Characterization of Leucaena (*Leucaena leucephala*) oil by direct analysis in real time (DART) ion source and gas chromatography

M. Alam[a,✉], N.M. Alandis[b], E. Sharmin[c], N. Ahmad[b], B.F. Alrayes[d] and D. Ali[e]

[a]Research Center-College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia
[b]Department of Chemistry, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia
[c]Department of Pharmaceutical Chemistry, College of Pharmacy, Umm Al-Qura University, P.O. Box 715, Makkah Al-Mukarramah 21955, Saudi Arabia
[d]Central Laboratory, College of Science, Riyadh-11451, King Saud University, Kingdom of Saudi Arabia
[e]Department of Zoology, College of Science, Riyadh-11451, King Saud University, Kingdom of Saudi Arabia

[✉]Corresponding author: malamiitd@gmail.com

SUMMARY: For the first time, we report the characterization of triacylglycerols and fatty acids in Leucaena (*Leucaena leucephala*) oil [LUCO], an unexplored nontraditional non-medicinal plant belonging to the family Fabaceae. LUCO was converted to fatty acid methyl esters (FAMEs). We analyzed the triacylglycerols (TAGs) of pure LUCO and their FAMEs by time-of-flight mass spectrometry (TOF-MS) followed by multivariate analysis for discrimination among the FAMEs. Our investigations for the analysis of LUCO samples represent noble features of glycerides. A new type of ion source, coupled with high-resolution TOF-MS was applied for the comprehensive analysis of triacylglycerols. The composition of fatty acid based LUCO oil was studied using Gas Chromatography (GC-FID). The major fatty acid components of LUCO oil are linoleic acid (52.08%) oleic acid (21.26%), palmitic acid (7.91%) and stearic acid (6.01%). A metal analysis in LUCO was done by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The structural elucidation and thermal stability of LUCO were studied by FT-IR, ^{1}H NMR, ^{13}C NMR spectroscopic techniques and TGA-DSC, respectively. We also measured the cytotoxicity of LUCO.

KEYWORDS: Cytotoxicity; Gas Chromatography; Leucaena oil; Thermal analysis; TOF-MS

RESUMEN: *Caracterización del aceite de Leucaena (***Leucaena leucocephala***) por análisis directo en tiempo real (DART) y cromatografía de gases.* Se presenta por primera vez la caracterización de triacilgliceroles y ácidos grasos del aceite de Leucaena (*Leucaena leucephala*) [LUCO], una planta no medicinal, no tradicional y no explorada, perteneciente a la familia Fabaceae. Se analizaron triacilgliceroles (TAGs) de LUCO y sus FAMEs por espectrometría de masas de tiempo de vuelo (TOF-MS) seguido de análisis multivariante para discriminación entre los FAME. Nuestras investigaciones para el análisis de muestras de LUCO presentaron características propias de los glicéridos. Un nuevo tipo de fuente de iones, junto con alta resolución TOF-MS se aplicó para el análisis exhaustivo de triacilgliceroles. La composición de aceite de LUCO basado en ácidos grasos se estudió usando Cromatografía de Gas (GC-FID). Los principales componentes de ácidos grasos del aceite LUCO fueron, linoleico (52,08%), oleico (21,26%), palmítico (7,91%) y esteárico 6,01%. El análisis de metales se realizó mediante Espectrometría de Plasma Acoplado Inductivamente a Masas (ICP-MS). La elucidación estructural y la estabilidad térmica de LUCO se estudiaron mediante FT-IR, ^{1}H NMR, técnicas espectroscópicas de ^{13}C NMR y TGA-DSC, respectivamente. También se midió la citotoxicidad de LUCO.

PALABRAS CLAVE: Aceite de Leucaena; Análisis Térmico; Citotoxicidad; Cromatografía de gases; TOF-MS

ORCID ID: Alam M http://orcid.org/0000-0001-9540-8532, Alandis NM http://orcid.org/0000-0002-2098-9800, Sharmin E http://orcid.org/0000-0002-3262-0162, Ahmad N http://orcid.org/0000-0002-2913-1763, Alrayes BF http://orcid.org/0000-0002-5319-2785, Ali D http://orcid.org/0000-0002-8034-4473

1. INTRODUCTION

The precise word 'leucocephala' derived from 'leu', meaning white, and 'cephala', meaning head, refers to flowers. Leucaena leucocephala is essentially a tropical species requiring warm temperatures (15–25 $^{\circ}$C) for optimum growth (Mullen et al., 2003). It is found in regions of Australia, US, Philippines, Thailand, China, Pacific islands, Mexico and India. In India, it is commonly known as Subabul / Kababul. It belongs to the family Fabaceae (Leguminosae) and sub-family Mimosaceae. The legume size is 10–17cm long containing 15–25 seeds each (Muthukrishnan et al., 2013). L. leucocephala is a fast growing hedgerow, medium-sized shrub. It is used in alley cropping and can release enough nitrogen to satisfy the requirement of maize grown in the alley crops (Mulongy et al., 1998). Its pod extract is a cheap, nontoxic and environmentally friendly natural product, used as corrosion inhibitor in an acidic medium (Muthukrishnan et al., 2013). The need for vegetable oils characterization arises in many aspects such as product development, quality assurance, product shelf life, and detection of adulteration. The industrial value of vegetable oils depends on their fatty acid composition and the ease by which these can be modified (Bhatnagar et al., 2009).

