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Fatty Acid Methyl Esters Composition of *Trichilia Emetica* Shell Oil

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Abstract

The formation of fatty acid methyl esters from *Trichilia emetica* shell oil by trans-esterification with methanol was monitored by ^{1}H nuclear magnetic resonance spectroscopy. The percentage triacylglycerol conversion to its corresponding methyl esters was calculated to be 98 %. A total of five fatty acid methyl esters (FAMEs) were identified in the oil sample by the retention time and the fragmentation pattern data of GC/MS analysis. The identified FAMEs were hexadecanoic (palmitic), octadecanoic (stearic), eicosanoic (arachidic), 9,12-octadecadienoic (linoleic) and 9,12,15-octadecatrienoic (linolenic) acid methyl ester.

Keywords: Transesterification; Fatty acid methyl esters; Retention time; Resonances; Triacylglycerol.

1. Introduction

The search for alternative sources of energy is on-going in a bid to find a suitable replacement to fossil fuel, which is fast diminishing due to high demand for industrial and domestic purposes. The increasing consumption of fossil fuel in the world has resulted in the increasing environmental pollution. Consequently, there is a need to explore all renewable resources that will be economically competitive and more environmentally friendly. Among all the renewable resources, biodiesel has attracted much attention [1,2]. And it requires assessing the potential of different plant species for their oil content and suitability for biodiesel production, nutritional supplements or industrial chemicals [3,4,5].

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Presently, more than 70% biodiesel are produced from food sources and this has made biodiesel production economically unfeasible [6,7]. To avoid this situation, non-edible oil as well as the seed shells or hulls of both edible and non-edible oil should be screened to assess their suitability for biodiesel production.

The oil extracted from any plant parts cannot be used directly as fuel in diesel engine, this is because of it high molecular mass and kinematic viscosity. And it can also result into several operational problems in the engine. Hence, the oil has to be transesterified into biodiesel to make it useful in diesel engine [8,9,10]. Biodiesel is a fuel that consists of monoalkyl esters of fatty acids derived from the transesterification of vegetable oils or animal fats. The reaction is normally catalyzed by NaOH, KOH or H_2SO_4. Biodiesel is biodegradable, nontoxic and can be used either pure or blended with petro diesel fuel [8,11,12]. Here we report the fatty acid methyl esters composition of *T. emetica* shell oil which has not been reported previously.

$$\mathrm{RCOOR' + R''OH \xrightarrow{Catalyst} RCOOR'' + R'OH} \qquad (1)$$

T. emetica is an evergreen shrub or tree that sometimes reaches up to 35m in height. The tree has a non-aggressive root system and the trunk is swollen at the base. The bark is grey-brown with shallow striations and smallish scales [13]. Inflorescences are either terminal or produced on short congested axillaries, and the flowers are unisexual [14]. The fruit is pear-shaped with a long stripe about 2-4 cm with three valve capsules, which are split into three or four parts to reveal 3-6 shiny black seeds which are almost completely concealed in a scarlet sarcotesta [13,14]. They are propagated by cuttings and regenerate naturally by root suckers, and seeds [13]. Plant maturity depends on the location where the tree is planted, with thirteen years for those planted in the open, and twenty four for those in the shade [15]. Fruits are harvested when the capsules open up. They are dried in the shade and the seeds are shaken out [13]. The seeds weighed between 0.35 to 1.0 g and consist of approximately 23% of an oily shell-like husk and 77% kernel [16].

In this study, we determined the composition of FAMEs in *T. emetica* shell oil which is the first stage of the bio refining of the oil.

2. Materials and Methods

T. emetica seeds used in this study were collected from Kumasi-Ghana. The seeds and the shells were manually separated, and spread out to dry at ambient temperature and were stored in plastic containers at room temperature until further analysis.

2.1. Extraction of Seed Oil

T. emetica shells (50.0 g) were flaked using Eschenfelder Seed and Grain Flaker 1200. The flaked shells were stirred in n-hexane (3×200 cm^3) at room temperature for 1 hour each. The extracts were combined, dried over $MgSO_4$ and then filtered under gravity. The n-hexane was removed by rotary evaporator at 40^oC to give yellowish brown oil. The oil obtained was stored at room temperature for further analysis.

