

Physicochemical, Toxicity and Antioxidant Activity of *Terminalia catappa* Kernel Oil in Mice

Emmanuel Mshelia Halilu^{1,4,*}, Chinenye Jane Ugwah-Oguejiofor², Göksu Oduncuoğlu¹, Solomon Gamde Matthias³

¹Department of Pharmacognosy, Faculty of Pharmacy, Cyprus International University, Nicosia, North Cyprus, Mersin 10, TURKEY.

²Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, NIGERIA.

³Department of Histopathology, Faculty of Health Sciences and Technology, University of Jos, Plateau, NIGERIA.

⁴Department of Pharmacognosy and Ethnopharmacy, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, NIGERIA.

ABSTRACT

Background: *Terminalia catappa* Linn. is an ornamental tree belonging to the family Combretaceae. This study was aimed at evaluating the toxicity profile and antioxidant activity of the oil. **Materials and Methods:** Oral acute and sub-acute toxicity were studied in mice using the Organization for Economic Co-operation and Development (OECD) guidelines. The antioxidant activity was assessed using DPPH free radical. **Results:** *Terminalia catappa seed* oil demonstrated antioxidant activity by scavenging DPPH free radical with IC₅₀ of 1.425 mg/mL while tannic acid (standard) was 5.005 mg/mL. The oral LD₅₀ of the oil in mice was greater than 5000 mg/kg. The sub-acute toxicity study, produced no significant difference in the weight and biochemical parameters in treated animals compared to the control. **Conclusion:** The oil was also non-toxic to the liver, kidney, heart and lungs on histopathological examination. Our results indicated that *Terminalia catappa seed* oil is not toxic and contains beneficial phytochemical that demonstrated antioxidant activity.

Keywords: Antioxidant, DPPH, Phytochemical, *Terminalia catappa*, Toxicity.

Correspondence:

Dr. Emmanuel Mshelia Halilu
Department of Pharmacognosy
and Ethnopharmacy, Faculty of
Pharmaceutical Sciences, Usmanu
Danfodiyo University, Sokoto, NIGERIA.
Email id: emshelia2002@gmail.com

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INTRODUCTION

An oil is a chemical compound that is mainly made of hydrocarbons and it has both lipophilic and hydrophobic characteristics. Plants are the main source of vegetable oils and have been used as major component of human diet.^[1,2] These vegetable oils are obtained mainly from plant seeds and other parts of fruits.^[3] Edible vegetable oils are liquids at room temperature and are mainly mixtures of triglycerides that comprises of up to three fatty acids attached to a propan-1,2,3-triol via ester linkage.^[4] Oils are a key source of nutrient as they been reported to offer higher calories when compared with carbohydrates.^[3,5] Some oils from the plant origin have been reported to contain high amount of cholesterol and may be a potential health risk to humans.^[6] The vegetable oils are composed of various fatty acids.^[7,8] The oils also have different

compositions in non-glyceride components including phenolics, steroids, squalene and tocopherols-Vitamin E.^[2]

Human beings have used plants and their products in raw and prepared forms without evaluation of their toxic effects on the liver, kidney, heart and lungs.^[9] The consumption of these products without thorough evaluation of their toxicity is based on the premise that they are safe. Oils have been used since the time immemorial by the ancient civilizations for their therapeutic properties. Antioxidant, anticancer, gastro protective and antimicrobial properties of oils have been reported.^[10,11]

Terminalia catappa Linn. belongs to the family Combretaceae.^[12] It is a tree mainly planted for ornamental purpose.^[12] The tree produces an edible fruit with one hard nut each.^[13] The hard nut also contains an edible kernel. The leaves (Figure 1-A) have been reported to demonstrate anti-*Helicobacter pylori* activity and gastroprotective property.^[14] *Terminalia catappa* fruits (Figure 1-B) and kernels (Figure 1-C) have been utilized for nutritional purposes but with no detailed assessment of toxicological profile especially that of the oil. Therefore, this study was aimed at investigating the acute and sub-acute toxicity profile of the seed oil in mice.



