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# Analysis of lipids from methyl ester of sapota seed oils

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**Abstract**
Sapota Kalli Patti seeds were used for oil extraction through Soxhlet apparatus. The Maximum seed oils were measured from these seeds as compared to other horticultural crop seeds. Seed oil used for making the methyl ester through standard protocol of GC MS- FID and analyzed individual fatty acids. The total seventeenth peak were observed from RT of 7.378 to 29.512. The qualitatively it was observed that 48.31 Area%, of Cholestane-3,5-diol, 5-acetate, (3. beta.,5. Alpha) which was recognized as the naturally occurring steroid hormones because of their saturated ring structures and then next was 9-Octadecenoic acid with an area of 15.08%. Because of higher valued in composition of this fatty acids, it showed more number (17) of peaks while analyzing. But actually, six fatty acids and two phenolics and one steroid in composition of sapota seed oils was determines through GCMS-FID. Therefore through profiling it can feasibility of extracting oil from Chikoo seeds and utilized other than food purpose.

**Keywords:** Sapota Kalli Patti seeds, oil extraction, soxhlet apparatus, horticultural crop seeds

## Introduction

Sapota (*Manilkara achras* Forb.) is an evergreen tree, the fruit used as fresh and processed in many juices. Sapota, also known as sapodilla, Chikoo contains high levels of ascorbic acid and phenolic compounds therefore it has numerous uses in human health benefits. (Madani *et al*.,2018) [1]. Chikoo, a tropical fruit native to Central America and widely cultivated in various regions, is not only valued for its sweet, flavourful flesh but also for its seeds, which are often discarded as waste. In general, it is known that the seeds of the fruits, because of their biological functions, are rich in nutrients and are a source of vegetable fats, starches, proteins, and many compounds which are useful as raw or supplementary materials for elaborating other products in the food, pharmaceutical and cosmetics industries (Solis-Fuentes, *et al*. 2015). [2] Many studies have highlighted the feasibility of extracting oil from Chikoo seeds and converting it into biodiesel through transesterification processes. (Rajkumar *et al*.,2016) [3].

This method involves the reaction of the seed oil with an alcohol (typically methanol) in the presence of a catalyst, resulting in the formation of fatty acid methyl esters (FAMEs) and glycerol. The optimization of this process was crucial to maximize yield and ensure that the resultant methyl ester meets industry standards for various application in industries especially biodiesel as potential as a fuel source, Furthermore, utilizing agricultural by-products like Chikoo seeds contributes to waste reduction and promotes sustainable agricultural practices. This analysis aim was to explore the extraction methods, characterization, and potential applications of methyl ester derived from Chikoo seed oils. By examining these aspects, assess the viability of Chikoo seed oil as an alternative source for oil uses in contributing valuable insights

## Materials and method

Soxhlet extracted oils from chikoo seed were used for methyl ester formation and injected in autosampler of GC MS with standardize condition with column size and temperature given below.

## GCMS conditions:

Extracted oils were used for derivatives, made methyl ester, and applied in autosampler tubes. GC-MS analyses were performed using a DB-5MS capillary column (30 m × 0.25 mm i.d., film thickness 0.25 µm, Agilent Technologies, Santa Clara, CA, USA). The chromatographic runs were started per the following detailed conditions [GC-2010]. The GCMS conditions as per the table indicated: ionization mode, electron ionization (EI) mode; ionization current, 60 µA; ionization voltage, 70 eV. Each sample was analysed. Eluted compounds were identified using the NIST (http://www.nist.gov/srd/nist1a.cfm), the Wiley NBS, the mass spectral data as well as used pubmed and other data sources for detection.

Fatty acids and compounds were used for google search, NIST library for the potential application for food and industrial application.

