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Determination of the Edibility of Thevita Peruviana Seed Oil Using GC-MS, FTIR and UV-VIS Analysis

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ABSTRACT

The objective of the present work is to characterize and derive added utility value of thevetia seed oil extracted from seeds obtained from within our local environment. The search for complimentary edible oils prompts the research into the inherent properties of seed oils from ornamental plants in order to added utility status to these plants and encourage the sustenance of their plantation and agronomy. Thevetia plant is presently an ornamental plant used for hedge and beautification. A yellow to golden colour oil was extracted using Petroleum ether and proximate analyses were carried out on the sample obtained. The seed oil yield was 62%, having a pH of 4.1, congealing temperature of -13^{0}C, burning with non-sooty, Specific gravity of 0.9018g/cm @ 29^{o}C and Viscosity of 126.1mpa.s @ 28^{o}C. was obtained from the plant(can be omitted). FTIR result showed a strong Aldehyde (HC=O) peak at 2859.56 and a medium strength peak at 2677.9. Medium strength cyanide (=C=N-) peaks were observed at 2035.93 and 2343.59. UV-VIS analysis confirmed the presence of Vitamins A, B_2, B_9, C and E (-5.1x 10^{-6}, 0.047, 0.00197, 6.751 and 0.0719 mg/ml respectively), with vitamin E & C having the highest concentrations (concentrations of AAAA and BBBB respectively). The GC-MS spectra results show a high degree of unsaturation and the presence of reasonable amounts of free fatty acids with the presence of Palmitic acid and its methyl ester Linoleic acid, trans-oleic acid, Arachidic acid methyl ester and Behenic acid methyl ester among others as peaks 1,2,3,6,8 and 9. The presence of more than one fatty acid in the oil gives it the status of a mixed triglyceride or compound glyceride. The result buttresses the nutritional potentials of the oil when adequately processed to remove the traces of cyanide contained in it. The low value of the congealing temperature of the oil is an indication that the oil can be used both in tropical and temperate areas.
KEYWORDS: Thevetia, oil, edible, toxicity, potentials.

INTRODUCTION

Thevetia peruviana is a plant native to central and southern Mexico and central America. It is an evergreen tropical shrub that belongs to *Apocyanacease* family [1] or small trees which is cultivated as an ornamental plant and planted as large flowering shrub or small ornamental tree in temperate climate. It tolerates most soils and is drought tolerant. In Nigeria, *Thevetia Peruviana* has been grown for over fifty years as an ornamental plant in our environment [2]. *Thevetia* seed has a similar chemical composition to soybean seed but the *Thevetia Peruviana* yields an oil that contain (Omit) 60-65% oil with a residual cake that comprises of 30-37% of proteins. Notwithstanding the high level of oil and proteins in the seed, it remains a non-edible lesser known (omit 'lesser known') oil seed. The bark of the *Thevetia Peruviana* is a powerful anti-periodic and febrifuge. It is sited that the seed has a nutritional potential that compares favourably to conventional oilseeds and be (omit 'and be') which make it suitable for use in humans and animals[3]. According to [4] and [5], the crude protein content of the defatted seed ranges from 42.72-47.50/100g of the seed cake while crude lipid ranges from 4.40-4.80/100g. The *Thevetia* oilseed contains toxic glycoside, a bitter principle with a powerful cardiac action [6] and[7]. The toxic glycoside include thevetin, theveridoside, digitoxiginin, cerberin, peruvoside and theveside with thevetin reported by [8] to be the major toxic glycoside with about 3.6-4.0% in the thevetia kernel. The toxicity of the glycoside is reflected in accidental poisonings that occur among children that feed on the seed of the plants [9] It is also reported that the kernel of about ten fruits may be fatal to an adult while the kernel of one fruit may be fatal to a child [10]. The glycoside extract of the *Thevetin* seed is used as a heart stimulant but in its natural form is extremely poisonous, as are all parts of the plant especially the seed. Attempts to improve the nutritive value of *Thevetia* were made by using various methods, however fermentation presented some advantages. Fermentation reduced the cardiac glycoside and gave about 20% improvement in broiler growth as compared to unfermented cake when applied as animal feed. The aim of this study is to determine the edibility of *Thevita Peruviana* seed oil using GC-MS, FTIR and UV-VIS analysis.

