSAIDs with *in vivo* model [4]. *T. indica* also investigated its application on biomedical material in pichlorohydrin cross-linked mucoadhesive patches [5], colon-specific drug delivery using biodegradable carriers [6]. Tamarind seed polysaccharide is an effective solid pharmaceutical excipient. Furthermore, extracted seed polysaccharide has been shown to be a natural gelling agent in pharmaceutical formulations [7]. In Vietnam, the research group of Bui Ngoc Tan also published the antioxidant activity, anticoagulation of tamarind seed polysaccharides and its sulfated and acetylated derivatives [8]. The ethanol extract from tamarin seeds also exhibited high IC_{50} (6.91 µg/mL) in DPPH model, as well as 113,8 µg/mL in the H_2O_2 inhibition activity [9].

By-products of tamarind fruits include seeds such as coats or testa and kernels or endosperms. Tamarind seed contains protein, crude fiber, carbohydrates, and fatty acids, among its many nutrients. Among the phenolic compounds found in tamarind, coat seed is oligomeric procyanidin tetramer,

oligomeric procyanidin hexamer, oligomeric procyanidin trimer, oligomeric procyanidin pentamer, with lower amounts of procyanidin B2 and (-)-epicatechin [10]. Another study displayed coat seeds containing 2-hydroxy-3',4'-dihydroxy-acetophenone, methyl-3,4-dihydroxybenzoate, 3,4-dihydroxy phenylacetate, and (-)-epicatechin [11]. The primary component fatty acids in the tamarind seed oil are linoleic, palmitic, oleic, and stearic acids [12]. Tamarin seeds were still being isolated from Vietnam, and the components of their fatty acids were not widely reported. By using by-products of the food industry, if the fatty acids are of high quality and quantity, there is a new economic approach for efficacy.

2. Materials and Methods

2.1. Plant Materials and Chemicals

Kennel seeds of plant materials were collected in Ho Chi Minh city, separated the outer layer to collect seeds in August 2018. After collecting the seeds, 8 kg of *T. indica* seeds were ground and rinsed at 60 ^{o}C and yielded powder.

Acetyl acetate, *n*-hexane, methanol, chloroform, and acetone were purchased from Chemsol (Vietnam), Milli-Q deionized water was used (Millipore, Billerica, MA,). Silica gel normal phase was purchased from Merck (Germany).

2.2. Extraction and Fractionated T.indica seeds

The seed powder of T. indica (8 Kg) was soaked in 8 L of methanol three times. The methanol extract was evaporated under pressure to yield crude extract (1 kg). Then, the crude extracted was dissolved in water to partitioned with solvents to collect n-hexane, chloroform, ethyl acetate, and water extracts and evaporate solvents to yield similar partitioned extracts.

The ethyl acetate extracts were used to further fractionate by its most promising activity on DPPH experiment. Then, the ethyl acetate extract was subjected to a normal-phase column and run with a mobile phase with the increasing ratio of *n*-hexane and ethyl acetate (9:1 -0:10). After running the chromatography column, seven sub-fractions were collected by TLC testing and labelled as TI-A to TI-G.

2.3. GC-MS applied method

Tamarind seed oil was transformed using a methanol boron trifluoride catalyst (MeOH/BF3). A fatty acids sample was hydrolyzed with 1 mL of 1 M KOH in 70% ethanol (v/v) at 90°C for one hour in a screw-capped glass tube. Acidified by adding 0.2 mL of 6 M HCl and then adding 1 mL of water, the reaction mixture was then diluted with water. Then, the fatty acids released were extracted with 1 mL of n-hexane. A solution of 10% BF3 in methanol was used to methylate the fatty acids after evaporation of the hexane in vacuo. After one mL of hexane had been added to the solution, the fatty acid methyl esters were extracted with 1 mL of water.

The composition of methyl esters of fatty acids was analyzed using gas chromatography-mass spectrometry (GC-MS). This study used an Agilent 6890N GC-MS system equipped with an HP-5 MS column (30 m x 0.25 mm). Helium was the carrier gas with a 1.0 mL/min total flow rate. It was injected at 300 oC, and the oven temperature was set as follows: Initial temperature was 50 oC, held for 4 minutes, then increased to 290 oC, and maintained for 8 minutes. Mass spectra were obtained at 70 eV. The fragmentation analysis was performed on each peak to interpret these spectra, and there were confirmations and comparisons by Library.Wiley229.LIB.

3. Results and Discussion

3.1. The partitioned and fractioned extracts of T. indica

After collecting and evaporating all the solvents, the total weight of solvent extracts was calculated as extraction yield, represented in Table 1.