2.2. Trans-esterification of the Oils

The method of Thoss et al., (2012) was used with modification. *Trichilia emetica* shells oil (2.0 g) was dissolved in sodium methoxide (0.5M, 20 ml) and heated under reflux with constant stirring for 50 minutes to produce fatty acid methyl esters (FAMEs). On cooling to room temperature, the mixture was transferred to a separating funnel. Deionised water (50 ml) was then added, and the solution extracted with n- hexane (50 ml × 3). The lower glycerol layer was discarded and the top hexane layers was combined, dried over $MgSO_4$, filtered under gravity and the solvent removed using rotary evaporator at 40^oC. The transesterified sample was diluted (1:1000) with hexane and analyzed by gas chromatography-mass spectrometry (GC- MS).

2.3. GC-MS Method

The fatty acid methyl esters (FAMEs) contents of *T. emetica* shell oil were determined using Thermoquest Finnigan Trace GC 2000, with a Chrompack silica fused DB-5 column (L 25 m × ID 0.32 mm × DF 0.25µm). The carrier gas was nitrogen with a flow rate of 1.5 ml/min. The initial oven temperature was 150^oC (held for 2 min), ramped at 5^oC min^{-1} up to 250^oC (held for 10 min). The phase transfer line temperature was held at 250^oC. A sample volume of 1µl in hexane was injected using a split mode, with the split ratio of 1:10. The mass spectrum was collected on a Thermoquest Voyager Qp MS. Electron impact (70 eV) ionisation was used for fragmentation with a rate of 1 scan per second.

2.4. NMR Analysis

^{1}H NMR (400.13 MHz) spectra were recorded on a Bruker BioSpin spectrometer, equipped with 5 mm PABBO gradient probe head, using $CDCl_3$ as solvent. The chemical shift (δ) is given in part per million (ppm) relative to tetramethylsilane (TMS) as an internal reference, with the solvent residual peaks of deuterated chloroform at δ_H 7.27 as reference. All sample runs were carried out at 294.7 K, with a relative time of 2.0 seconds. The spectra were processed and analysed using MestReNova version 6.0.

3. Result and discussion

3.1. Extraction of T. emetica shell oil

The *T. emetica* shell oil extracted with hexane was yellowish brown. The yield is 23 ± 1.3 % by mass for dry shell. And it is within 22-25 % reported for *T. emetica* shell [16].

3.2. ^{1}H NMR analysis of crude T. emetica shell oil

The ^{1}H NMR of crude *T. emetica* shell oil is shown in (Figure 1). The spectrum showed a terminal methyl proton that appears as a broad superposition of triplet at δ_H 0.89. The signals of the methylene chain (CH_{2n}) resonate at δ_H 1.26. The protons attached to allylic and bis-allylic carbon are found at δ_H 2.02 and 2.77. The resonance for the unsaturated fatty acids (CH=CH) is also found at δ_H 5.35 ppm. Other significant resonances are those appearing at δ_H 5.27 ppm and that between δ_H 4.14 - 4.29 ppm which were assigned for α and β glycerol, they are the main components of the triacylglycerol fraction and serve as a backbone to which fatty

acid chains are attached [17,18].

3.3. ^{1}H NMR analysis of transesterified T. emetica shell oil

The ^{1}H NMR spectrum (Figure 2) of transesterified oil sample shows the presence of two distinct peaks, a singlet at B (δ_H 3.67) which is due to methoxy groups and a triplet of α- carbonyl methylene groups in the methyl esters at A (δ_H 2.31 ppm). The presence of these peaks confirm the presence of methyl esters in the oil sample [1,18]. And the integration of these peaks was used to quantify the yield of transesterification reaction. In the spectrum, the glyceryl-related signals found at δ_H 4.14-4.29 and 5.27 ppm were absent, while the remaining signals are similar to those in the crude sample (Figure 1). The yield of transesterification reaction was quantified using the equation below:

$$Z\% = 100 \times (2B/3A) \quad (2)$$

Where, A and B are the integration values of the methoxy and α-carbonyl methylene group.