The most common method for the analysis of vegetable oils is gas chromatography. This technique is based on the selection of stationary phases, mobile phases, detectors, column temperature program and an appropriate resolution of carbon chain. Several scientific instruments are used to develop rapid, reliable and cost effective techniques for the analysis of vegetable oils such as gas chromatography-mass spectroscopy (GC-MS), high performance liquid chromatography (HPLC), matrix assisted laser desorption/ionization mass spectrometry (MALDI) and others (Dugo et al., 2012; Mercy et al., 2016; Kubo et al., 2013). TOF-MS techniques are advanced, efficient, much faster, have high data acquisition rate and a small internal volume for mass analysis (Vaclavik et al., 2009). Other advantages include rapid screening for target analytes, identification of non-target analytes by accurate-mass determination of protonated or de-protonated molecules and their product-ion mass spectra, quantification capability or performance, and reproducibility. The most frequently employed MS-related technique for the direct analysis of samples is direct analysis- in-real- time (DART) coupled with quadrupole time-of-flight mass spectrometry (Q-TOFMS).

In TOF analysis, molecules are charged by applying voltage between orifice and optics lens of ion source; the mass profile produced by applied voltage can be varied with respect to the amount of charged fragmentation. Typically, at low potential difference, positively charged species pass into the flight tube while at higher potential difference,

induced-collision takes place which provides more spectral and isomeric information through fragmentation. TOF software has the ability to collect various orifice voltages consecutively, giving a full profile of a sample (Mess et al., 2013; Gómez-Gonza et al., 2011).

The present study investigated the triacylglycerols (TAGs) of pure L. leucocephala oil [LUCO] and their FAMEs by TOF-MS, fatty acid composition by GC, structural analysis by FT IR, ^{1}H NMR,^{13}C NMR and thermal stability by TGA-DSC. We also measured the cytotoxicity of LUCO.

2. MATERIALS AND METHODS

2.1. Materials

We collected ripe legumes from the L. leucocephala plant (University Campus, King Saud University) and ginning seeds. Seeds were powdered, and the oil was extracted from powdered seeds through a soxhlet apparatus using petroleum ether as solvent. After extraction, petroleum ether was removed by a vacuum rotatory evaporator and yield was found as 5.67%. Petroleum ether, sulphuric acid (BDH, Poole, England), and methanol (Sigma-Aldrich, Steinheim, Germany) were used without any further purification.

2.2. Instrumentation and testing conditions

For the experiments, an Accu TOF LC plus (JMS-T 100LP) system consisting of a DART ion source (IonSense, DART), an Accu TOF LC plus a higher solution time-of-flight mass spectrometer (JEOL, Japan) and an auto injection valve were used. The operating conditions of the DART ion source were as follows: positive ion mode; discharge needle voltage: 3.0kV; perforated and grid electrode potentials: +100 and -100 V, respectively. Conditions of TOF-MS: cone voltage: 2000 V, monitored mass range: m/z 50–10000; acquisition rate: 1600 spectra min^{-1}; resolving power: approx. 6000 FWHM (full width at half maximum). The distance between the DART gun exit and mass spectrometer inlet was 7mm. The sampling glass rod was immersed for 1s into the sample containing approx. 500μL of the respective sample and was transferred to the optimized position in front of the DART gun exit and desorbed for 5s, while the spectral data were recorded. For each sample/PEG standard, a minimum of three repeated measurements were carried out. At the end of each run, the mass spectra of polyethylene glycol (PEG, average relative molecular weight 600/1000, Sigma–Aldrich, USA) methanol solution (200μL/mL^{-1}) were acquired to perform mass drift compensation (Easter J L et al., 2014; Wang Wang Y et al., 2014). The influence of gas beam temperature on signal intensity, LUCO and FAMEs were analyzed at 200 $^{\circ}$C. The final spectra were obtained after the

background was subtracted. The operating conditions were as follows: Gas Chromatography, Clarus 500 GC with FID, Perkin Elmer, USA, Column: Supelco Wax, 30 m long, 0.32 mm id, phase thickness 0.25μm, injection mode: split; split ratio, 1:100, 1.0μL injection used, diluted (1/10 v/v, FAMEs/hexane), Column temperature 140–240 °C, 4°C/min, an initial temperature hold at 140 °C for 5 min, FID temperature 260 °C, Helium carrier gas flow 1.0 mL/min, Hydrogen 30 mL/min, Air 300 mL/min. The FAMEs mix standard was analyzed under the same operating conditions to determine the peak identity. The FAMEs were expressed as relative area percentage (Dodds *et al.,* 2005; Fardin-Kia *et al.,* 2013).

LUCO spectra were taken on a FT IR spectrometer, Spectrum 100, Perkin Elmer, USA. ^{1}H and ^{13}C NMR spectra were recorded on Jeol DPX400 MHz using deuterated chloroform as solvent and tetra methyl silane as an internal standard. The thermal analysis of LUCO was carried out by simultaneous thermogravimetric analysis-differential scanning calorimetry (TGA-DSC) (Model TGA/DSC1, Mettler Toledo AG, Analytical CH-8603, Schwerzenbach, Switzerland) in nitrogen atmosphere (99.997 purity). The metal analysis of acid digested LUCO oil was analyzed with Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS); ThermoScientific, Instrument, USA.