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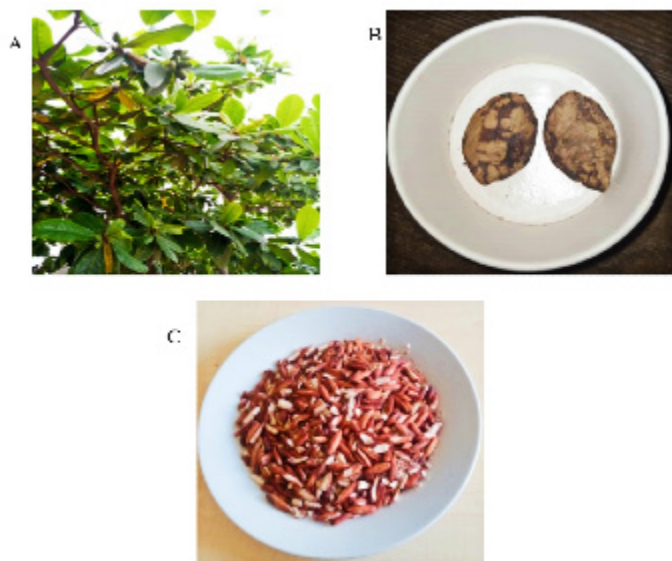


Figure 1: A) *Terminalia catappa* tree (B) *Terminalia catappa* fruits (C) *Terminalia catappa* Kernel.

MATERIALS AND METHODS

Plant collection, identification and preparation

Terminalia catappa seeds were collected at Tamaje Area, Sokoto-Nigeria by Dr. H.E. Mshelia in January, 2021. The plant was identified via taxonomic means and herbarium specimen with number PCG/UDUS/COMB/0005 was prepared and then kept for future reference at the Herbarium of the Department of Pharmacognosy and Ethnomedicine, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto. The seeds were allowed to dry for 28 days and the kernel was obtained by cracking the hard nut with stone. The kernels were powdered using a blender.

Evaluation of physicochemical parameters of the powder kernel

These include the determination of the moisture content, total ash value, acid insoluble ash value, alcohol soluble extractive value and water soluble extractive value. These parameters were evaluated according the methods described by Halilu Mem, et al.^[15] The methods are described briefly.

The moisture content

The moisture content was determined by loss of weight on drying method. The powder sample (2 g) was transferred to an already weighed empty porcelain evaporating dish and the total weight was noted. The porcelain with its content was placed in an oven at 105°C for 1 hr. It was removed and then allowed to cool in a desiccator. Thereafter, the weight was noted. This procedure was repeated three times at the interval of 30 min until a stable weight was obtained. The moisture content in percentage was determined as follows:

$$\% \text{ Moisture content} = \frac{\text{Weight loss (g)}}{\text{Initial weight (g)}} \times 100$$

Determination of total ash content

The total ash content was determined by transferring 2 g of the powder sample to an already weighed clean crucible. The weight of clean crucible and its content was determined and then heated in a furnace at 550°C for 8 hr. The crucible and contents were cooled and the final weight was noted. The percentage ash content was calculated as follows:

$$\% \text{ Total ash content} = \frac{\text{Weight of ash (g)}}{\text{Initial weight of sample (g)}} \times 100$$

Determination of acid insoluble ash

The total ash obtained was transferred to 25 mL of 2N dilute hydrochloric acid in 250 mL conical flask. The mixture was boiled for 5 min and then filtered through filter paper. The washing of the residue with 2N HCl through the filter paper was repeated 3 times. The filter paper with the residual ash were dried in an oven at 105°C in order to remove any trace of moisture. The filter paper with its content was folded and then transferred to an already weighed clean crucible and the weight was determined. The crucible with its contents was placed in the furnace and then heated at 550°C for 8 hr until the filter paper was completely burnt to ash and the final weight noted. The percentage acid insoluble ash was calculated as follows:

$$\% \text{ Acid insoluble ash} = \frac{\text{Weight of ash (g)}}{\text{Initial weight of sample (g)}} \times 100$$

