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Article

Profiling the physicochemical and solid state properties of edible *Tetracarpidium conophorum* oil and its admixtures for drug delivery

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The study is aimed at investigating the functional physicochemical and solid state characteristics of food-grade *Tetracarpidium conophorum* (*T. conophorum*) oil for possible application in the pharmaceutical industry for drug delivery. The oil was obtained by cold hexane extraction and its physicochemical properties including viscosity, pH, peroxide, acid, and thiobarbituric acid values, nutrient content, and fatty acid profile were determined. Admixtures of the oil with Softisan®154, a hydrogenated solid lipid from palm oil, were prepared to obtain matrices which were evaluated by differential scanning calorimetry, fourier-transform infrared spectroscopy, and x-ray diffractometry. Data from the study showed that *T. conophorum* oil had Newtonian flow behaviour, acidic pH, insignificant presence of hyperperoxides and malondialdehyde, contains minerals including calcium, magnesium, zinc, copper, manganese, iron, selenium, and potassium, vitamins including niacin (B3), thiamine (B1), cyanocobalamine (B12), ascorbic acid (C), and tocopherol (E), and long-chain saturated and unsaturated fatty acids including *n*-hexadecanoic acid, 9(*Z*)-octadecenoic acid, and *cis*-13-octadecenoic acid. The lipid matrices had low crystallinity and enthalpy values with increased amorphicity, and showed no destructive intermolecular interaction or incompatibility between *T. conophorum* oil and Softisan® 154. In conclusion, the results have shown that, in addition to *T. conophorum* oil being useful as food, it will also be an important excipient for the development of novel, safe, and effective lipid-based drug delivery systems.

Keywords: *Tetracarpidium conophorum* oil. Softisan®154. Lipids. Nutritional. Physicochemical. Drug delivery

INTRODUCTION

In today's world, there is intense renewed interest in the application of natural products of plants as source of raw materials in the food, cosmetics, and pharmaceutical industries because these green products are readily available, abundant, and eco-friendly. In addition, the safety, efficacy, and quality of natural products need not be demonstrated before they become commercially available because they are considered safe for human consumption. Many medicines we use today trace their origins from plant sources e.g. quinine, digoxin, aspirin, artemisinin, to name a few. Currently, vegetable oils from plants account for more than 90 % of the lipids and fats used in various regions of the world because they play

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prominent roles in human and veterinary health (Egbuonu *et al.*, 2015). Vegetable oils are made up of complex mixtures of triacylglycerols, diacylglycerols, tocopherols, and phytosterols. They are used in the manufacture of nutritional lipid emulsions in parenteral nutrition where they serve as vital source of calories and essential fatty acids for patients who are not able to swallow food. Unfortunately, a large amount of these oils are wasted during processing leading to their underutilization for solving important domestic, health, and industrial needs (Ajayi, 2010).

T. conophorum oil (also called walnut oil) is edible oil extracted from a species of African walnut belonging to the family, Euphorbiaceae, which is popular in Western and Central Africa (Amaeze *et al.*, 2011; Djikeng *et al.*, 2017). In Southern Nigeria, *T. conophorum* is a perennial climbing shrub whose nuts are processed by roasting or boiling, cracked and consumed by the local populace as a snack because it is rich in nutrients (Babalola, 2011). In addition, the Yoruba tribe of South-West Nigeria processes the nut as flour and uses it in cooking soup as a thickener (Nkwonta *et al*., 2015). Ethnomedicinally, decoctions of the nut has been reported to contain phenolic antioxidants with various biological activities including antipyretic, anti-inflammation, anti-diabetic, anti-cancer, anti-malaria, anti-diarrhoeal, and anti-hypertensive effects (Aladeokin, Umukoro, 2011). However, despite these practical evidences of the huge potentials of the nut for the food and pharmaceutical or biotechnological industries, available literature on the physicochemical properties and lipid profile of oil extracted from the nut remains relatively few (Uhunmwangho, Omoregie, 2017). In addition, to the best of our knowledge, evaluation of the infrared spectroscopic and solid state characteristics of *T. conophorum* oil and its admixtures have not been claimed elsewhere in terms of its applicability in drug delivery systems especially lipid-based drug delivery systems (LBDDS). The admixtures were prepared with Softisan®154; a hydrogenated solid lipid from palm oil which has been reported in our previous studies as an important excipient for the formulation of lipid-based drug delivery systems (Umeyor *et al.*, 2016). This forms an important aspect of the research subject because LBDDS represents an important strategy for enhancing intraluminal solubility and oral bioavailability of lipophilic or poorly-water soluble drugs (Obitte *et al*., 2018). Since oil is an important constituent of LBDDS such as lipid nanoemulsions, solid lipid nanoparticles (SLN), self-emulsifying formulations (SEFs) and the use of synthetic and semi-synthetic oils for the development of LBDDS often present safety concerns with attendant stringent regulations, it becomes imperative to explore the drug delivery potentials of natural lipids from plants which are generally regarded as safe (GRAS) as candidates for the formulation of LBDDS.

In the present study, we extracted *T. conophorum* oil by cold hexane extraction method and investigated its physicochemical properties such as pH, viscosity, acid, peroxide, and thiobarbituric acid values, nutritional composition as well as fatty acid profile without further refinement or treatment. In addition, the oil was structured using a solid lipid, Softisan®154 to prepare phytolipid matrices by fusion which was characterized by thermal analysis, fourier-transform infrared spectroscopy, and x-ray diffractometry so as to ascertain the applicability of the matrix system for improved and efficient drug delivery.