2.2.1. Preparation of FAMEs of LUCO

Initially LUCO was derivatized by the alkaline saponification of vegetable oil (to break down glycerides because of their low volatility in nature) and was further esterified in the presence of methanol and a catalyst. LUCO (500mg) was refluxed with a 5mL methanolic sodium hydroxide (0.5M) solution at 150±5 °C for 5 minutes on a hot plate. 5 mL of a boron trifluoride methanol solution were added and the whole content was further heated at 150±5 °C for 5 minutes. The flask was removed from the hot plate; 5 mL of n-hexane and 10 mL of a saturated aqueous sodium chloride solution were added and the contents were agitated thoroughly. An aliquot of supernatant hexane layer was transferred into anhydrous sodium sulphate to remove moisture. The above 10 μL filtrate was analyzed by GC-FID (Lee *et al.,* 1998; Reiter *et al.,* 2001; Řezanka *et al.,* 2001).

2.2. Digestion of LUCO for ICP-MS

A certain amount (250mg) of LUCO sample was put into a digestive vessel of 60 mL capacity, with 8 mL of 65% nitric acid and the mixture was shaken carefully with a clean polytetrafluroethylene (PTFE) bar. After 30 minutes, the vessel was closed. The sample and blank (without oil) vessels were placed in the Topwave Analytik Jena microwave for digestion and the program temperature was set at

140 °C, 50 bar pressure for 8 minutes (ramp). The vessels were then left to cool at room temperature. The digested aliquots were filtered with a whatman no. 42 filter paper and the filtrate was diluted with 50 mL deionized water (Pehlivana *et al.,* 2008; Hsu W H *et al.,* 2013). ICP-MS calibration was carried out by external calibration using 10 ppm of multi element standard solutions (Aristar grade, BDH laboratory supplies, England) for the trace elements. The sample and blank were analyzed in triplicate. The results are summarized in Table 1.

2.3. Cytotoxicity studies 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

The MTT viability assay was performed with slight modifications as previously described (Mosmann T, 1983). Human hepatocarcinoma (HepG2) cells (2 $\times 10^3$) were seeded in 96 well plates and incubated in a CO_2 incubator at 37 °C for 24 hour. The cells were exposed to the culture medium and LUCO (0, 50, 100, 300, 700 μL/mL) for 24 h. After exposure, 100 μL of a MTT solution was added to each well and incubated for 4 hour at 37 °C. After incubation the formed formazon crystal was dissolved in DMSO (100 μL). Absorbance was taken at 530 nm using the enzyme-linked immune sorbent assay (ElISA) reader and the cell viability was calculated as:

$$\text{Cell viability (\%)} = \frac{\text{Total cells} - \text{Viable cells}}{\text{Total cells}} \times 100$$

3. RESULTS AND DISCUSSION

3.1. TOF-MS spectra of LUCO

The representative TOF-MS spectra of LUCO are shown in Figure 1. The spectra were divided into three categories according to ion fragmentations. The elemental (C, H, O) compositions of the major peaks were calculated by a software based on the exact mass number of the elements. The mass differences of identified compounds differed from their theoretical mass numbers by less

TABLE 1.   List of trace metals present in LUCO

Metals	Concentration (ppb)
Mn	2.940
Fe	16.80
Co	0.414
Ni	12.81
Zn	28.50
Cd	0.013
Pd	0.603

than 5.0 mmu (Table 2). Our target focused only on triglycerides, diglycerides and monoglycerides, and no other molecules. We tentatively identified triacylglycerol ion peaks of palmitoyldilinolein (PLnLn, m/z 854.736), trilinolein (LnLnLn, m/z 874.780), linolyldilinolein (LLnLn, m/z 875.767), dipalmitoylbehenene (PPB, m/z 892.783), trilinolein (LLL, m/z 879.743), linolinediolein (LOO, m/z 883.773), triolein (OOO, m/z 886.767), stearinedilinolien (SLnLn, m/z 879.743), gadolyldilinolein (GLnLn, m/z 906.782), archadoyldilinolien (A LnLn, m/z 907.748), gadoyldiolein (GLL, m/z 910.758), archedoyldilinolein (ALL,m/z 912.770), gadoyldiolein (GOO, m/z 913.778), behenendilinolein (BLnLn, m/z 936.806), behenendiolein (BOO, m/z 936.806), lenolyldistearine (LSS, m/z 884.749), linolene digadolein (LnGG, m/z 938.803), olyldigadolein (OGG, m/z 942.821), linolyldiarchidein (LAA, m/z 944.827), and olyldiarchidiene (OAA, m/z 946.816). However, the differentiation of

positional isomers of TAGs with two or three different fatty acids is not possible by TOF-MS, due to the absence of separation of the isobaric molecules prior to ionization. The relative intensities of molecular adducts, as well as molecular ions are present in TOF-MS spectra (Vaclavik *et al.,* 2009; Hajslova *et al.,* 2011; Bosque-Sendra *et al.,* 2012; Xu *et al.,* 2015).

In the TOF-MS spectra (Figure 1), the extensive fragmentation of TAG acquisition resulted in the occurrence of numerous types of ions under the experimental conditions. The most abundant were diacylglycerol fragment ions created by the loss of one fatty acid molecule from the glycerol backbone.