Determination of alcohol soluble extractive value

The powder (2 g) was soaked in 30 mL of ethanol for 24 hr and then filtered. The filtrate was transferred to an evaporating dish with a known weight. The crucible with its content was heated in an oven at 105°C and then cooled in a desiccator and the final weight was noted. The percentage alcohol soluble extractive value was calculated as follows:

$$\% \text{ Alcohol soluble extractive value} = \frac{\text{Weight of extract (g)} \times 100}{\text{Initial weight used (g)}} \times 100$$

Determination of water soluble extractive value

The powder (2 g) was soaked in 30 mL of chloroform water for 24 hr and then filtered. The filtrate was transferred to an evaporating dish with a known weight. The crucible with its content was heated in an oven at 105°C and then cooled in a desiccator and

the final weight noted. The percentage water soluble extractive value was determined as follows:

$$\% \text{ Water soluble extractive value} = \frac{\text{Weight of extract (g)}}{\text{Initial weight used (g)}} \times 100$$

Oil Extraction

The oil was extracted according to the method described by, Emmanuel MH *et al.*^[3] The method is briefly explained. The *Terminalia catappa* oil was extracted from the blended seed with the aid of Soxhlet apparatus. The powder (234.03 g) was extracted with 700 mL of petroleum ether for four (4) h at 60°C. The oil obtained was concentrated using the rotary evaporator and was kept in an oven at 68°C for 12 h in order to obtain an oil free from petroleum ether. The yield of the oil in percentage was calculated using the following formula:

$$\% \text{ Yield of oil} = \frac{\text{Weight of oil (g)}}{\text{Initial weight sample (g)}} \times 100$$

Solubility/miscibility studies

The solubility/miscibility of the oil was tested in water, ethanol, methanol, acetone, benzaldehyde, ethyl acetate, chloroform, cyclohexane, acetic acid and phenol according to the method of Emmanuel MH, *et al.*^[3]

Organoleptic evaluations

The method described by of Emmanuel MH, *et al.*^[3] was used. The colour, odour, taste and texture of the oil sample were determined.

Physico-chemical evaluation of the oil

Specific gravity determination

Specific gravity of the oil was determined according to the method.^[3]

Determination of acid, saponification and ester values

The acid value, saponification value and ester value were obtained according to the method outlined by of Emmanuel MH, *et al.*^[3]

Preliminary phytochemical screening

Paper test, Sudan II test and Salkowski's test

The tests were carried out in accordance with the procedure outlined by Halilu ME, Halilu ME, *et al.*^[15,16]

GC-MS Analysis

The GC-MS was carried out as outline by Samira AG, *et al.*^[7] The oil was analysed on SHIMADZU GC-2010 Plus Series obtained

at the instrument laboratory, Cyprus International University, North Cyprus.

Antioxidant studies

The DPPH free radical assay was used for the antioxidant studies and was conducted in accordance with described by Halilu ME, *et al.*^[15]

Ethical Approval and laboratory animals

The research ethical approval was given by the Ethical Committee on Animal Research of the Department of Pharmacology and Toxicology, Usmanu Danfodiyo University, Sokoto. The Ethical approval number given was: PTAC/Tc/(He)OT/47-22 was given. Twenty-five mice of both sexes (25±5 g) was obtained from the Animal House, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto-Nigeria. The animals were acclimatized to laboratory conditions for 14 days prior to the experiments and maintained at a room temperature (25±2°C) with a 12 hr light/dark cycle. Animals were given standard chow and water *ad libitum*. All experimental procedures were conducted in accordance to the Animal Research Ethical Committee, Usmanu Danfodiyo University, Sokoto and in adherence with the established public health guidelines in the Guide for Care and Use of Laboratory Animals.