The extraction yield of powder of T. indica seed after extraction with methanol and partition with ethyl acetate, chloroform, and n-hexane. The solvent extracts displayed low percentages of compounds in the seed of this material (ethyl acetate extract (0.9%), chloroform extract (0.2%), and *n*-hexane extract

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(0.1%), whereas water extract comprised the highest percentage (11.2%). It is entirely reasonable because carbohydrates are the most component in kennel seed overall. [13]

Table 1. *Weight and extraction yield of extraction and solvent extracts of seed of T. indica*

No	Extracts	Weight (g)	Extraction Yield (%)
1	Crude material	8000	-
2	Methanol Crude Extract	1000	12.5
3	Hexane extract	0.25	<0.1%
3	Chloroform Extract	15	0.2
4	Ethyl acetate Extract	70	0.9
5	Water Extract	700	11.2

Table 2. *Weight of fractions from ethyl acetate layer of T. indica seed*

No	Fractions	Weight (g)
1	TI-A	31.8
2	TI-B	5.34
3	TI-C	10.47
3	TI-D	3.94
4	TI-E	8.54
5	TI-F	4.16
6	TI-G	2.02

Since the ethyl acetate extract exhibited the best antioxidant activity in the previous study, it was used to fractionate further. By deploying column chromatography normal phase, seven fractions (TI-A to TI-G) were collected and grouped using thin layer chromatography (Table 2). The TI-A (31.8 g) comprised nearly 50% of the total amount weight of ethyl acetate extracts. Therefore, the compounds in this fraction might be the main components of ethyl acetate extracts. In this study, the oil part of TI-A was investigated for its components containing fatty acids.

3.2. Fat acid composition identified by gas chromatography-mass spectrometry

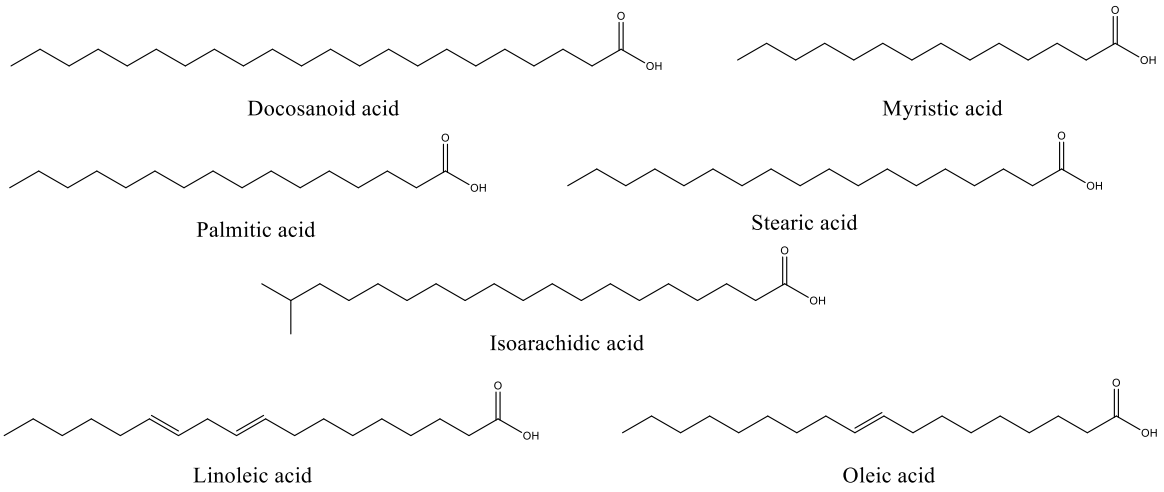

Figure 1. *Fatty acid structures from ethyl acetate fraction of T. indica seed identified by GC-MS*

The saturated and unsaturated fatty acids in TI-A fraction of ethyl acetate extract (Figure 1) were detected and analyzed by GC-MS. The retention times, the content of oil in fraction, as well as the

molecular weights of seven fatty acids, are also exhibited in Table 3. In there, five saturated acids comprised myristic acid, palmitic acid, stearic acid, isoarachidic acid, docosanoic acid, and two unsaturated acids, oleic acid and linoleic acid. In the whole oil extracted, linoleic acid (54.17%) was a predominant unsaturated fatty acid; it is completely relevant to previous publications [14]. All fatty acids were detected in one study on the seeds of *T. indica* from Indonesia, with the exception of isoarachidic acid (0.86%) [14]. As a result, the GC-MS method can be applied to distinguish the originals of *T. indica* seed.