Ions at m/z 593 to 709 were identified as markers for diacylglycerols fragment ions: palmitolinolein (PL, m/z 593.493), oleyllinolein (OL, m/z 620.530), diolein (OO, m/z 623.530), palmitoylgadolein (PG, m/z 624.551), linolylarchidin (LnA, m/z
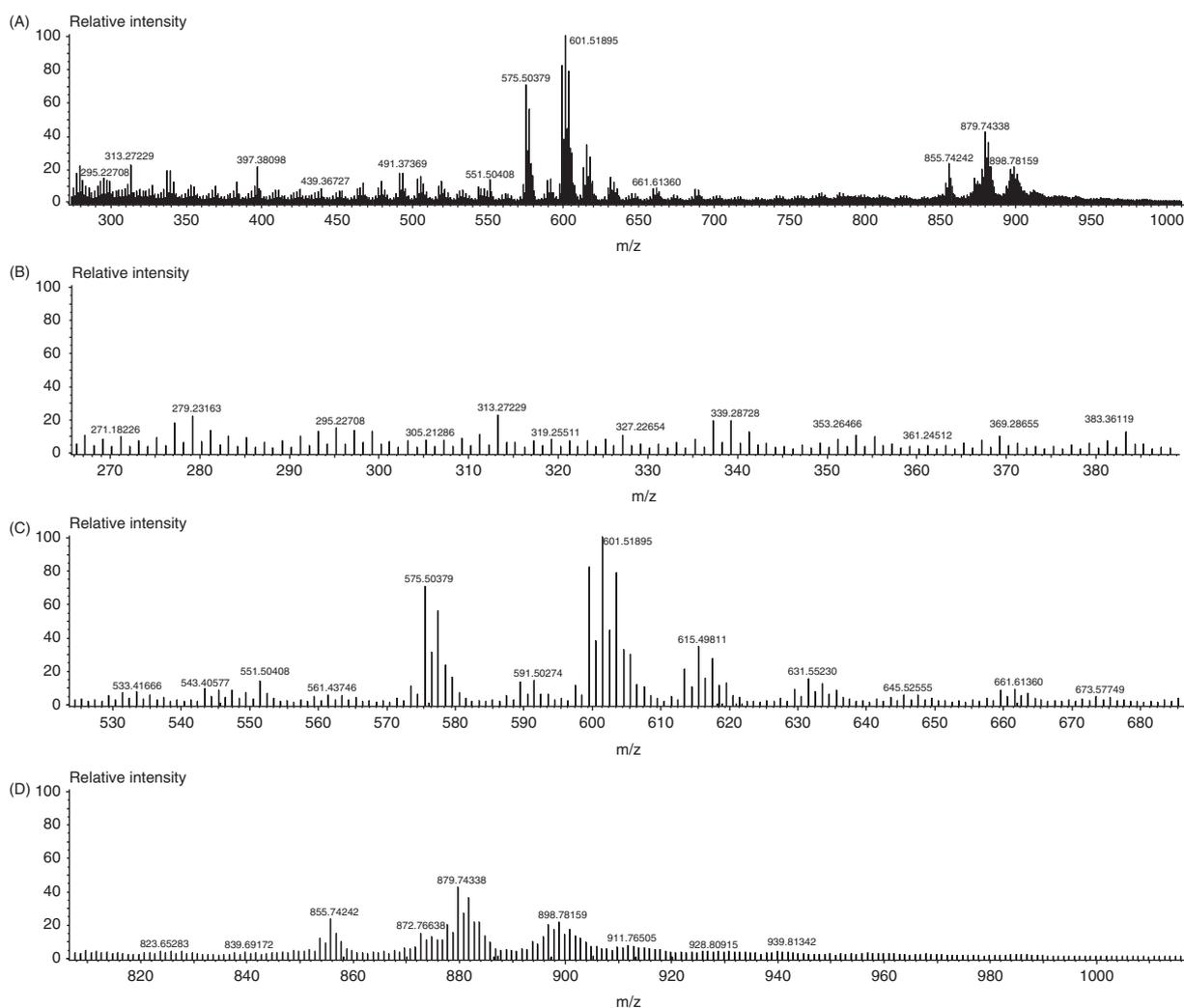

FIGURE 1.   TOF- MS spectra of LUCO; (A) Full spectra; (B) monoglycerols; (C) diacylglycerol; (D) triacylglycerols.