Acute toxicity determination

The acute toxicity of *T. catappa* seed oil in Mice was carried out orally using the "Up-and- Down" method in mice at single doses of 500, 2000, and 5000 mg/kg in line with the OECD guideline no. 425 (Organization for Economic Development, 2001) at the Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. Five (5) mice were picked, weighed, and dosed with the equivalent volume of the oil. The oil was administered by oral gavage using gastric feeding tube. Similar dose level of *T. catappa* seed oil was administered to the next animals in each group after 48 hr and observed for behavioral signs of toxicity such as changes in fur, eyes, diarrhoea, convulsion, tremors, salivation, lethargy, and motor activity of the central nervous systems according to the specification of the OECD Organization for Economic Development.^[18]

Sub-acute toxicity studies

From the Acute toxicity study of *T. catappa* seed oil, a total of twenty mice were randomly selected and then divided into four (4) groups ($n=5$). Group 1, normal control received distilled water while Groups II-IV received daily doses of 200, 400, and 800 mg/kg of oil for a period of 28 days. The animals were dosed by oral gavage, using a curved, ball tipped stainless steel feeding needle for 28 days as described by Ugwah-Oguejiofor CJ, *et al.*^[19] Signs of toxicity such as body weight, fur appearance, and mortality were monitored. At the end of the experiment, blood was withdrawn under anesthesia, via cardiac puncture for

biochemical analysis. The livers, kidneys, hearts, and lungs were excised for histopathological analysis using standard methods.

Biochemical analysis

The biochemical analyses were carried out using validated analytical method for the quantification of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein (TP), albumin (ALB), bilirubin (BIL), and serum electrolytes; potassium (K^+), sodium (Na^+), bicarbonate, and chloride (Cl^-) using an autoanalyzer machine (Raytochemray 120, Germany).

Histological analysis

The lungs, livers, kidneys, and hearts were excised and observed for any gross lesions. Organs about 3 mm thick were taken and fixed in 10% formal saline and processed by the paraffin wax technique. The organ paraffin sections were cut at $3\mu m$ using Rotary microtome (Surgicare microtome, model 335A, USA), stained with hematoxylin and eosin (H&E) method to demonstrate the general tissue structures.

Statistical Data Analysis

All statistical data were expressed as the mean \pm standard deviation. The values were analyzed statistically by one-way Analysis of Variance (ANOVA) followed by the Bonferroni *post hoc* test with the aid of SPSS version 23 and $p < 0.05$ compared to the control group was taken to be significant. The IC_{50} was determined using Graph pad prism 7.

RESULTS

Physico-chemical evaluation of powder sample

The physico-chemical parameters primarily ascertain the quality of the kernel used for the oil extraction and is presented in presented in Table 1.

Oil Extraction, Organoleptic and Solubility studies

The mass of the oil obtained from 234.03 g of the powder sample was 111.95 g. The percentage yield was 47.84 %. The organoleptic evaluation revealed that the oil had a yellowish colour, odourless, smooth touch and tasteless. The oil was found to be soluble in acetone, ethyl acetate, chloroform, cyclohexane and benzaldehyde (Table 2).

Table 1: Physico-chemical parameters.

Parameter	Result (%)
Moisture content value	4.5
Total ash value	3.5
Acid insoluble ash value	1.0
Alcohol soluble extractive	14.5
Water soluble extractive	6.0

Physico-chemical properties of the oil

The results of the physico-chemical studies are presented in Table 3. These data are critical for the establishment of quality of an oil.

Phytochemical studies

Preliminary phytochemical screening

The paper test and Sudan III tests showed the presence of oil. The Salkowski's test indicated the presence of a steroidal/triterpenoidal nucleus.

GC-MS Analysis

The GC-MS experiment (Table 4) showed the presence of hexadecanoic acid, 9-octadecenoic acid (E), oleic acid, cis-vaccenic acid, octadecanoic acid, tricosanoic acid and 9-octadecenoic acid (Z)-,2,3-dihydroxypropyl ester.