MATERIAL AND METHODS

Chemical materials

Softisan®154 (Fa.Condea Chemie GmbH, Germany), *n*-hexane (JHD, Guangdong Guanghua Sci-Tech Co., Ltd, China). All other reagents were of analytical grade and used without further treatments.

Plant material

Pods of *Tetracarpidium conophorum* were bought from a rural market in Diogbe town in Igbo-Etiti Local Government Area, Enugu State, Nigeria in June, 2017. The pods were authenticated by a plant taxonomist in the Department of Botany, Faculty of Biological Sciences, University of Nigeria, Nsukka, Enugu State, Nigeria, and a voucher specimen was deposited at the herbarium. The

plant name was checked on http://www.theplantlist.org/ on 10 August, 2019, and confirmed to be the official name. The pods were packed in net sacks to permit adequate air movement in the sacks until processing.

Processing of plant material

The pods were cleaned manually by removing particles of leaves and other organic matters. The pods were de-husked to release the seeds which were unshelled using a seed cracker to obtain the nuts. The nuts were cut to tiny bits to facilitate drying and air-dried at room temperature (27 ± 2 °C) to constant weight. After drying, the dried nuts were crushed to fines using 40 Fl.oz capacity blender (Qasa blender and grinder, Qlink Group, China), and stored in an air-tight polythene bag until use.

Extraction of oil

Extraction of oil from the milled nuts was performed by maceration technique using *n*-hexane (cold hexane extraction). This method was adapted because it is less destructive and guarantees the integrity of the extracted oil. Precisely, 50 g of milled nuts was macerated in 300 mL of *n*-hexane per extraction for 7 runs with constant stirring provided by a magnetic stirrer (Coslab, Cosmo Laboratory Equipment, Haryana, India) at 2,000 rpm for 12 h and allowed to stand for 10 min at room temperature (27 ± 2 °C). After extraction, the oil was filtered with the aid of a filter paper (Whatman No. 1). The entire oil was combined and the organic solvent (*n*-hexane) was evaporated at reduced pressure using a rotary evaporator (Stuart, Barloworld Scientific Limited, Stone, Staffordshire, UK) at 40 °C and rotation time of 1 h. Clear, golden-yellow oil was collected in screw-cap, air-tight amber bottles and stored in a refrigerator until use.

Physicochemical characterization of oil

Measurement of viscosity

The flow rate of the oil was measured using a digital rotational viscometer (NDJ-5S, Searchtech Instruments, China) with the spindle number 4 at 6, 12, 30, and 60 rpm speed levels. The spindle attached to the coupling nut was immersed in the oil up to the groove on the spindle shaft. At each shear rate, the mean of triplicate determinations was taken as the viscosity at the ambient temperature of 27 ± 2 °C.

Measurement of pH

A digital pH meter (Labtech, India) previously calibrated using buffer solutions with pH 4 and 8, was used with temperature compensation at 27 ± 2 °C to determine the pH of the oil in triplicate, and the average pH and standard deviation were calculated. Measurement was done by direct immersion of the pH meter electrode into the oil. The test was repeated after 90 days.

Determination of specific gravity

The specific gravity of the oil was determined with slight modification at 25 °C using a 50 mL density bottle which was thoroughly washed with detergent, water, and dried (USP 40, 2017). After drying, the density bottle was weighed (Pec Medical Instruments, USA) empty and its weight was recorded as W0. Then, the bottle was filled with boiled water at 25 °C and its stopper was inserted and reweighed, W1. The temperature of the oil was adjusted to 20 °C, filled in the density bottle and the overall temperature brought to 25 °C. The weight of the oil in the pycnometer was determined while removing any excess oil, and the weight obtained was recorded as W2. The specific gravity of the oil was calculated using the formula:

$$\text{Specific gravity} = \frac{W_2 - W_0}{W_1 - W_0} \quad (1)$$

Where: Wo = Weight of empty density bottle, W1 = Weight of water + density bottle, and W2 = Weight of oil + density bottle

Determination of peroxide value

This was carried out according to the method prescribed in the USP 40 (2017). An amount of the oil (5 g) was accurately weighed and placed in a 250-mL

conical flask fitted with a glass stopper. Thereafter, 30 mL of a mixture of glacial acetic acid and chloroform in the ratio (3:2) was added to the conical flask, shaken to dissolve, and 0.5 mL of saturated potassium iodide solution was added to the mixture. The mixture was shaken for exactly 1 min, and 30 mL of water was added to it. The mixture was titrated slowly with 0.01 N sodium thiosulphate with continuous shaking until the yellow colour is almost discharged. This was followed with the addition of 5 mL of starch solution, and the titration was continued with intermittent vigorous shaking until the blue colour is discharged. A blank determination was also performed under the same conditions. The peroxide value was calculated using the formula:

$$\text{Peroxide value} = \frac{[1000\,(V_t - V_b) \times N]}{W} \quad (2)$$

Where Vt = volume of 0.01 N sodium thiosulphate used in the actual test (mL), Vb= volume of 0.01 N sodium thiosulphate used in the blank test (mL), N= exact normality of the sodium thiosulphate solution, W= weight of the oil used for the test (g).