TABLE 2. Mass spectral statistics MS-TOF analysis of LUCO

S No.	Formula	Measured (M+H)$^+$	Difference (mmu)	Identification
1.	$C_{19}H_{38}O_4$	330.2406	3.81	a[P]$^+$
2.	$C_{20}H_{38}O_4$	341.2539	-2.3	a[L]$^+$
3.	$C_{20}H_{41}O_4$	345.5414	-2.8	a[S]$^+$
4.	$C_{21}H_{36}O_4$	352.2378	2.89	a[Ln]$^+$
5.	$C_{21}H_{40}O_4$	357.2277	-1.98	a[O]$^+$
6.	$C_{22}H_{45}O_4$	373.601	-3.10	a[A]$^+$
7.	$C_{23}H_{42}O_4$	382.2437	-1.28	a[E]$^+$
8.	$C_{23}H_{44}O_4$	384.2857	2.74	a[G]$^+$
9.	$C_{44}H_{49}O_4$	401.6514	-2.64	a[B]$^+$
10.	$C_{37}H_{68}O_5$	593.4934	0.81	b[PL]$^+$
11.	$C_{37}H_{72}O_5$	597.4925	0.90	b[PS]$^+$
12.	$C_{39}H_{72}O_5$	620.5301	0.47	b[OL, LS,]$^+$
13.	$C_{39}H_{74}O_5$	623.5327	3.90	b[OO,OS]$^+$
14.	$C_{39}H_{76}O_5$	624.5512	-0.38	b[PG]$^+$
15.	$C_{41}H_{74}O_5$	647.5403	-0.74	b[LnA, LnG]$^+$
16.	$C_{41}H_{76}O_5$	649.5978	-0.37	b[,LG]$^+$
17.	$C_{41}H_{78}O_5$	651.5591	-1.54	b[OG, LA,OA, GS]$^+$
18.	$C_{41}H_{80}O_5$	653.0447	-2.83	b[AS]$^+$
19.	$C_{43}H_{80}O_5$	677.6691	-2.08	b[LnB]$^+$
20.	$C_{43}H_{82}O_5$	680.5545	0.04	b[LB]$^+$
21.	$C_{45}H_{88}O_5$	709.6644	-4.37	b[AB,GB]$^+$
22.	$C_{55}H_{96}O_6$	854.7364	0.05	c[PLnLn]$^+$
23.	$C_{55}H_{102}O_6$	862.6908	0.89	c[PSS]$^+$
24.	$C_{57}H_{92}O_6$	874.7808	-2.87	c[LnLnLn]$^+$
25.	$C_{57}H_{94}O_6$	875.7674	2.84	c[LLnLn]$^+$
26.	$C_{57}H_{98}O_6$	879.7434	-0.79	c[LLL, SLnLn]$^+$
27.	$C_{57}H_{100}O_6$	881.7084	-2.70	[SOO]$^+$
28.	$C_{57}H_{102}O_6$	883.7738	-1.7	c[LOO]$^+$
29.	$C_{53}H_{102}O_6$	884.7497	-3.77	c[LSS]$^+$
30.	$C_{57}H_{104}O_6$	886.7672	4.69	c[OOO, SSS, OSS]$^+$
31.	$C_{57}H_{110}O_6$	892.7832	0.94	c[PBP]$^+$
32.	$C_{59}H_{100}O_6$	906.7822	-0.75	c[GLnLn]$^+$
34.	$C_{59}H_{102}O_6$	907.7449	3.41	c[ALnLn,ELL]$^+$
35.	$C_{59}H_{104}O_6$	910.7580	1.35	c[GLL]$^+$
36.	$C_{59}H_{106}O_6$	912.7708	-1.56	c[ALL]$^+$
37.	$C_{59}H_{108}O_6$	913.7784	-1.75	c[GOO]$^+$
38.	$C_{59}H_{108}O_6$	914.7453	0.17	c[GSS]$^+$
39.	$C_{59}H_{110}O_6$	916.7543	-0.62	c[ASS],
40.	$C_{59}H_{114}O_6$	920.7368	2.23	c[PBB]$^+$
41.	$C_{61}H_{106}O_6$	936.8060	-2.41	c[BLnLn]$^+$
42.	$C_{61}H_{108}O_6$	938.8034	3.67	c[GLnG]$^+$
43.	$C_{61}H_{112}O_6$	942.8215	2.21	c[LnAA, SGG, OGG]$^+$
44.	$C_{61}H_{114}O_6$	944.8279	2.30	c[LAA, BSS]$^+$
45.	$C_{61}H_{116}O_6$	946.8167	2.53	c[OAA,BOO]$^+$
46.	$C_{63}H_{116}O_6$	970.8408	-0.44	c[GGG]$^+$
47.	$C_{63}H_{118}O_6$	972.8525	1.53	c[EAA, AGG]

(*Continued*)

TABLE 2. (*Continued*)   Mass spectral statistics MS-TOF analysis of LUCO

S No.	Formula	Measured (M+H)+	Difference(mmu)	Identification
48.	$C_{63}H_{120}O_6$	974.8371	0.98	c[GAA]$^+$
49.	$C_{63}H_{122}O_6$	976.8440	-1.9	c[AAA]$^+$
50.	$C_{64}H_{120}O_6$	998.9008	1.03	c[LnBB]$^+$
51.	$C_{65}H_{122}O_6$	1000.9268	-0.94	c[BGG, LBB]$^{++}$
52.	$C_{65}H_{124}O_6$	1002.9171	1.35	c[OBB]$^+$
53.	$C_{65}H_{126}O_6$	1005.8879	1.57	c[BAA]$^+$

Abbreviations:
Fragment ions of LUCO are indicated by the following legend: P=Palmitic; Ln=Linolenic; L=Lenoleic; O=Oleic; G=Gondoic; B=Behenic; Stearic
a-monoglycerols fragment ion
b-diglycerols fragment ion
c-triglycerols fragment ion

TABLE 3. Fatty acid Composition of LUCO

S.No.	Fatty acid	Common Name	Abbreviation	%
1.	Hexadecanoic acid	Palmitic acid	C16:0	13.91
2	Octadecanoic acid	Stearic acid	C18:0	6.01
3.	Octadecenoic acid	Oleic acid	C18:1	21.26
4.	Octadecadieneoic acid	Linoleic acid	C18:2	52.08
5.	Octadecatrienoic acid	Linolenic acid(Ln)	C18:3	1.21
6	Eicosanoic acid	Arachidic acid	C20:0	1.78
7.	Eicosenoic acid	Gondoic acid	C20:1	0.98
8.	Docosanoic acid	Behenic acid	C22:0	1.75

647.540), oleinecosadien (OS, m/z 623.532), oleylgadolein (OG, m/z 651.559), Linolylbehenine (LnB, m/z 677.669), Linolylbehenene (LB, m/z 680.5545), archideinstearine (AS, m/z 653.0447), gadolein stearine (GS, m/z 651.559), and archadeinbehenein (AH, m/z 709.6644). The main fragment ion (m/z 601.51) was the base peak in all spectra of LUCO; the least intensive was the monoglycerol ion of its corresponding fatty acid ion (Wang *et al.,* 2014). It is, therefore, TOF-MS followed by an appropriate method for the differentiation of TAGs for the identification of compounds.