Antioxidant Activity

Scavenging activity of *Terminalia catappa* oil and Tannic acid (standard) against DPPH

The percentage free radical scavenging activity of *Terminalia catappa* oil is presented in Table 5.

Acute toxicity studies

The LD_{50} determination using the Up and Down method at doses of 500, 2000, and 5000 mg/kg of the oil in mice caused no mortality and no toxicity signs in the mice throughout the study period.

Table 2: Solubility of the oil in organic solvents.

Solvent	Solubility
Water	Insoluble
Ethanol	Insoluble
Methanol	Insoluble
Acetic acid	Insoluble
Phenol	Insoluble
Acetone	Soluble
Benzaldehyde	Soluble
Ethyl acetate	Soluble
Chloroform	Soluble
Cyclohexane	Soluble

Table 3: Physico-chemical properties.

Analysis	Value
Specific gravity	0.947
Acid value	10.66 mgKOH/g of oil
Saponification value/index	10.94 mgKOH/g of oil
Ester value/index	0.28 mgKOH/g of oil
Iodine value/index	63.45 $gI_2/100 g$ of oil

Sub-acute toxicity and effect of *T. catappa* seed oil on body weight of mice

The mice treated with oral doses of the oil at survived throughout the 28 days of treatment. There were no observable signs of toxicity in the group of mice when compared with control group.

Evaluation of biochemical parameters

This results in Table 6 showed the functions of the liver and kidney as measured by serum biochemical analyses which are critical parameters in the toxicological investigation of pharmaceuticals.

Histopathological examination

To confirm the safety of the *T. catappa* seed oil on body tissues, histopathological analysis was undertaken. The result showed no changes in the liver, kidney, heart and lung tissues in the mice when compared to that of the control (Figure 2). This implies that the weight changes observed in the course of the 28 days study may not be due to the toxicity of the seed oil.

Table 4: GC-MS Analysis.

Fatty acid/ Fatty acid ester	Quality
n-hexadecanoic acid	99
9-octadecenoic acid	99
Oleic acid	99
cis-Vaccenic acid	99
Octadecanoic acid	99
Tricosanoic acid	92
9-octadecenoic acid (Z)-, 2,3-dihydroxypropyl ester	81

DISCUSSION

According to WHO,^[20] physico-chemical evaluation of plants is a key step that is required towards ascertaining standards on purity and quality of crude plant drug to be used for consumption or for medicinal purposes. The presence of moisture in plants materials encourages the growth of micro-organisms (bacterial, fungal or yeast) and this leads to the deterioration of the active constituents following enzymatic hydrolysis.^[15] The moisture content of 4.5 % found in the kernel indicated that the kernel from which the oil was extracted was properly kept away from moisture/properly dried. The total ash was found to be 3.5%. This result agrees with^[12] who reported total ash of 3.4 % in castor seeds. The acid insoluble ash obtained was 1 % and it indicates that the kernels have not been contaminated with inorganic substances such as sand. The alcohol soluble extractive value was 14.5 % and water extractive was 6.0 %. This indicates that the kernel consists more of alcohol soluble substances than water.^[16]

The percentage yield of the oil was 47.84 % which indicates has high concentration of oil in the kernel.^[22] suggested that since the kernel has high percentage of oil, then the oil have a great potential for application as an edible vegetable oil.

Vegetable oils can be adulterated easily by addition of cheaper, inferior, harmful, or undesired substance(s) to the oil which may affect its nature and quality.^[23] Hence, the need for the evaluation of their quality. The yellowish colour may be attributed to the presence of dissolved organic lipophilic substances.^[3]

Oil's solubility in a wide range of solvents determines its suitability and scope of application. Hence, the solubility profile of the oil in different solvents was tested. The solubility of an oil determines the spectrum of its application in the industry. The solubility of vegetable oils especially in aqueous ethanol depends on the