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METHODS AND MATERIALS

Preparation and Extraction of Samples

The thevetia seeds were dried, deshelled and kernels were further cleaned to remove dirt and any contaminant that might have been picked up during the processing. They were then grounded using a mechanical blender. Seed oil was extracted from the kernel by Soxhlet extraction. The thevetia seeds were placed inside a "thimble" made from thick filter paper, which was loaded into the main chamber of the Soxhlet extractor. The Soxhlet extractor was placed onto a flask containing the extraction solvent. The Soxhlet was then equipped with a condenser. The solvent was heated on a traditional hotplate to reflux. The solvent vapour travelled up the distillation arm, and flooded into the chamber housing the thimble of seeds. The condenser was in place to ensure that solvent vapour was cooled, causing the condensate to fall back down into the chamber housing the seeds. The chamber containing the seeds was slowly filled with solvent. The desired oil was dissolved in the solvent. When the Soxhlet chamber was full, the chamber was automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. This cycle was allowed to repeat many times. During each cycle, a portion of the non-volatile compound was dissolved in the solvent. After many cycles the desired compound was concentrated in the distillation flask. The advantage of this system is that instead of many portions of solvent being passed through the sample, just one batch of solvent is recycled.
The extract solvent was removed by simple distillation to obtain the crude oil sample. The oil obtained was then subjected to analysis as indicated below.

FTIR

The FTIR analysis was carried out using SHIMADZU FTIR-8400S Spectrophotometer with a NaCl cell.

UV-VIS

The UV-VIS analysis was obtained with SHIMADZU UV-2500PC series. The analytical condition was set as wavelength range (nm) 200.00 to 900.00, sampling interval of 0.5. Auto scan mode, measuring mode (absorbance), slit width of 2.0 and light source change wavelength of 360.0nm.

GC-MS

GC / MS. Shimadzu Model (QP-2010 Plus) Mass Spectrometer.

Rtx1(100% Dimethyl Polysiloxane) coated fused silica capillary column.
Helium Carrier Gas (1.34 ml/minute). 220°C injection temperature.
250°C interface temperature. 200°C Ion Source Temperature.
Colunm (30m x 0.25mm x 0.25µm)
Column oven temperature programmed at 60°C/Hold 5mins with 5°C / min rise to 140°C rise @ 15^0C/min to 280^0C Hold for 10mins. Column Flow 1.61mL/min
Threshold 3000. Solvent cut time 2.50min. GC/MS detection ionization energy 70ev

Flame Nature And The Congealing Temperature.

The tests were carried out as reported in [11].

pH

pH of the oil was determined according to the official method of analysis of the Society of Leather Trades' Chemists SLTC [12].