These observations confirmed that the results obtained from TOF-MS data were good and it was the choice of instrument for the screening of vegetable oil. Therefore, it is necessary to resort to multivariate techniques, which are important and proven techniques for complex data analysis (Vaclavik *et al.,* 2009). The fatty acids involved may all be the same or different number of acids in many possible combinations. It is precisely this variation which gives rise to the wide spectrum of vegetable oils. It is not possible here to write the exact fatty acid sequence of the triglycerides. TOF-MS provides us a new way for vegetable oil analysis with speed of analysis, simplicity of sample introduction, and high sensitivity (Lesiak A D *et al.,* 2014). The TOF-MS technique has been applied for the first time for the profiling of LUCO and was done successfully.

3.2. Fatty acid composition of LUCO

The result of compositional determination based on peak area of LUCO is given in Table 3. Vegetable oils have a characteristic fatty acid composition that is useful for product authentication (Tavassoli-Kafrani *et al.,* 2016). On the other hand, fatty acid composition not only depends on species but on region, climate, degree of ripeness, harvesting and processing conditions. LUCO is rich in Linoleic acid (52.08%); the fraction analyzed contains palmitic, stearic, oleic, linolenic, arachidic, gadoleic, and behenic acid (Nehdi *et al.,* 2013, 2014).

3.3. Spectral Analysis

FTIR(cm^{-1}): 3472.20, 3008.54 (-CCH=CH-C); 2926.07 (CH$_2$.symmetrical); 2854.82, asymmetrical); 1746.51 (C=O,ester); 1661.00 (-HC=CH-); 1465.33 (vibration of deformation–CH-), 1377.63 (deformation vibration of methylene group), 1238.14 (deformation vibration in the plane of =CH from the unconjugated *cis* double bonds), 1163.72 (-CCOO-C); 1099.93,722.79 (vibration of C-C) (Figure 2) (Barison *et al.,* 2010).

^{1}H NMR, CDCl$_3$, δ, ppm: 0.869–0.878 (**-CH$_3$**), 1.241–1.291 (**-CH$_2$-**), 1.597 (**-O-CO-CH$_2$-CH$_2$-CH$_2$**), 2.011–2.046 (**-CH$_2$-CH=CH**)-,2.280–2.299 (**-OCO-CH2-**), 2.742–2.774 (**=CH-CCH$_2$-CCH=**), 4.141–4.156 (**CCH$_2$-COCO-CH$_2$-C**), 4.264–4.273 (**>CHOCO-CH$_2$-C-**), 5.330–5.362 (**-CCH=CH-C-**) (Figure 3).

^{13}C NMR, CDCl$_3$, δ, ppm: 14.158–14.204 (**-CH$_3$**), 25.704, 27.279, 29.206–29.787, 31.607 (**-CH$_2$-**), 62.177 (**>CHOCO-CH$_2$-**), 68.944 (**-OCH$_2$-CH<**), 127.966–130.298 (**-CH=CH-**), 172.911–173.332 (**C=O**) (Figure 4) (Alam *et al.,* 2014).

FTIR shows –CH$_2$, -CH$_3$ symmetrical and asymmetrical absorption bands at 2926.07cm^{-1} and at 2854.82cm^{-1}. The absorption bands for **-CH=CH-** str appear at 3008.54cm^{-1} and also the carbonyl ester band at 1746.51cm^{-1}. The presence of unsaturation is also supported by characteristic peaks in the ^{1}H NMR and ^{13}C NMR spectra at 5.330–5.362 ppm and 127.966–130.298ppm, respectively. The rest of the characteristic peaks for typical functional groups in the LUCO backbone have been mentioned above.
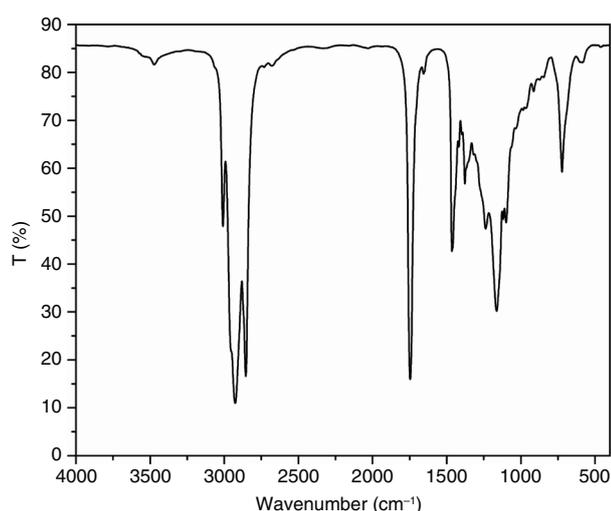
3.4. Thermal stability