## Instruments protocol for analysis of GCMS-FAME

| | | |
|---|---|---|
| Column Oven Temp. | 40.0 °C | MS table: |
| Injection Temp. | :250.00 °C | Start Time :3.50min, |
| Injection Mode | : Split | End Time :54.67min, |
| Flow Control Mode | : Pressure | ACQ Mode: Scan, |

| | | |
|---|---|---|
| Pressure | :49.5 kPa | Event Time :0.50sec, Scan Speed 1428, Start m/z :50.00, End m/z :700.00, Sample Inlet Unit: GC "[GCMS-QP2010 Plus], Ion Source Temp :200.00 °C, Interface Temp.:290.00 °C, Solvent Cut Time :2.00 min, Detector Gain Mode: Relative Detector Gain: +0.00 kV, Threshold 100" |
| Total Flow | :54.0 mL/min | |
| Column Flow | :1.00 mL/min | |
| Linear Velocity | :36.1 cm/sec | |
| Purge Flow | :3.0 mL/min | |
| Split Ratio | :50.0 | |
| High Pressure Injection | : ON | |
| High Press. Inj. Pressure | :250.0 kPa | |
| High Press. Inj. Time | :1.00 min | |
| Carrier Gas Saver | : OFF | |
| Splitter Hold | : OFF | |
| Oven Temp. Program | | |
| Rate | Temperature(°C) | Hold Time(min) |
| - | 40.0 | 3.00 |
| 6.00 | 290.0 | 10.00 |

**Results and discussion**

The data for compounds detected through GC MS in seed oils of sapota are presented in figure1. Table.1 & description of compounds with their standardize name are presented in class of fatty acids and carbon chain (Table 2).

Experiments with dry seeds showed variation in fixed oils during extraction because of varying in size of seeds in sapota fruits. But normally it was varied between 15.7 to 23.58 % on dry weight basis was observed. The optimized processing of peak identification showed higher amount of palmitic oleic and linoleic acids in seeds without outer cover but after addition of with seed coats showed higher amount of sterols derived steroids in composition that was 48.31% Cholestane-3,5-diol, 5-acetate, (3. beta.,5. alpha.)-. The results from chromatogram showed the total seventeenth peak identified based on RT (Retention Time). It was ranges between 7.378 to 29.512. The greater number of peaks as palmitic acids was detected many times but over all seven fatty acids were common in all fraction of seeds but seed coats contained higher amounts of steroids and phenolics compounds derivatives too as shown in peak table.

These results are in agreements with the fatty acids in sapota seed determined by Solis-Fuentes (2015) [2]. was studied *Pouteria sapota* and its oil extracted from the seed (MSSO) of ripe and unripe fruits.

Out of seventeenth peaks most were founds to be fatty acids. Thus, the oils without seeds coat can be useful for foods supplements but with seed cover it can not be utilized as food ingredients. Thus, it can be utilized for fuel purpose only.