RESULTS

The FTIR spectroscopic results,

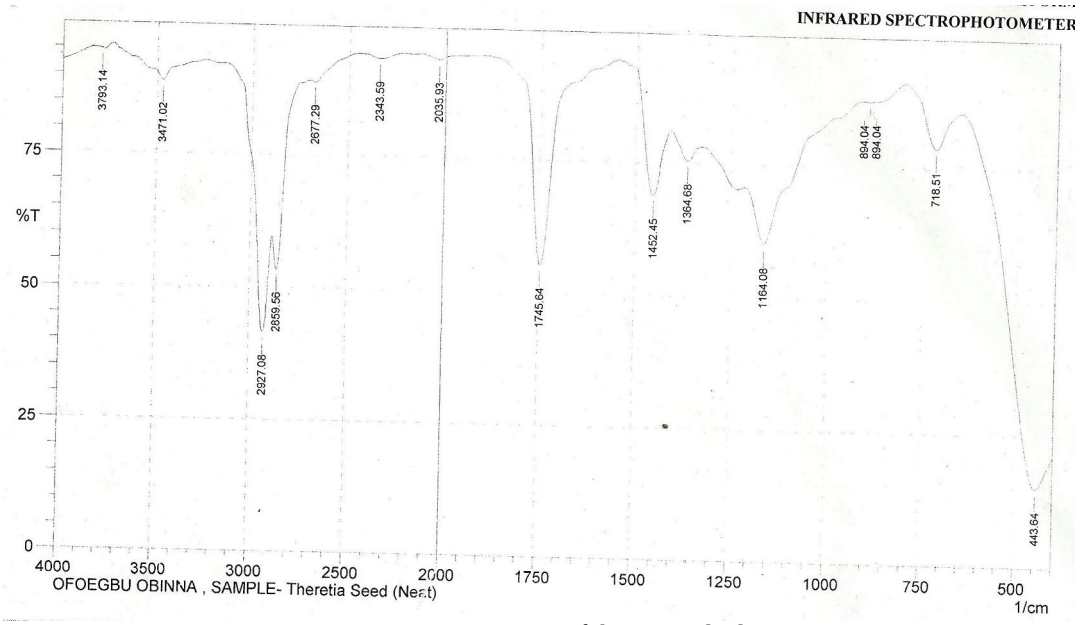

Figure 1: FTIR spectra of thevita seed oil

Table I, presents nine functional groups contained in the sample with expected alkanes, aldehydes and carbonyl functional groups but with a cyanide functional group present at the 2035.93 and 2343.59 wavelengths of absorption.