Determination of acid value

This was carried out according to the method described in the USP 40 (2017). An amount of the oil (10 g) was accurately weighed and added in 50 mL of a mixture of equal volumes of ethanol and petroleum ether (which has been neutralized to phenolphthalein with 0.1 N potassium hydroxide) contained in a flask. The mixture was warmed slowly with frequent shaking until dissolution. Then, 1 mL of phenolphthalein was added to the mixture, and titrated with 0.1N potassium hydroxide until the solution remains faintly pink after shaking for 30 s. The acid value of the oil was calculated using the relationship:

$$\text{Acid value} = (M_r \times V) \times \frac{N}{W} \quad (3)$$

Where Mr = molecular weight of potassium hydroxide, V= volume (mL), N= normality of the potassium hydroxide solution, W= weight of the oil taken (g).

Evaluation of thiobarbituric acid value

A quantity of 10 g of oil was macerated with 50 mL distilled water for 2 min; washed and transferred into a distillation flask with 47.5 mL of distilled water. An amount of 2.5 mL 4N hydrochloric acid (antifoam liquid) to bring the pH to 1.5 and a few glass beads were added to the mixture. The flask was heated by means of an electric mantle and the mixture was distilled at the rate of 5 mL/min. The distillate (5 mL) was taken in a glass-stoppered tube and 5 mL of TBA reagent (containing 90 % glacial acetic acid), shaken and heated in boiling water for 35 min. The sample was allowed to cool and absorbance measured against blank at 538 nm using 1 cm cells. Each absorbance was used to calculate the TBA value as follows:

$$\text{Thiobarbituric acid (TBA) value} = \frac{50 \times (A - B)}{M} \quad (4)$$

Where A – absorbance of the test sample, B – absorbance of the reagent blank, M – Mass of the test sample.

Determination of minerals composition

This evaluation was performed by wet digestion method. An amount of oil (1 g) was weighed into 50 mL conical flask. 20 mL of a freshly prepared mixture of concentrated nitric acid and hydrogen peroxide (2:1) and 3 mL of sulphuric acid solution were added to the flask and kept for 10 min at room temperature. The sample solution was heated on a mantle in a fume hood at 250 °C for 1 h until a clear solution was obtained. Then, the sample was evaporated and the semi-dried mass was dissolved in 5 mL 0.2 M nitric acid, then filtered and made up to final volume of 50 mL with distilled water and metal contents was determined in the diluted solutions by atomic absorption spectrometer (Varian AA240, France) and quantification was done using calibration curves of the standard minerals in triplicate.

Vitamins content analysis

The presence of ascorbic acid, tocopherol, niacin, thiamine, and cyanocobalamine in the oil was determined

and quantified. Ascorbic acid, niacin, and thiamine were quantified by titrimetry; content of tocopherol and cyanocobalamine were analyzed spectrophotometrically at 410 nm by the Futter-Mayer method, and at 361 nm respectively, as previously described (Kirk, Sawyer, 1991)

Determination of fatty acid profile

The fatty acid profile of the oil was investigated by gas chromatography coupled mass spectroscopy (GC-MS) using Agilent 6890N gas chromatograph coupled to Agilent 5973N mass selective detector (Agilent Technologies, Palo Alto, CA, USA) equipped with a cross-linked 5 % PH-ME siloxane HP5-MS capillary column (30 m × 0.25 mm, film thickness of 0.25 mm). During the analysis, the following conditions were adapted as follows: carrier gas, helium with a flow rate of 2 mL/min; column temperature, 60 – 275 °C at 4 °C min^{-1}; split injector and detector temperatures at 280 °C; sample injected volume 2 mL; split ratio of 1:50. The MS operating conditions were as follows: ionisation potential, 70 eV; ionisation current, 1A; ion source temperature, 200 °C; and resolution of 1000. Identification of fatty acid components of the oil was based on comparison of the retention times and computer matching of total ion chromatogram (TIC) of MS fragments with the National Institute of Standards and Technology (NIST) 14L spectral library. Measurement of peak areas and processing was executed using XCALIBER software while the chemical structures of the lipids were drawn using ChemOffice Ultraversion 8.0, Cambridge Soft Corporation.

Preparation of lipid matrices

The lipid matrices were prepared by fusion employing a portion of *T. conophorum* oil and Softisan®154 (S154) at oil:S154 ratios of 1:1 and 1:2. Appropriate quantities of the lipids were weighed, melted together in a thermostatically controlled water bath at 70 °C and stirred until a homogenous melt of each admixture was obtained, which was then stirred at room temperature (27 ± 2 °C) until solidification. The lipid matrices were thereafter stored in airtight and moisture-resistant plastic containers away from light.

Evaluation of drug delivery properties of the lipid matrices

Thermal analysis

Melting transitions and crystallization properties of the lipid matrices were determined using a differential scanning calorimeter (DSC) (Mettler-Toledo, Beaumont Leys, Leicester, UK). About 13.0 and 6.4 mg respectively of 1:1 and 1:2 lipid matrices were separately weighed into an aluminium-plated crucible, hermetically sealed and the thermal behaviour determined in the range of 80 – 280 °C at a heating rate of 5 °C/min. Baselines were determined using an empty crucible, and all the thermograms were baseline-corrected. Similarly, thermal analysis was extended to *T. conophorum* oil and Softisan®154 in the thermal ranges of 80 – 280 and 40 – 280 °C respectively at a heating rate of 5 °C/min.