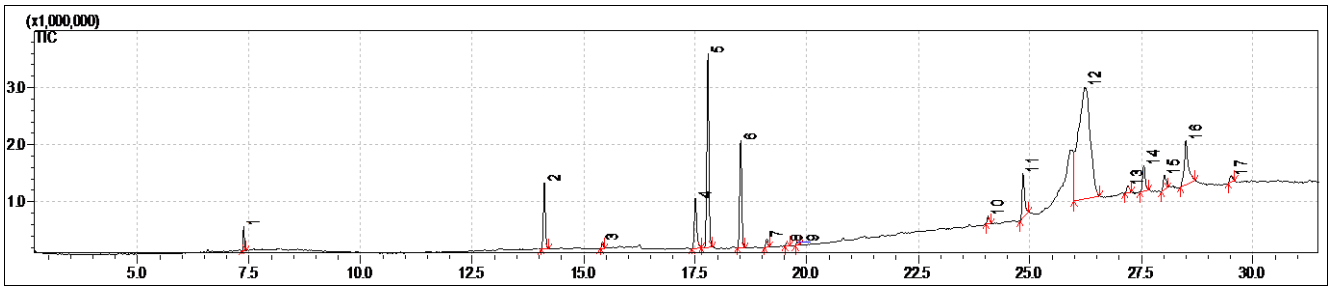


**Fig 1:** Chromatogram on Methyl ester detected through GC-MS-FID in oils of sapota seed

**Table 1:** Number of Peak, Peak Area % and compound detected through GC MS from Sapota seed oils

| Peak No | RT (min) | Area | Area (%) | Compound Identified |
|---|---|---|---|---|
| 1 | 7.378 | 739606 | 0.99 | Naphthalene |
| 2 | 14.116 | 3637375 | 4.86 | Hexadecanoic acid |
| 3 | 15.423 | 296729 | 0.4 | Phenol, 2,4-bis(1,1-dimethylethyl)- |
| 4 | 17.503 | 3363018 | 4.5 | Octadecanoic acid |
| 5 | 17.784 | 11280885 | 15.08 | 9-Octadecenoic acid (Z |
| 6 | 18.517 | 6135295 | 8.2 | 9,12-Octadecadienoic acid |
| 7 | 19.104 | 460953 | 0.62 | 1,2-Benzenedicarboxylic acid |
| 8 | 19.558 | 215771 | 0.29 | 11,14,17-Eicosatrienoic acid |
| 9 | 19.788 | 172876 | 0.23 | Methyl 3-(3,5-di-tert-butyl-4-hydroxyphenyl) propionate |
| 10 | 24.064 | 419108 | 0.56 | Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester |
| 11 | 24.844 | 3044356 | 4.07 | n-Hexadecanoic acid |
| 12 | 26.233 | 36156150 | 48.31 | Cholestane-3,5-diol, 5-acetate, (3. beta.,5. alpha.)- |
| 13 | 27.196 | 486212 | 0.65 | Hexadecanoic acid, 2-hydroxy-1,3-propanediyl ester |
| 14 | 27.553 | 1838054 | 2.46 | 9-Octadecenoic acid, 1,2,3-propanetriyl ester, (E,E,E)- |
| 15 | 28.014 | 1135478 | 1.52 | Octadecanoic acid |
| 16 | 28.497 | 4943218 | 6.61 | Oleic Acid |
| 17 | 29.512 | 489015 | 0.65 | 9,12-Octadecadienoic acid |

**Table 1:** Name of peak with standardize name and subclass of compounds detected from Sapota seed oils

| Peak No | Input name | Standardized name | Formula | Exact mass | Sub class |
|---|---|---|---|---|---|
| 1 | Naphthalene | Naphthalene | $C_{10}H_8$ | 128.0626 | SFA |
| 2 | Hexadecanoic acid | Naphthalene | $C_{16}H_{32}O_2$ | 256.2402 | SFA |
| 3 | Phenol | Phenol | $C_6H_6O$ | 94.0419 | UnSFA |
| 4 | Octadecanoic acid | Stearic acid | $C_{18}H_{36}O_2$ | 284.2715 | UnSFA |
| 5 | 9-Octadecenoic acid | Elaidic acid | $C_{18}H_{34}O_2$ | 282.2559 | UnSFA |
| 6 | 9,12-Octadecadienoic acid | Linoleic acid | $C_{18}H_{32}O_2$ | 280.2402 | SFA |
| 7 | 1,2-Benzenedicarboxylic acid | Phthalic acid | $C_8H_6O_4$ | 166.0266 | SFA |
| 8 | 11,14,17-Eicosatrienoic acid | Dihomo-gamma-linolenic acid | $C_{20}H_{34}O_2$ | 306.2559 | SFA |
| 9 | Methylpropionate 3-(3,5-di-tert-butyl-4-hydroxyphenyl) | - | | | UnSFA |
| 10 | Hexadecanoic acid | Palmitic acid | $C_{16}H_{32}O_2$ | 256.2402 | SFA |
| 11 | n-Hexadecanoic acid | Palmitic acid | $C_{16}H_{32}O_2$ | 256.2402 | UnSFA |
| 12 | Cholestane-3,5-diol, 5-acetate | | $C_{29}H_{50}O_3$ | 446.375 | UnSFA |
| 13 | Hexadecanoic acid, | Palmitic acid | $C_{16}H_{32}O_2$ | 256.2402 | SFA |
| 14 | 9-Octadecenoic acid | Elaidic acid | $C_{18}H_{34}O_2$ | 282.2559 | SFA |
| 15 | Octadecanoic acid | Stearic acid | $C_{18}H_{36}O_2$ | 284.2715 | UnSFA |
| 16 | Oleic Acid | Oleic acid | $C_{18}H_{34}O_2$ | 282.2559 | UnSFA |
| 17 | 9,12-Octadecadienoic acid | linoleic acid | $C_{18}H_{32}O_2$ | 280.2402 | UnSFA |

**Conclusion:** GC MS from Sapota seed oils showed the fatty acids viz., Elaidic acid Stearic acid Linoleic acid Phthalic acid Dihomo-gamma-linolenic acid Oleic acid and Naphthalene too.

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