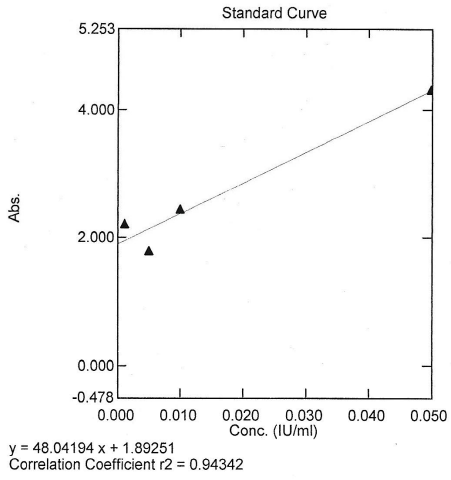

Fig2: Vitamin A

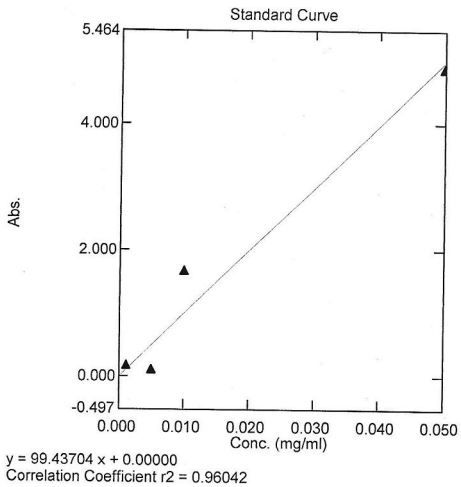

Fig 3: Vitamin B2

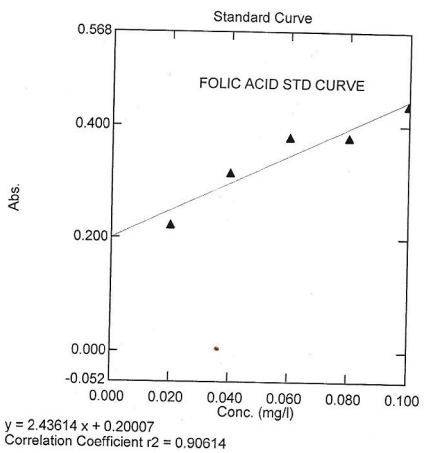

Fig 4: Vitamin B9

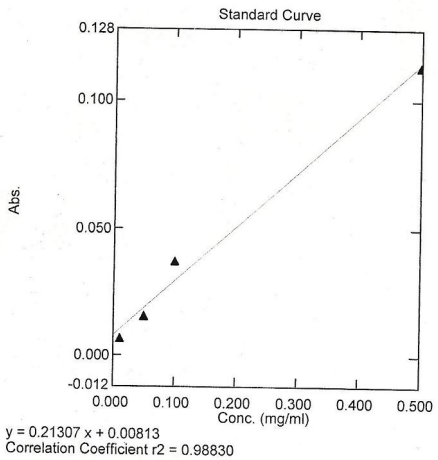

Fig 5: Vitamin C

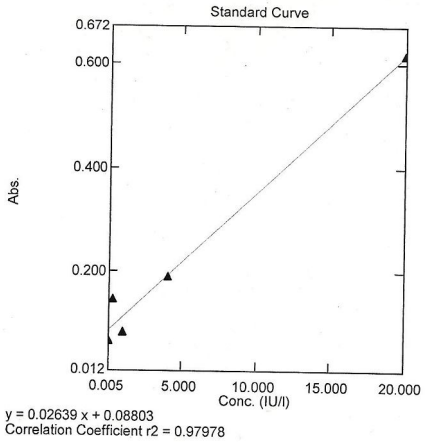

Fig 6: Vitamin E

Figures 2-6: UV-VIS Spectra of thevita seed oil with plot of absorbance against concentration for various vitamins.
Table II, confirms the presence of Vitamins A (- 5.1x 10^{-6}), B (0.047), B_9(0.00197),
C(6.751) and E (0.0719).

Table 3 presents the values of the physiochemical analysis result showing the oil to have a viscosity at 28^0C of 126.1mpa. specific gravity of 0.9018g/cm at 29^0C, e.t.c. The oil has a congealing temperature range of – 13^0C to - 22^0C and when burnt gave a non sooty flame nature.
The GCMS analysis result,

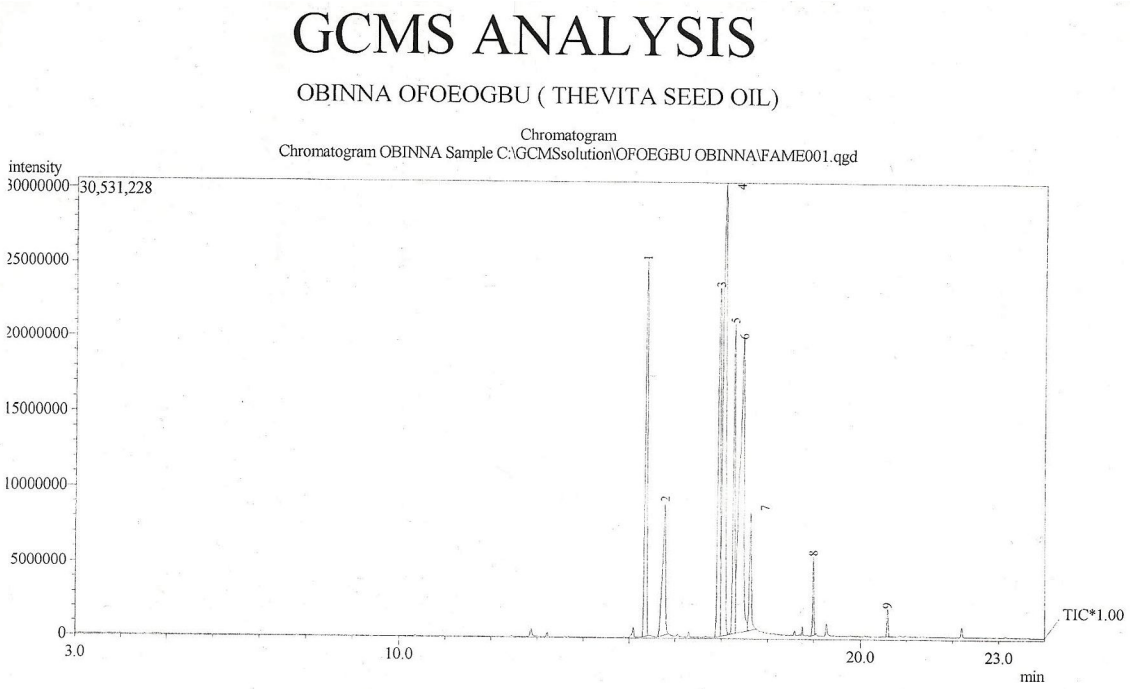

Figure 7: GC-MS spectra of thevita seed oil

Table 4 shows the presence of Palmitic acid methyl ester, Palmitic acid, Linoleic acid methyl ester, Elaidic acid methyl ester, Steric acid methyl ester, Trans-Oleic acid, Stearic acid, Arachidic acid methyl ester and Behenic acid methyl ester.