The percentage conversion of triglycerides to corresponding methyl esters using equation (2) above was found to be 98% and it is an excellent yield.

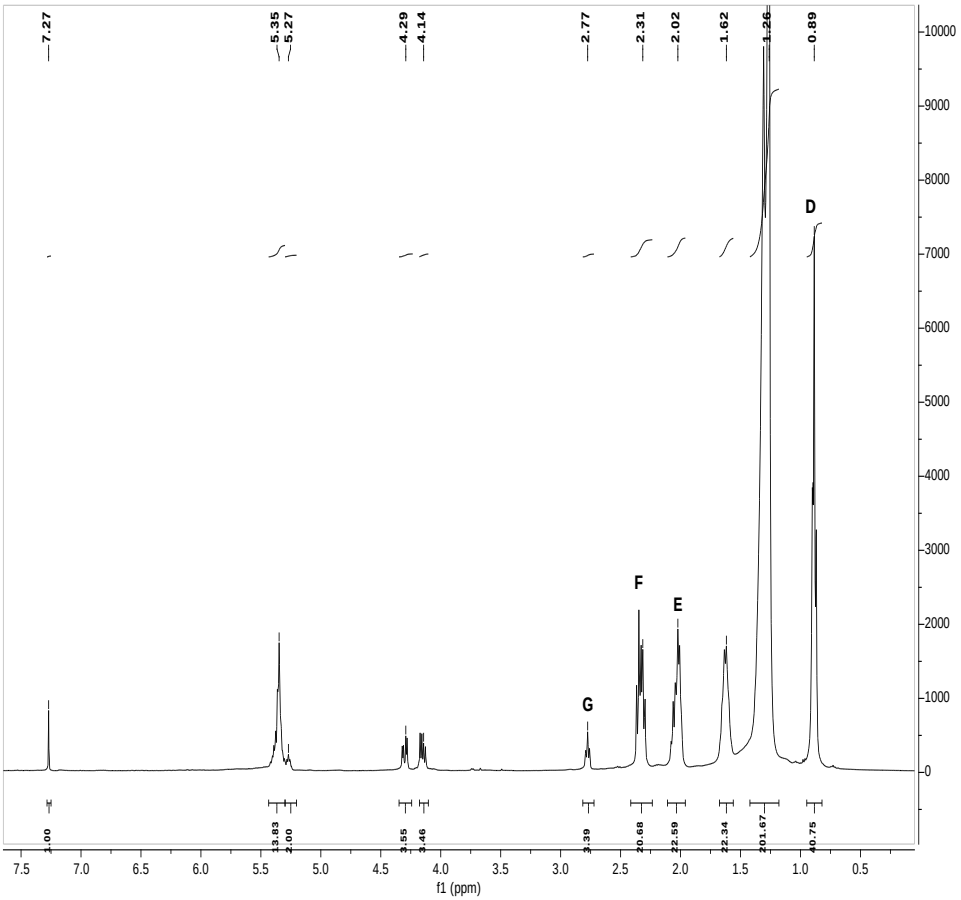

Figure 1: ^{1}H NMR of spectrum of crude *T. emetica* seed oil.