DSC (Figure 5) thermogram of *L. leucocephala* oil shows two endotherms, first at -30.25 to -13.53 °C, centered at -24.51 °C, the second endotherm starting at -11.50 to 8.57 °C, centered at 3.33 °C. The first endotherm belongs to phase transition and the second to melting. The TGA-DTG (Figure 6) thermogram shows the onset of degradation at 350 °C, no weight loss was observed at this temperature; only 4% weight loss occurs at 380 °C due to moisture and volatile impurities. 50% weight loss occurs at 460 °C. The TGA thermogram shows single step degradation.
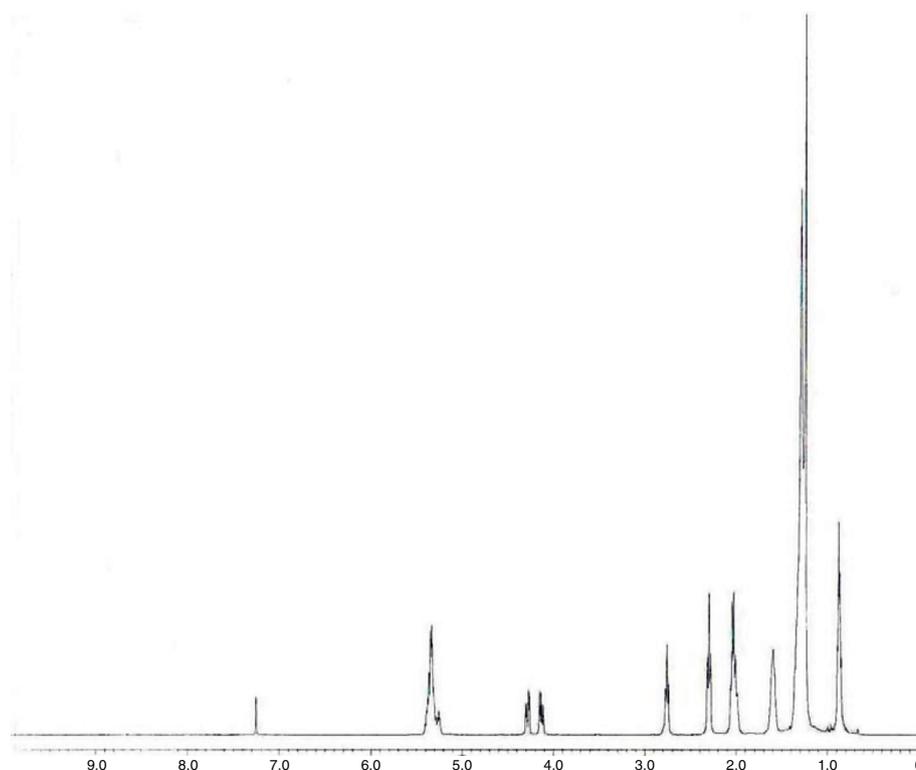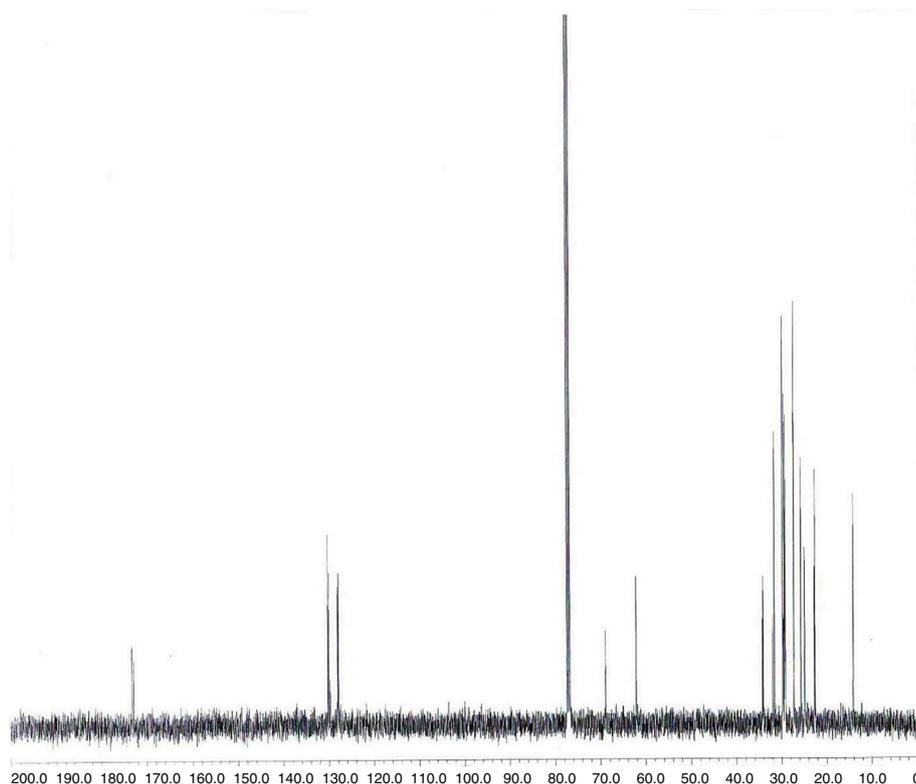

FIGURE 2. FTIR spectra of LUCO.

FIGURE 3. ^{1}H NMR Spectra of LUCO.

FIGURE 4. ^{13}C NMR Spectra of LUCO.

At 525 °C, a complete decomposition of oil occurs. This decomposition is assigned to the degradation of the hydrocarbon chain of the oil. In the DTG curve, an endothermic transition between 375 °C and 525 °C assigned to the hydrocarbons was also observed. The maximum weight loss of the sample according to the DTG curve was observed at 375 °C to 525 °C.