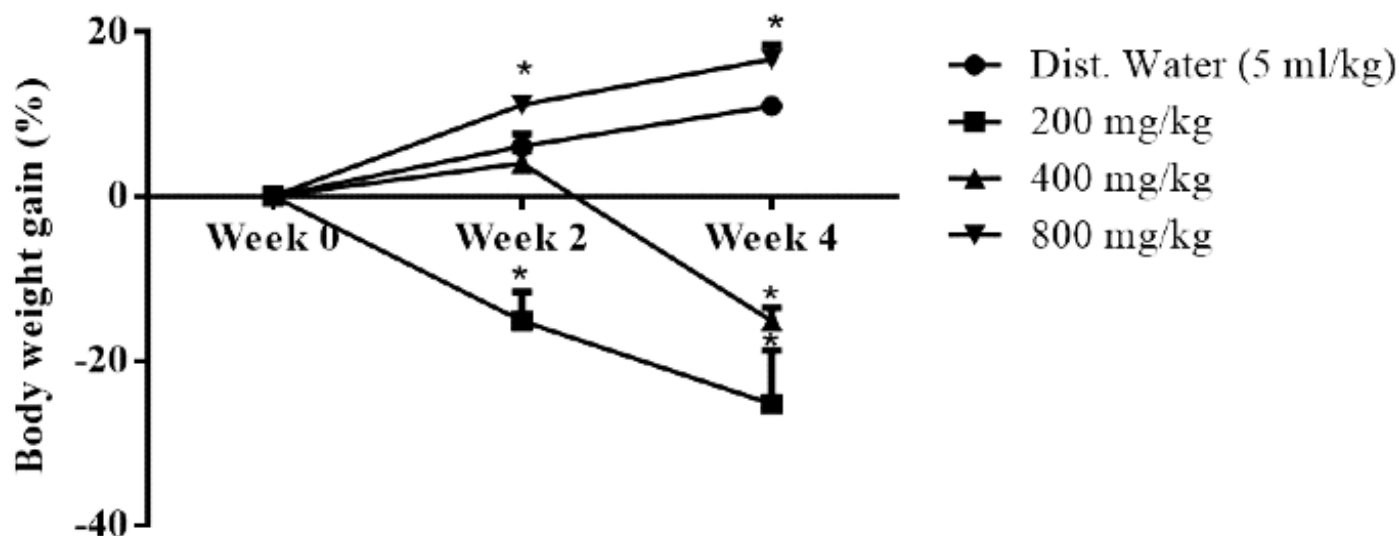


Figure 2: Representative histological sections of hearts, lungs, kidneys, and livers of animals treated with 200, 400, and 800 mg/kg extract of *T. catappa* seed oil indicated no histopathological changes when compared to the control. (H&E x 100).

Table 5: Percentage scavenging activity of *Terminalia catappa* oil and tannic acid (standard).

Conc. of <i>Terminalia catappa</i> (mg/mL)	% Scavenging activity <i>Terminalia catappa</i>	Conc. of tannic acid (mg/mL)	% Scavenging activity of tannic acid
0.78	51	0.3125	100
1.5	31	0.625	100
3.13	23	1.25	100
6.25	13	2.5	100
25.0	13	5.0	75
50.0	50	10.0	49

Table 6: Effect of *T. catappa* seed oil on biochemical parameters.