DISCUSSION

The FTIR peaks (Fig 1), show very strong and broad hydroxyl peaks at wavelengths of 3471.02 and 3793.14. A variable alkene C=C bond at wavelength of 1452.45, a medium strength cyanide bond at wavelengths of 2035.93 and 2343.59 which confers a degree of toxicity to the oil confirming the effect of the compositional presence of thevitin (toxic component of the oil). At wavelengths of 2677.9 and 2859.56 the oil showed medium and strong bond strengths respectively of Aldehyde functionality.

The UV-VIS analysis results (Figs 2-6 and Table 2) confirm the presence of vitamins A, B_2, B_9, C and E which are important vitamins essential for the proper functioning of the brain and other parts of the body. Interesting is the combinatory composition of water soluble vitamin C (6.751mg/ml) and the oil soluble vitamin E (0.0719mg/ml) in the oil. The amount of Vitamin E reported for thevetia is high compared to some edible oils for example refined peanut oil 212-217 µg/g (as tocopherol) and 295-636 µg/g (as tocopherol) for Corn oil. [13]

The proximate analysis results obtained (Table 3), are in close values with reported values by earlier researchers (Ibeyimi et.al.) though with difference in oil yield of 0.99%. The results show the tendency of the oil to be applied to various culinary uses but not before further thermal treatment because of the observed presence of cyanide in the oil.

The identification of Linoleic acid, Palmitic acid, Trans-Oleic and Behenic acids among the nine identified methyl esters from the GC-MS spectra (Fig 7 and Table 4) creates a utility status for the oil as a good source of edible long chain fatty acid triglyceride. Oleic acid lowers the risk of a heart attack, arteriosclerosis an aid in cancer prevention, a combination of omega 3, omega 6, omega 9 which make up the oleic acid fatty acid, and essential lipids contribute to the healthy living condition of consumers by improving cell membrane integrity, assist in restoring cellular and tissue damage, optimize neurological transmission, improve heart and circulatory function, produce supple and moist skin, where consistent consumption is maintained.

The non sooty flame nature of the oil is also a direct correlation of the content of free fatty acids present. The higher the relative percentage of free fatty acid, the lower the smoke point for instance with the data, a free fatty acid content of 0.01 %, a corresponding smoke point of 232^0C is obtained, while for a free fatty acid content of 100 %, a smoke point of 93^0C,[11]. This gives an indication that the smoke point of the oil acid value of 1.26 and free fatty acid of 0.63 is about 50% which is between 232^0C and 93^0C but definitely close to 93^0C. The implication of t

A congealing temperature range of – 13^0C - 22^0C makes it useful in both the tropics and temperate regions of the world, with underpinning constant fluidity at even relatively cold environment. This is a quick assertion of the fact that it contains no saturated fat and therefore healthy for consumption.

The low pH value corresponds to the observed presence of reasonable amount of fatty acids in the oil. This is a desirable property in edible oils because it indicates the presense of fatty acids like Palmitic and Oleic acids. [13] Acid value represents free fatty acid content due to

Enzymatic activity and is usually indicates spoilage. The maximum acceptable level permissible for edible oils is 4mgKOH/g, below which the oil is acceptable for consumption. The FFA value for the oils was determined to be 0.63mgKOH/g, which is well within the stated limited for edible oils [14].

The iodine value is a measure of the unsaturation of fats and oils; therefore, the value obtained in analysis: 78.94gI_2/100g oil does not suggest high unsaturation. Oils with values less than 112gI2/100 g oil may find use in the confectionery industry. Oils having high unsaturation of fatty acids, when heated are prone to polymerization of the glycerides, causing formation of deposits, and thereby compromising oxidative stability.27

In comparison to [14], peroxide values of 3.92meq/kg of the oil impacts stability on the oil ensuring a reasonable shelf life and durability which is required for edible oils (that is the oil does not easily become rancid).