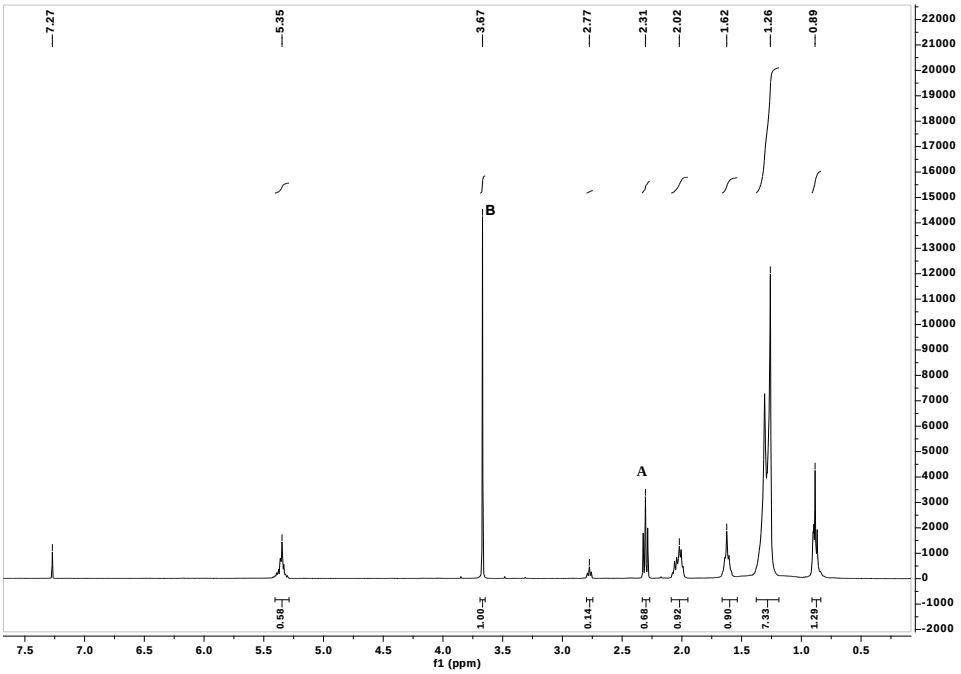

Figure 2: ^{1}H NMR spectrum of transesterified *T. emetica* shell oil.

3.4. GC-MS analysis of transesterified oil

Crude *T. emetica* shell oil was first derivatized in order to improve volatility and subsequent resolution in the GC-MS [3]. The transesterified sample and alkane standard were run simultaneously under the same experimental conditions. Their respective retention time and mass spectrometric data analysis was compared and the results obtained are shown in Table 1. Five FAMEs were identified: hexadecanoic (palmitic, C16:0), octadecanoic (stearic, 18:0), 9,12-octadecadienoic (linoleic, C18:2), 9,12,15-octadecatrienoic (linolenic, C18:3) and eicosanoic (arachidic, C20:0) acid methyl ester. And each of these FAMEs was further verified by using the library match software (NIST MS Search 2.0). The saturated, diunsaturated and polyunsaturated fatty acids present in the oil were identified by their base peak, with m/z 74, 67 and 79 [1]. The GC–MS fragmentation of peak A, D and E with retention time (RT) 9.23, 19.88 and 24.29 were confirmed to be saturated FAMEs and identified as hexadecanoic (palmitic), octadecanoic (stearic) and eicosanoic (arachidic) acid methyl esters respectively (Figure 3). Apart from their individual molecular ions (M^+) at m/z 270, 298 and 326, other ions common to them are 213($M^+ - C_2H_5$), 199($M^+ - C_3H_7$), 185($M^+ - C_4H_9$), 171($M^+ - C_5H_{11}$), 157($M^+ - C_6H_{13}$), 143($M^+ - C_7H_{15}$), 129($M^+ - C_8H_{17}$), 115 ($M^+ - C_9H_{19}$), 101($M^+ - C_{10}H_{21}$), 87($M^+ - C_{11}H_{23}$) and 74 ($M^+ - C_{12}H_{25}$) which is a base peak occurring due to McLafferty rearrangement and α-cleavage. The mass spectrum of octadecanoic acid methyl ester (C18:0) with important fragmentation ion is shown in (Figure 4). Peak C with retention time 18.86 showed a molecular ion (M^+) at 294. Also, the fragment at 262 occurs as a result of α-cleavage of methoxy (-OCH_3) group, as well as γ-hydrogen atom transfer and i-induced cleavage. The base peak at 67 was as a result of a double-bond transfer and α-cleavage [1]. Other ions was thus observed at m/z 55, 67, 81, 95, 109, 121, 135, 149, 164, 178, 191, 205, 220, and 234. Based on these fragmentation patterns, this FAMEs was identified as 9,12-octadecadienoic acid methyl ester (linoleic acid methyl ester).