X-ray diffraction (XRD) study

Wide angle X-ray diffraction (WAXD) analysis was performed using an x-ray diffractometer (Empyrean® Nano Edition diffractometer, Malvern Panalytical Ltd, Royston, UK) to study the crystalline structures of Softisan®154 and the lipid matrices (1:1 and 1:2) which were placed in flat sample holders respectively. The diffractometer was equipped with camera length of 480 mm, sealed high-resolution x-ray tube, temperature-controlled capillary holder, low-noise, and hybrid pixel area detectors. The diffraction patterns were measured using a Cu-based anode material (40 mA and 45 kV), and obtained data were analyzed using NGRL® Flat Programme software and filtered using Ni filter with 2θ angle of 10 - 70°.

Fourier-transform infrared (FT-IR) spectroscopic analysis

FT-IR spectroscopic analysis was conducted on a representative lipid matrix, Softisan® 154 and conophor oil using FT-IR M530 Spectrophotometer (Buck Scientific, Connecticut, USA) and the spectrum was recorded in the wavelength region of 3500 to 1000 cm^{-1} with threshold of 1.303, sensitivity of 50 and resolution of

2 cm^{-1} range. A smart attenuated total reflection (SATR) accessory was used for data collection. The potassium bromated (KBr) plate used for the study was cleaned with a tri-solvent (acetone–toluene–methanol at 3:1:1 ratio) mixture for baseline scanning. A 0.1 g of each sample was mixed with 0.1 mL nujol diluent. The solution was introduced into the KBr plate and compressed into discs by applying a pressure of 5 tons for 5 min in a hydraulic press. The pellet was placed in the light path and the spectrum obtained. Spectra were collected in 60 s using Gram A1 spectroscopy software, and the chemometrics were performed using TQ Analyzer1.

Statistical analysis

Data from each individual experiment are expressed as the mean ± standard deviation (SD) of at least triplicate determinations. All statistical analyses were performed using student *t*-test. Differences were considered to be statistically significant at $p<0.05$.

RESULT AND DISCUSSION

Viscosity

Viscosity is a measure of the resistance of a fluid to flow under shear stress. Result of the viscosity measurement showed that *T. conophorum* oil has very low viscosity ranging from 0.01 ± 0.0 to 0.02 ± 0.0 mPaS with zero twisting force (torque). The non-viscous nature of *T. conophorum* oil indicates that there was no significant ($p>0.05$) resistance to its flow under stress. It was observed that increased stirring speed (shear rate) did not cause any continuous decrease in the viscosity of the oil, suggesting that the oil has Newtonian flow character. This property is important since it might significantly influence the syringeability, extrudability, and pourability of the oil especially in the formulation of liquid and semi-solid dosage forms.

pH

The pH study was carried out to determine the pH stability of *T. conophorum* oil with time on storage at room temperature. Result of the test showed that the oil recorded acidic pH ranging from 2.1 ± 0.1 to 2.2 ± 0.0. Since significant deviation in pH with time is an indication of degradation due to rancidity as a result of peroxidation and release of aldehydic products, the result of the pH study indicates that the oil will remain stable on storage with time at room temperature. This information suggests that food production or drug formulation using *T. conophorum* oil might not necessarily require a stabilizer or an antioxidant due to the stable pH of the oil.

Specific gravity

Specific gravity is the ratio of the mass of a given volume of the oil to the mass of an equal volume of water. From the study, the obtained specific gravity of the oil was 0.9 ± 0.01 g mL^{-1}. The result is consistent with the report that most oils have specific gravity ranging from 0.91 – 0.94 g mL^{-1} (Barkatullah, Adbur, Ur-Rahman, 2012). This indicates that the oil is less dense than water and could easily flow and spread on surfaces, and will be an excellent excipient in the formulation of liquid and semi-solid dosage forms.

Peroxide value

Peroxide value (PV) is a measure of storage stability of the oil used to determine the content of primary oxidation products (mainly hydroperoxides) in oils due to oxygen, light, and temperature. The result of the test showed that the peroxide value of the oil was 8.0 ± 0.5 meq O2/kg. The obtained peroxide value (PV) of the oil is lower than the recommended peroxide value of 10 meq O2/kg for oils and fats (Djikeng *et al*., 2017). This implies that the oil is stable and contains an insignificant ($p>0.05$) amount of oxidation products especially hyperperoxides. The result also suggests that when the oil is incorporated in LBDDS, it might contribute to the prolonged shelf-lives and stability of dosage forms.

Acid value

The increase in acid value (AV) of oils might be an important measure of rancidity of foods. Result obtained

from the study showed that the acid value of the oil was 5.1 ± 0.3 mg KOH/g. The measured acid value of *T. conophorum* oil is slightly higher than 4 mgKOH/g recommended acid value for oils (Djikeng *et al*., 2017). This acid value signifies that the triglycerides of the oil could be susceptible to hydrolysis; and therefore, if the oil is not properly stored especially in air-tight, moisture-free environment, it could undergo rancidity. Furthermore, dosage forms prepared using the oil should be packaged in moisture-proof containers so as to improve their storage stability.