Table 3. *The fatty acids components from TI-A analysis by GC-MS method.*

N₀	Fatty acid	*m/z*	Retention time	Content of oil (%)
1	Myristic acid $C_{14}H_{28}O_2$	228.37	28.37	0.54
2	Palmitic acid $C_{16}H_{32}O_2$	256.42	32.55	13.43
3	Stearic acid $C_{18}H_{36}O_2$	248.5	36.35	3.88
4	Isorachidic acid $C_{20}H_{40}O_2$	312.5	39.84	0.86
5	Docosanoic acid $C_{22}H_{44}O_2$	340.6	43.07	0.77
6	Linoleic acid $C_{18}H_{32}O_2$	280.4	35.77	54.17
7	Oleic acid $C_{18}H_{32}O_2$	282.5	35.87	20.32

It has been shown that fatty acids have broad antibacterial properties, long-chain unsaturated fatty acids, including linoleic acid, palmitoleic acid, oleic acid, linolenic acid, and arachidonic acid, have antibacterial properties through inhibition of bacterial enoyl-acyl carrier protein reductase (FabI). But the antibacterial activity of saturated has not been shown clearly [15]. Antibacterial activity of linoleic acid is observed against *Bacillus cereus*, *Bacillus pumilus*, *B. subtilis*, *Micrococcus kristinae*, and *Staphylococcus aureus* with MIC values ranging from 0.01 to 1.0 mg/mL. [16]. Only short-carbon chain molecules possess antitumor properties in saturated fatty acids. The anticancer activity of saturated FAs with carbon chains longer than C10 has not been reported. As the carbon chain in the molecule elongates and the unsaturation level increases, so does unsaturated FA activity [17]. Linoleic acid is the most component in fraction TI-A exhibited its anti-proliferation of different cancer cell lines, which were KPL-1, Caco-2, BT-474, A-549, MAC16, DU145 [18-22]. Besides, oleic acid (20.32%) also investigated its bioactivity on prostatic cancer, lung cancer, breast cancer, and liver cancer [23] – [26]. Therefore, the bioactivity of the oil fraction extract from the ethyl acetate layer of *T. indica* seed should be studied well soon.

4. Conclusions

From the most promising antioxidant solvent layers, the fatty acids in the lowest polarity part of *T. indica* were fractionated. After, they were hydrolyzed to create derivatives and the GC-MS method to identify the fatty acid components. The unsaturated fatty acid (oleic acid, 20.13% and linoleic acid 54.16%) was the predominant fatty acid in the tamarin seed collected in Vietnam. The result was different from the *T. indica* seeds collected in Indonesia, in which saturated acids were the major components in the oil part. As the result of the antioxidant activities of tamarin seeds, the fraction of the fatty acids will be tested in the same model and optimized the extraction procedure for efficient use of by-products in the food industry.

REFERENCES

[1] S.S. Bhadoriya, et al., "Tamarindus indica: Extent of explored potential," Pharmacognosy Review, vol. 5, no. 9, pp 73-81, Jan. 2011
[2] A. P. Landi Librandi et al., "Effect of the extract of the tamarind (Tamarindus indica) fruit on the complement system: studies in vitro and in hamsters submitted to a cholesterol-enriched diet," *Food Chemistry Toxicology*, vol. 45, no. 8, pp 1487-1495, Aug. 2007, doi: 10.1016/j.fct.2007.02.008.
[3] T. Tsuda *et al*., "Antioxidative components isolated from the seed of tamarind (Tamarindus indica L.)," *Journal of Agricultural and Food Chemistry*, vol. 42, pp. 2671-2674, Dec. 1994.
[4] A. A. Suralkar, *et al*., "Evaluation of anti-inflammatory and analgesic activities of Tamarindus indica seeds," *International Journal of Pharmaceutical Sciences and Drug Research*, vol. 4, pp. 213-217, 2012.
[4] S. Jana, D. Lakshman, K. K. Sen, and S. K. Basu, "Development and evaluation of epichlorohydrin cross-linked mucoadhesive patches of tamarind seed polysaccharide for buccal application," *International Journal of Pharmaceutical Sciences and Drug Research*, vol. 2, pp. 193-198, 2010.
[5] M. Mishra, J. J. Khandare, "Evaluation of tamarind seed polysaccharide as a biodegradable carrier for colon specific drug delivery," *International Journal of Pharmacy and Pharmaceutical Sciences*, vol. 3, pp. 139-142, Nov. 2010