Table 1: FAMEs compositions of *T. emetica* shell oil

Peak	Retention Time (min)	Name	Corresponding Acid	Molecular Weight
A	9.23	Hexadecanoic (Palmitic) acid methyl ester	C16:0	270
B	12.65	9,12,15-Octadecatrienoic (linolenic) acid methyl ester	C18:3	292
C	18.86	9,12-Octadecadienoic (linoleic) acid methyl ester	C18:2	294
			C18:0	298
			C20:0	326
D	19.88	Octadecanoic (stearic) acid methyl ester		
E	24.29	Eicosanoic (arachidic) acid methyl ester		

The only polyunsaturated FAME identified in this oil sample was 9,12,15-octadecatrienoic (linolenic) acid methyl ester (peak B, RT = 12.65 min). It is characterized by the molecular ion (M^+) at m/z 292 and the loss of a methoxy group resulted in the peak at 261 (M^+ - CH_3OH). The base peak is found at m/z 79 which occurred as a result of double bond transfer and α-cleavage of the carbonyl [20]. Other peaks were those found at m/z 55, 67, 79, 95, 109, 121, 135, 149, 173, 191, 222 and 236.

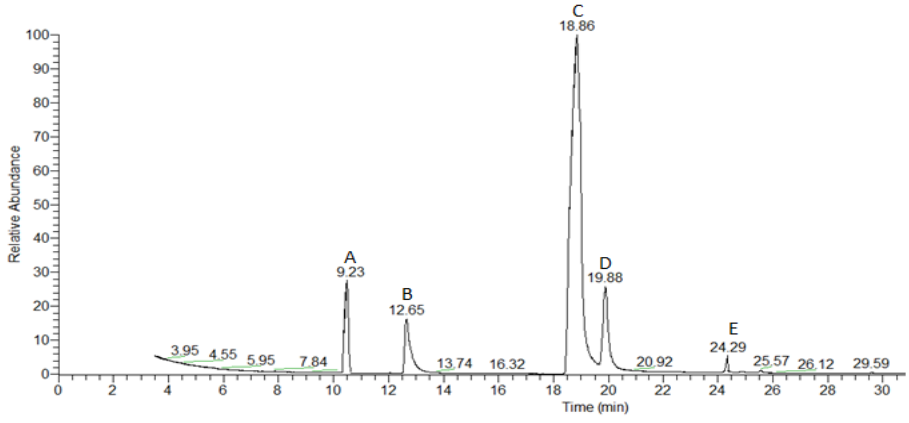

Figure 3: Total ion chromatogram of *T. emetica* shell oil showing composition of FAMEs

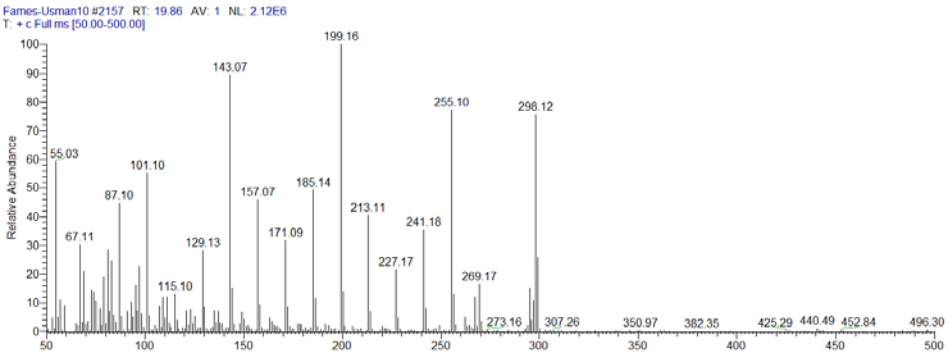

Figure 4: Mass spectrum of octadecanoic acid methyl ester from *T. emetica* shell oil

4. Conclusion

The *T. emetica* shell oil was first derivatized with sodium methoxide and the yield was confirmed by ^{1}H NMR analysis. The chemical composition of methyl esters was determined by GC/MS. Five fatty acid methyl esters were identified in the transesterified *T. emetica* shell oil, ranging from C16 to C20 by chromatographic retention time data and verified by mass fragmentation pattern.

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