Thiobarbituric acid value

Thiobarbituric acid (TBA) value measures secondary oxidation products mainly malondialdehyde, which may contribute off-flavour to oxidized oil. TBA is a marker for lipid peroxidation in food materials. The TBA value of the oil was 0.577 mg MDA/kg, and this indicates that the rate of primary and secondary oxidation in the oil was significantly low. This is the consequence of low concentration of malondialdehyde, which is a secondary oxidation product obtained from the decomposition of hydroperoxides (Djikeng*et al*., 2017).

Minerals content of oil

Result of the assay showed that essential elements including calcium, magnesium, zinc, copper, manganese, iron, selenium, and potassium were found in the oil sample at varying amounts as shown in Table I. From the result, calcium content of the oil was highest with 80.51 ppm recorded, and this was followed by magnesium with a total content of 17.36 ppm, while copper was the least metal measured with an overall content of 0.039 ppm. It is important to note that the body requires these essential elements daily in small amounts in diets for optimum systemic functioning because the deficiency of these elements results in structural and functional abnormalities. The implication of this finding is that *T. conophorum* oil is food-grade oil that is safe for use as an excipient in the formulation of LBDDS.

TABLE I - Mineral content of T. Conophorum oil

Mineral	Abundance ([a]ppm) ± SD
Calcium	80.51 ± 2.1
Magnesium	17.37 ± 1.1
Zinc	0.96 ± 0.1
Copper	0.04 ± 0.0
Manganese	0.12 ± 0.0
Iron	1.12 ± 0.0
Selenium	0.19 ± 0.0
Potassium	5.65 ± 1.2

[a]ppm – parts per million. Data presented as Mean ± standard deviation (SD) at $p<0.05$

Vitamins content

The result showed the presence of niacin (vitamin B3), thiamine (vitamin B1), cyanocobalamine (vitamin B12), ascorbic acid (vitamin C), and tocopherol (vitamin E). Analysis of vitamins content of the *T. conophorum* oil showed the presence of water-soluble [B3, B1, B12, and C], and fat-soluble [E] vitamins. Ascorbic acid content at 140.8 mg/g was the highest in the oil followed by cyanocobalamine which was quantified at 16.475 mg/g, while thiamine was the lowest vitamin found in the oil at 0.27 mg/g. The water-soluble vitamins are easily absorbed while the absorption of fat-soluble vitamins is aided by pancreatic lipase enzyme or bile. It is also possible that the high content of vitamin C in the oil might have contributed to the observed stability of the oil against peroxidation due to its anti-oxidant activity.

Fatty acid profile

GC-MS analysis of the *T. conophorum* oil revealed the presence of long-chain saturated and unsaturated fatty acids at different retention times and percentage area (Table II) with their structures shown in Figure 1 and mass spectra shown in Figure 2. Precisely, three major fatty acids including *n*-hexadecanoic acid, 9(*Z*)-octadecenoic acid, and *cis*-13-octadecenoic acid were found in abundance in the oil. *N*-hexadecanoic acid

(palmitic acid) is a saturated fatty acid (SFA) found in animals, plants and microorganisms. The anti-inflammatory and cytotoxic potentials of *n*-hexadecanoic acid have been reported (Aparna *et al.*, 2012; Ravi, Krishnan, 2017). In addition, it is an important vehicle exploited in lipid-based formulations and drug delivery systems (Goon *et al.*, 2019). 9(*Z*)-octadecenoic acid (oleic acid) is an essential monounsaturated omega-9 fatty acid (MUFA) found in various animal and vegetable oils. It is a component of many foods and its consumption is associated with decreased low-density lipoprotein (LDL) cholesterol and increased high-density lipoprotein (HDL) cholesterol (Di Nicolantonio, O'Keefe, 2018). It has been reported as a penetration enhancer in topical delivery of drugs to provide sustained release effect (Pawan *et al.*, 2018). *cis*-13-octadecenoic acid is a long-chain MUFA prominently found in cell membranes, cooked food and fish. It also serves as an important adjuvant in the manufacture of cosmetics (Gupta, Kumar, 2017).

TABLE II - Fatty acid constituents identified in T. Conophorum oil by GC-MS analysis

Plant material	Compound name	Molecular formula	Molecular weight (g/mol)	RT (min^{-1})	PA (%)
Tetracarpidium conophorum oil	*n*-hexadecanoic acid	$C_{16}H_{32}O_2$	256.0	52.09	63.23
	9(*Z*)-octadecenoic acid	$C_{18}H_{34}O_2$	282.2	58.24	27.62
	cis-13-octadecenoic acid	$C_{18}H_{34}O_2$	282.3	58.46	9.15

The mass spectra of n-hexadecanoic acid, 9(Z)-octadecenoic acid, and cis-13-octadecenoic acid showed molecular ion peak at m/z 256.0, 282.2, and 282.3 respectively. RT – retention time, PA – peak area