CONCLUSION

Edible oil with potential essential fatty acids, can be obtained from the seed oil of thevetia plant after further processing which will involve moderate temperature application.

The presence of Linoleic acid having the alpha-linolenic acid (Omega 3) and linoleic acid (Omega 6), which are both sensitive to destruction by light, oxygen, and high temperature initiate damage to these compounds within the oil and production of relatively toxic components when the oil is used for frying. Consequently the oil is not recommended for cooking processes that involve deep frying. More so, such frying processes also destroy the Vitamins contained and reduce the nutritional potentials of the oil.

The growing of the plant both around the houses and as plantations should be encouraged with the setting up of cottage oil extracting units both in homes and on farms.

Non heat treatments should form the focus of further research in the treatment and removal of the toxic components of the oil considering the utility potentials of the oil with respect to it being introduced into the consumer food chain.

The planting of thevetia will boost the fight against drought, famine and will also provide good cover for carbon sequestration and protection of the environment.

Table 1. FTIR ANALYSIS OF THE THEVETIA SEED OIL

S/No	Peak	Functional Group	Intensity	Compound	Strenght
1	718.51	H-C-H	78.754	alkane	Weak
2	894.04	C-H	87.589	alkane	Strong
3	894.04	C-H	87.589	alkane	Strong
4	1164.08	R-O-R	60.069	ether	Strong
5	1364.08	H-C-H	75.479	alkane	Medium
6	1452.45	C=C	68.836	alkene	Variable
7	1745.64	RCOR	55.33	ketone	Strong
8	2035.93	= C=N-	93.693	Cyanide	Medium
9	2343.59	= C=N-	93.640	Cyanide	Medium
10	2677.9	HC=O	88.915	aldehyde	Medium
11	2859.56	HC=O	53.378	aldehyde	Strong
12	2927.08	C-H	41.457	alkane	Variable
13	3471.02	O-H	88.760	hydroxyl	Very strong & broad
14	3793.14	O-H	94.578	hydroxyl	Very strong & broad

Table 2. UV –VIS analysis of thevita seed oil showing the vitamins concentration.

Vitamins	mg/ml
1. $Vit._A$	$- 5.1x\ 10^{-6}$
2. $Vit._{B2}$	0.047
3. $Vit._{B9}$	0.00197
4. $Vit._C$	6.751
5. $Vit._E$	0.0719

Table 3. Physicochemical Analysis of Oil

Parameter	Value
1. Viscosity@28^{0}C	126.1mpa.s
2. Specific gravity @ 29^{0}C	0.9018g/cm
3. Acid value	1.26mgKOH/s
4. Free fatty acid	0.63mgKOH/g
5. Saponification value	123.92mgKOH/g
6. Iodine value	78.94
7. Oil yield	62.31%
8. Peroxide Value	3.92
9. pH	4.1
10. Congealing temperature	-13^{0}C to -22^{0}C
11. Flame nature	Sootless

Table 4. GC-MS spectra analysis of thevita seed oil

S/No	Retention Time	Name Of Compound	Molecular Formula	Molecular Weight	Mass Peak
1	15.36	Palmitic acid methyl ester	$C_{17}H_{34}O_2$	270	143
2	15.772	Palmitic acid	$C_{16}H_{32}O_2$	256	105
3	16.946	Linoleic acid methyl ester	$C_{19}H_{34}O_2$	294	184
4	17.065	Elaidic acid methyl ester	$C_{19}H_{36}O_2$	296	201
5	17.265	Steric acid methyl ester	$C_{19}H_{38}O_2$	298	151
6	17.461	Trans-Oleic acid	$C_{18}H_{34}O_2$	282	161
7	17.627	Stearic acid	$C_{18}H_{36}O_2$	284	133
8	18.984	Arachidic acid methyl ester	$C_{21}H_{42}O_2$	326	100
9	20.586	Behenic acid methyl ester	$C_{23}H_{46}O_2$	354	89

Acknowledgment

The authors declare that they have no conflicts of interest in this research.

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