[6] R. Singh, R. Malviya, P. Sharma, "Extraction and Characterization of Tamarind Seed Polysaccharide as a Pharmaceutical Excipient," *International Journal of Pharmacy and Pharmaceutical Sciences*, vol. 3, pp. 17-19, 2011
[7] T. N. Bui, "Nghiên cứu thành phần hóa học và khảo sát hoạt tính sinh học của polysaccharide từ hạt me Tamarindus indica L," Ph.D. dissertation, Dept. Org. Chem., Hanoi Univ. of Scien. and Tech., Ha Noi, Vietnam, 2016.
[8] P. T. A. Dao, at al., "Screening on Antioxidant Activities of By-Products from Vegetables and Fruits in Tay Nguyen Region and Applying for Shrimp Cold Storage," in *3rd International Conference on Green Technology and Sustainable Development (GTSD)*, Taipei, Taiwan, 2016, pp. 93-97.
[9] Y. Sudjaroen et al., "Isolation and structure elucidation of phenolicantioxidants from Tamarind (Tamarindus indica L.) seeds and pericarp," *Food Chemistry Toxicology*, vol. 43, pp. 1673-1682, 2015
[10] T. Tsuda, *et al*., "Antioxidative components isolated from the seed of Tamarind (Tamarindus indica L.)" *Journal of Agricultural and Food Chemistry*, vol. 42, pp. 2671-2674, 1994
[11] A.A. Ajayi, R.A. Oderinde, D.O. Kajogbola, J.I. Uponi, "Oil content and fatty acids composition of some underutilized legumes from Nigeria," *Food Chemistry*, vol. 99, no 1, pp. 115-120, 2006.
[12] M. Aguirre *et al*., "Carbohydrate reverses and seed development: an overview", *Plant Reproducttion*, vol. 31, no. 3, pp. 263-290, May 2018.
[13] Sutrisno *et al*., "Fatty Acids in Tamarindus indica L. Seeds Oil and Antibacterial Activity Assay," *Key Engineering Materials*, vol. 811, pp. 40-46, Mar. 2019.
[14] C.J. Zheng, *et al*., "Fatty acid synthesis is a target for antibacterial activity of unsaturated fatty acids," *FEBS Letters*, vol. 57, pp. 5157-5162, 2005.
[15] F. Dilika, P.D. Bremner, and J.J.M. Meyer, "Antibacterial activity of linoleic and oleic acids isolated from Helichrysum pedunculatum: a plant used during circumcision rites," *Fitoterapia*, vol. 71, pp 450- 452, 2000.
[16] M. Jóźwiak, *et al*., "Anticancer activities of fatty acids and their heterocyclic derivatives," *European Journal of Pharmacology*, vol. 871, 2020.
[17] H. Senzaki, *et al*. "Dietary effects of fatty acids on growth and metastasis of KPL-1 human breast cancer cells in vivo and in vitro," *Anticancer research GreeceInfo*, vol. 18, pp. 1621-1627, 1988
[18] Y.E.M. Dommels, *et al*., "The role of cyclooxygenase in n-6 and n-3 polyunsaturated fatty acid mediated effects on cell proliferation, PGE2 synthesis and cytotoxicity in human colorectal carcinoma cell lines," *Carcinogenesis*, vol. 24, no. 3, pp. 385-392, 2003.
[19] M. Mouradian, *et al*., "Key roles for GRB2-associated-binding protein 1, phosphatidylinositol-3-kinase, cyclooxygenase 2, prostaglandin E2 and transforming growth factor alpha in linoleic acid-induced upregulation of lung and breast cancer cell growth," *Prostaglandins Leukotrienes and Essential Fatty Acids*, vol. 90, no. 4, pp. 105-115, 2014.
[20] E.A. Hudson, S.A. Beck, and M.J. Tisdale, "Kinetics of the inhibition of tumour growth in mice by eicosapentaenoic acid-reversal by linoleic acid," *Biochemical pharmacology England*, vol. 45, no. 11, pp. 2189-2194, 1993.
[21] J.M. Connolly, M. Coleman, and D.P. Rose, "Effects of dietary fatty acids on DU145 human prostate cancer cell growth in athymic nude mice", *Nutrition Cancer*, vol. 29, no. 2, pp. 114-119, 1997.
[22] M. Jóźwiak, *et al*., "Anticancer effects of alloxanthoxyletin and fatty acids esters – In vitro study on cancer HTB-140 and A549 cells," *Biomedicine Pharmacotherapy*, vol. 110, pp. 618-630, 2019.
[23] V. Venepally, *et al*., "Synthesis and biological evaluation of some new N-fatty acyl derivatives of 4,5-dimethoxy tryptamine," *Indian Journal Chemistry*, vol. 56, no. 5, pp. 531-541, 2017.
[24] V. Venepally, *et al*., "Synthesis of novel ethyl 1-ethyl-6-fluoro-7-(fatty amido)-1,4-dihydro-4-oxoquinoline-3-carboxylate derivatives and their biological evaluation," *Bioorganic & Medicinal Chemistry Letters*, vol. 26, no. 2, pp. 613-617, 2016.
[25] D.S. Santos, *et al*., "Antiproliferative activity of synthetic fatty acid amides from renewable resources," *Bioorganic & Medicinal Chemistry Letters*, vol. 23, no. 2, pp. 340-347, 2015.

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