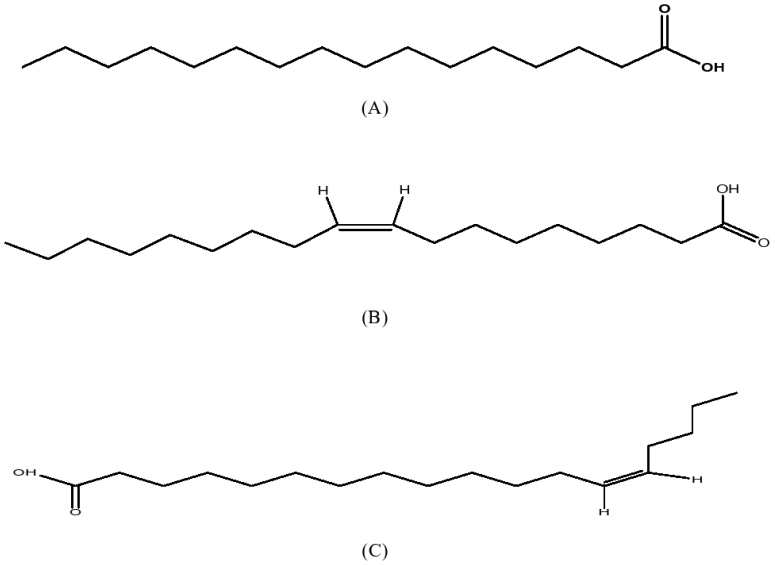
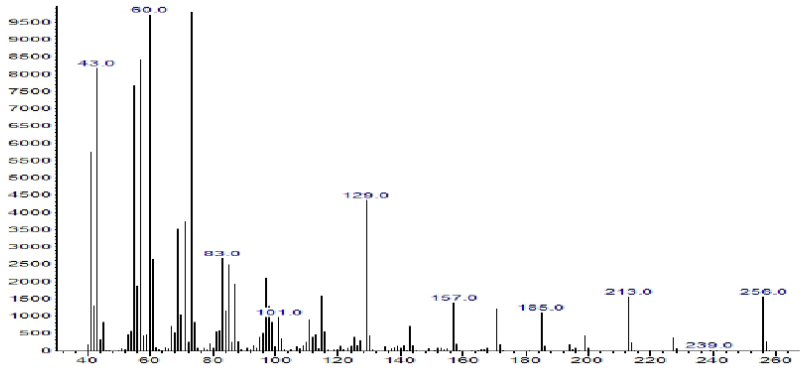
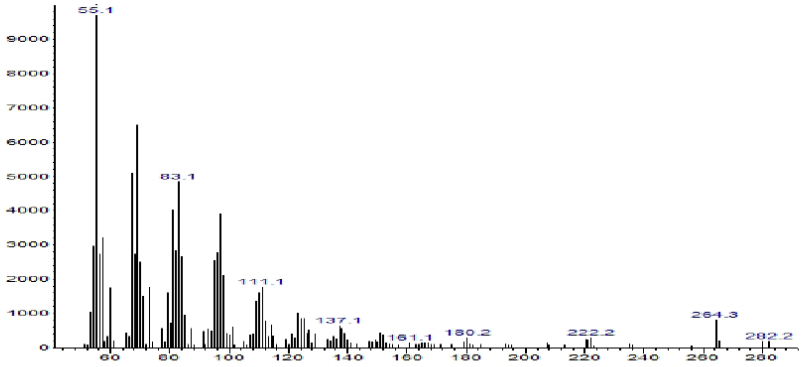


FIGURE 1 - Structures of fatty acids identified in *Tetracarpidium conophorum* oil:

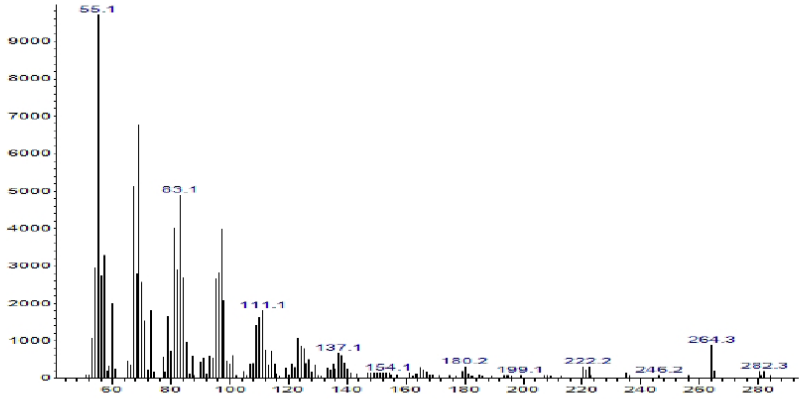
(A) *n*-hexadecanoic acid (B) 9(*Z*)-octadecenoic acid, and (C) *cis*-13-octadecenoic acid.



(A)



(B)



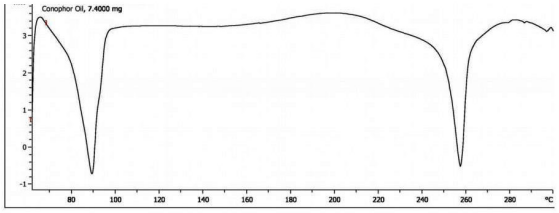
(C)

FIGURE 2 - Mass spectra showing the fatty acids identified in *Tetracarpidium conophorum* oil (A) *n*-hexadecanoic acid (B) 9(*Z*)-octadecenoic acid, and (C) *cis*-13-octadecenoic acid.

