GC-MS Analysis the Fatty Acid Components of Tamarindus indica Seeds 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.

KEYWORDS
Tamarindus indica seed; Fatty acid; GC-MS analysis; Linoleic acid; Vietnam.

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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 IC50 (6.91 µg/mL) in DPPH model, as well as 113.8 µg/mL in the H2O2 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 °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 extract 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
(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]

| 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                 |

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

![Diagram of fatty acid structures from ethyl acetate fraction of T. indica seed identified by GC-MS](image)

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.

| No | Fatty acid                | m/z     | Retention time | Content of oil (%) |
|----|---------------------------|---------|----------------|--------------------|
| 1  | Myristic acid C_{14}H_{29}O_{2} | 228.37  | 28.37          | 0.54               |
| 2  | Palmitic acid C_{16}H_{35}O_{2} | 256.42  | 32.55          | 13.43              |
| 3  | Stearic acid C_{18}H_{36}O_{2} | 248.5   | 36.35          | 3.88               |
| 4  | Isoarachidic acid C_{20}H_{41}O_{2} | 312.5   | 39.84          | 0.86               |
| 5  | Docosanoic acid C_{22}H_{45}O_{2} | 340.6   | 43.07          | 0.77               |
| 6  | Linoleic acid C_{18}H_{34}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 (FabB). 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 antimicrobial 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.

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