3.5. HepG2 cells toxicity

Figure 7 shows the growth inhibition graph of LUCO at different doses (0, 50, 100, 300, 700 μL/mL). Taking the results of all concentrations into account, the cell death in HepG2 cells was found to be higher at (700 μL/mL) by the MTT assay. This result shows that the cytotoxicity of HepG2 cells was oil dose dependent. In this study, we quantified the cytotoxicity of oil in human hepatocarcinoma cells for 24 hour by employing the MTT test. Finally, IC_{50} values were calculated for the oil according to the MTT test.

4. CONCLUSION

The TOF-MS technique was effectively applied to study the composition of the TAGs of LUCO. The applied operating conditions of the DART ion source were successfully investigated to obtain the best detection limit with high quality mass profile. The TOF-MS finger prints study could provide a useful technique for the rapid identification of vegetable oil, finding of adulteration, quality assurance, and the evaluation of similarities and improvement
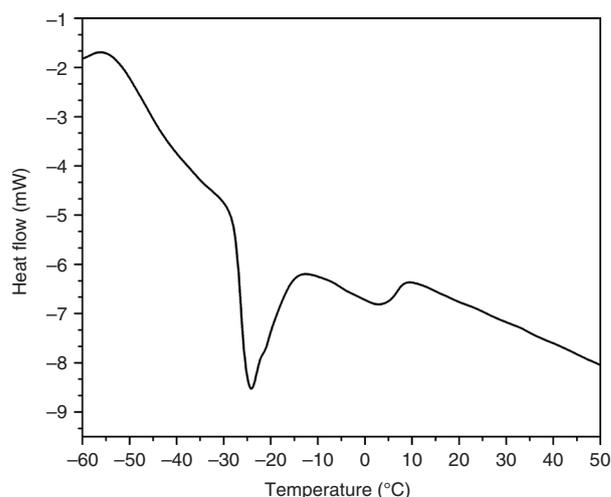

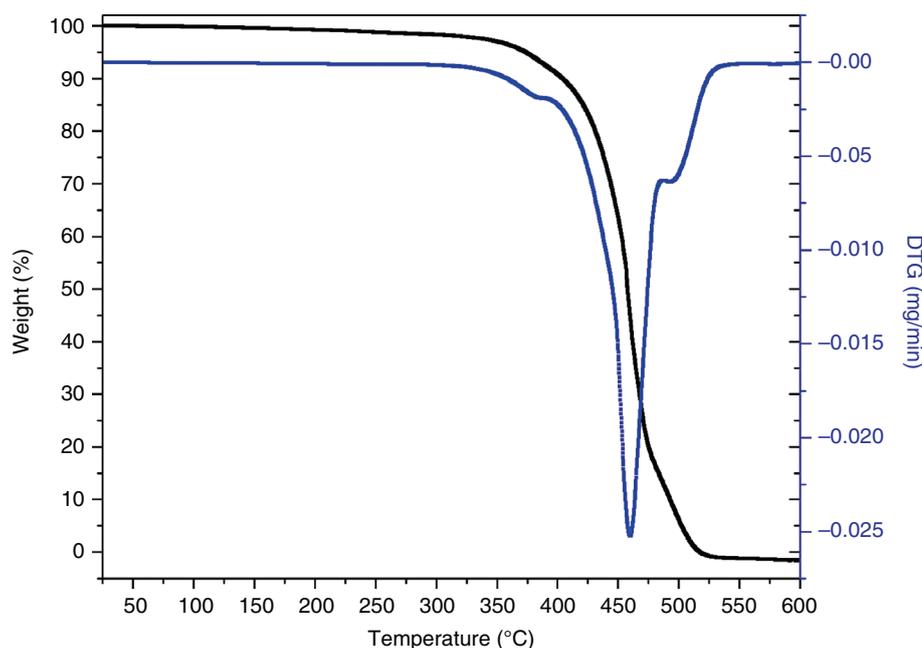FIGURE 5.   DSC thermogram of LUCO.
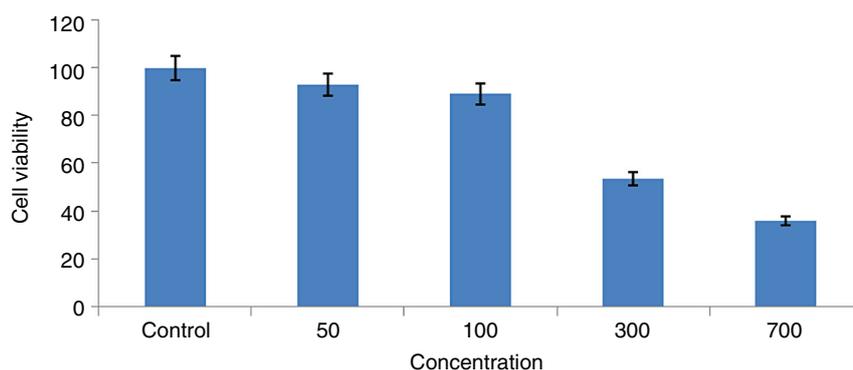
FIGURE 6.   TGA-DTG thermogram of LUCO.

FIGURE 7.   Cytotoxicity of HepG2 cells due to different concentrations of LUCO.

of product quality. The fatty acid profile plays a key role to the physico-chemical properties for further advanced research.

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