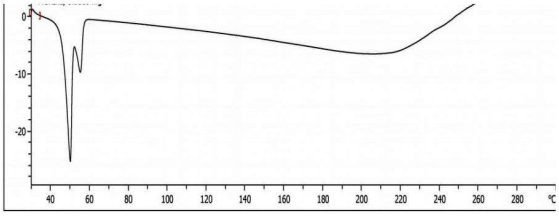
Thermal analysis

Result of thermal analysis is shown in Figure 3. The DSC thermogram of the oil showed dual sharp, endothermic peaks with an onset temperature at 89.61 °C which peaked at 257.47 °C with an enthalpy of -3.22 mW/mg. On the other hand, the DSC thermogram of Softisan® 154 gave a single, sharp endothermic peak at 51.69 °C with an enthalpy of –7.17 mW/mg. The DSC thermogram of LM1 showed single endothermic melting

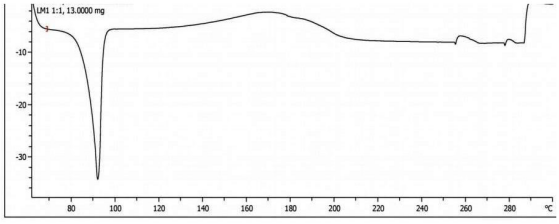
peak at 91.39 °C with an enthalpy of –4.36 mW/mg. At ratio 1:2 (LM2), the DSC thermogram also showed single endothermic peak at 85.22 °C with an enthalpy of – 3.56 mW/mg. The dual sharp, endothermic peaks observed in the DSC thermogram of *T. conophorum* oil attests to the purity of the oil. The high melting peak observed could be attributed to the presence of long-chain saturated and unsaturated fatty acids content of the oil. On the other hand, the single, sharp endothermic peak of the DSC thermogram of Softisan® 154 clearly revealed the crystalline nature and purity of the solid lipid, and the melting peak did not exceed melting point specified in the product information sheet. Fusing *T. conophorum* oil and Softisan® 154 yielded matrices with single endothermic melting peaks and low enthalpies. Since high enthalpies indicate more ordered crystal lattice structure (Kenechukwu *et al*., 2018), it was obvious from the data that the crystalline nature of the solid lipid in the matrices was rearranged following integration of the oil which led to their modification (perhaps acquiring enhanced amorphicity) generating an imperfect matrix structure in both matrices which might favour drug encapsulation.



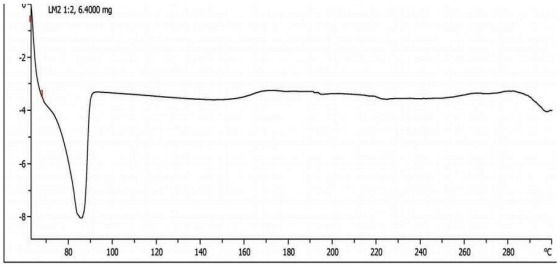
(A)



(B)



(C)

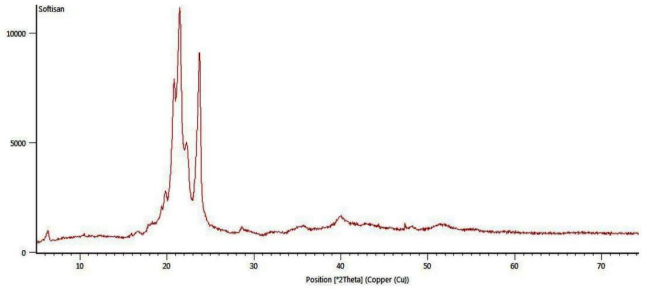


(D)

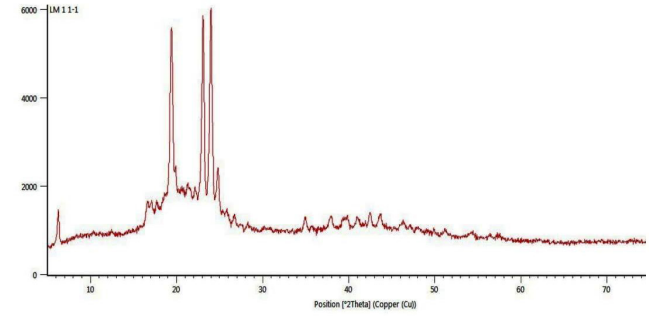
FIGURE 3 - Differential scanning calorimetry (DSC) thermograms of: (a) *T. conophorum* oil (b) Softisan®154 (c) Lipid matrix (LM) 1:1 (d) Lipid matrix (LM) 1:2.

X-ray diffractometry

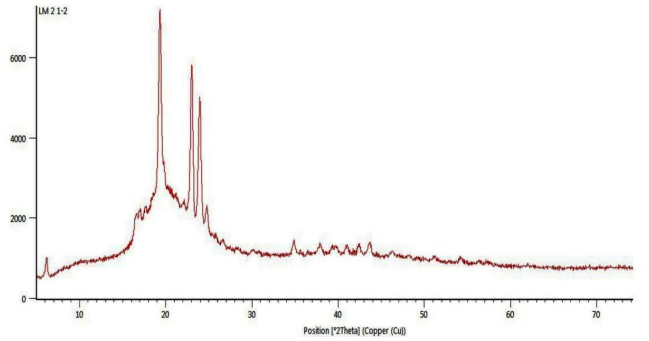
XRD diffractograms are shown in Figure 4. The XRD pattern of Softisan® 154 showed characteristic high intensity diffraction peaks at (2θ) 21°, 22°, and 24°. The XRD pattern of the lipid matrices (LM1 and LM2) showed sharp diffraction peaks at (2θ) 19°, 23°, and 24° respectively. The characteristic high intensity diffraction peaks observed in Softisan® 154 might be due to the presence of esters of low chain fatty acids (C10 – C18) and the absence of polymorphic forms in the solid lipid. In addition, there was the presence of other medium and low intensity reflections indicating the crystalline nature of Softisan® 154. The disappearance of the major diffraction peaks at (2θ) 21° and 22° observed in the XRD pattern of Softisan® 154 and the appearance of major diffraction peaks at (2θ) 19° and 23° in the XRD profile of the lipid matrices might be due to the molecular integration of the fatty acid chains in the solid lipid and *T. conophorum* oil resulting in a disordered and widened crystal lattice structure of the lipids. The widened crystal lattice gave lipid matrices with the existence of mixed crystals of Softisan® 154 and the fatty acid esters of the oil thereby creating disorders with high potential to favour drug entrapment.



(A)



(B)



(C)

FIGURE 4 - X-ray diffractograms of (a) Softisan®154 (b) Lipid matrix (LM) 1:1 (c) Lipidmatrix (LM) 1:2.

FT-IR analysis

FT-IR analysis is shown in Table III. FT-IR spectrum of *T. conophorum* oil showed principal peaks at wave numbers 3685.27 cm^{-1}, 3158.72 cm^{-1}, 2683.74 cm^{-1}, 2139.53 cm^{-1}, 1618.67 cm^{-1}, and 809.95 cm^{-1}. Softisan® 154 showed the following principal peaks: 3462.32 cm^{-1}, 2922.20 cm^{-1}, 2332.87 cm^{-1}, and 1735.80 cm^{-1}. The lipid matrix gave the following principal peaks: 3691.73 cm^{-1}, 3108.49 cm^{-1}, 2724.44 cm^{-1}, 2176.33 cm^{-1}, 1619.29 cm^{-1}, and 901.80 cm^{-1}. The existence of any adverse *T. conophorum* oil-Softisan® 154 interaction is detected by an appreciable shift of absorption bands or disappearance of important functional groups or appearance of new bands. For *T. conophorum* oil, the principal peaks observed correspond to –OH bond vibration (strong) in carboxylic acids, -OH bond (medium) in carboxylic acids, C–H stretching, C=N stretching, C=C bond stretching (strong), and aromatic C–H deformation respectively. In Softisan® 154, the principal peaks correspond to –OH bond vibration, C-H bond stretching, carboxylic acid C-OH bond vibration, and carboxylic acid C = O vibration respectively. Furthermore, the lipid matrix has principal peaks corresponding to –OH bond vibration (strong), -OH bond (medium), C–H stretching, C=N stretching, C=C bond stretching (strong), and aromatic C–H deformation respectively. The peaks found in the lipid matrix indicate there was no adverse intermolecular interaction or incompatibility between the starting excipients confirming the amorphicity of the matrices for effective drug delivery.

TABLE III - FT-IR profiles of the materials (T. conophorum oil, Sofisan® 154, and lipid matrix)

Material	Principal peak (cm^{-1})	Type of bond
T. conophorum oil	3685.27	-OH bond vibration (strong) in carboxylic acids
	3158.72	-NH stretching in carboxylic acids
	2683.74	C – H stretching
	139.53	C = N stretching
	1618.67	conjugated α, β unsaturated C = C bond
	809.95	aromatic C – Cl deformation
Softisan® 154	3462.32	-OH bond vibration
	2922.20 2332.87	C-H bond stretching
	1735.80	carboxylic acid C-OH bond vibration
		Carboxylic acid C = O vibration
Lipid matrix	3691.73	-OH bond vibration (strong)
	3108.49	-NH stretching
	2724.44	C – H stretching
	2176.33	C = N stretching
	1619.29	conjugated α, β unsaturated C = C bond
	901.80	aromatic C – Cl deformation

CONCLUSION

In conclusion, given the need to develop safe and eco-friendly excipients from plants for use in food and pharmaceutical manufacturing, investigation of the physicochemical, nutritional, infrared spectroscopic, and fatty acid profiles of *T. conophorum* oil forms an interesting research subject. Data from the study showed that *T. conophorum* oil had Newtonian flow behaviour, acidic pH, good stability due to insignificant hyperperoxides and malondialdehyde content, and long-chain saturated and unsaturated fatty acids.

Softisan® 154-templated lipid matrix of the oil showed low crystallinity and enthalpy values with increased amorphicity, no destructive intermolecular interaction or incompatibility between the oil and Softisan® 154, and imperfect crystal morphology with high potential for drug entrapment. These properties have shown that, in addition to the nuts of *T. conophorum* being useful as food; its oil is an important excipient for the development of safe and effective lipid-based drug delivery systems. Looking ahead, there may be need to treat *T. conophorum* oil with enzymes through lipase-catalyzed transesterification in order to produce structured triglycerides which will be applied in improving the solubility and absorption of poorly soluble drugs.

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