Parameter	Unit	Distil water (control)	200 mg/kg	400 mg/kg	800 mg/kg
Albumin	g/L	38.50 ± 1.56	37.50 ± 1.71	37.00 ± 0.71	39.00 ± 1.08
Total protein	g/L	68.50 ± 4.35	67.50 ± 2.90	64.75 ± 4.13	68.75 ± 2.29
Total bilirubin	µmol/L	2.15 ± 1.32	0.68 ± 0.05	2.03 ± 0.96	0.83 ± 0.05
Dir. Bilirubin	µmol/L	0.95 ± 0.68	0.20 ± 0.04	0.78 ± 0.54	0.28 ± 0.05
ALP	UI/L	65.00 ± 5.58	75.50 ± 6.74	91.25 ± 10.87	72.50 ± 10.19
ALT	UI/L	8.75 ± 1.25	11.25 ± 1.97	16.00 ± 5.76	11.00 ± 1.08
AST	UI/L	10.50 ± 1.85	12.25 ± 0.75	14.50 ± 3.01	12.50 ± 1.66
Chloride (Cl ⁻)	mmol/L	98.50 ± 2.99	92.00 ± 2.35	91.50 ± 2.26	93.75 ± 4.19
Sodium (Na ⁺)	mmol/L	139.50 ± 4.11	139.75 ± 3.33	131.50 ± 4.57	140.75 ± 1.93
Potassium (K ⁺)	mmol/L	3.43 ± 0.24	3.43 ± 0.25	3.28 ± 0.61	4.00 ± 0.39
Bicarbonate (HCO ₃)	mmol/L	26.75 ± 2.18	27.25 ± 1.11	26.25 ± 1.65	25.75 ± 2.25
Creatinine (Cr)	mmol/L	0.80 ± 0.17	0.88 ± 0.15	0.68 ± 0.08	0.63 ± 0.09
Urea (U)	mmol/L	5.05 ± 0.77	5.30 ± 0.79	4.78 ± 0.26	4.53 ± 0.54

Statistical data presented as Mean ± SD were analyzed by ANOVA followed by Bonferroni *Post hoc* Test. * $p \leq 0.05$ values are statistically significant.

concentration and temperature.^[24] The *Terminalia catappa* seed oil specific gravity was 0.947 at room temperature (25°C). This value is within the ranges of most commercial oil (groundnut oil 0.92, Palm oil 0.924, mustard oil 0.908, Sesame oil 0.923, Coconut oil 0.925 and Soya 0.928). Specific gravity is employed with other parameters to determine the purity of oil and its identification.^[25] Acid value is an important parameter and it was determined to be 10.66 mgKOH/g of oil; this value indicates that the oil consists of high proportion of fatty acids. Saponification value of 10.94 mgKOH/g indicates low molecular weight fatty acid. The ester value of the was 0.28 mgKOH/g of oil. The iodine indicates the extent of unsaturation of the fatty acids present in the oil. The iodine value increases as the unsaturation increases. The iodine value of the *Terminalia catappa* was 63.45 gI₂/100 g of oil and it indicates that the oil has low degree of unsaturation.^[3]

The phytochemical screening revealed the presence of free/esterified sterols. Most vegetable oils have been reported to contain sterols (e.g., cholesterol). This result agrees with Verleyen T, *et al.*^[26] who showed that free and esterified sterols occur in vegetable oils.

The GC-MS revealed the fatty acid composition of the oil. The study revealed the presence of oleic acid, n-hexadecanoic acid and octadecanoic acid. The data agrees with Santosa OV, *et al.*^[22]

The antioxidant activity was expressed in terms of the IC₅₀. The IC₅₀ of the oil was found to be 1.425 mg/mL and that of tannic acid (standard) was IC₅₀ 5.005 mg/mL. The IC₅₀ revealed that the oil had better antioxidant activity when compared with tannic acid (standard). The antioxidant activity demonstrated by the oil may be due to the presence of phenolics, terpenes and tocopherols Savva SC, Sibel K, *et al.*^[2,27] The lower the IC₅₀ value, the higher the antioxidant activity.

The acute toxicity revealed that *T. catappa* seed oil may be regarded as safe at the specified doses and the oral LD₅₀ considered was greater than 5000 mg/kg. The acute toxicity test is one of the most informative methods to figure out the effect of a toxicant on a particular organism. The outcome is fast and can be determined in the shortest possible time.^[28] Since the LD₅₀ of *T. catappa* seed oil is greater than 5000 mg/kg in mice, this suggest that the oil is safe. Documented evidence has shown that pharmaceuticals with oral LD₅₀ greater than 5000 mg/kg are relatively safe (OECD, 2001). However, the acute toxicity data have limited clinical applications

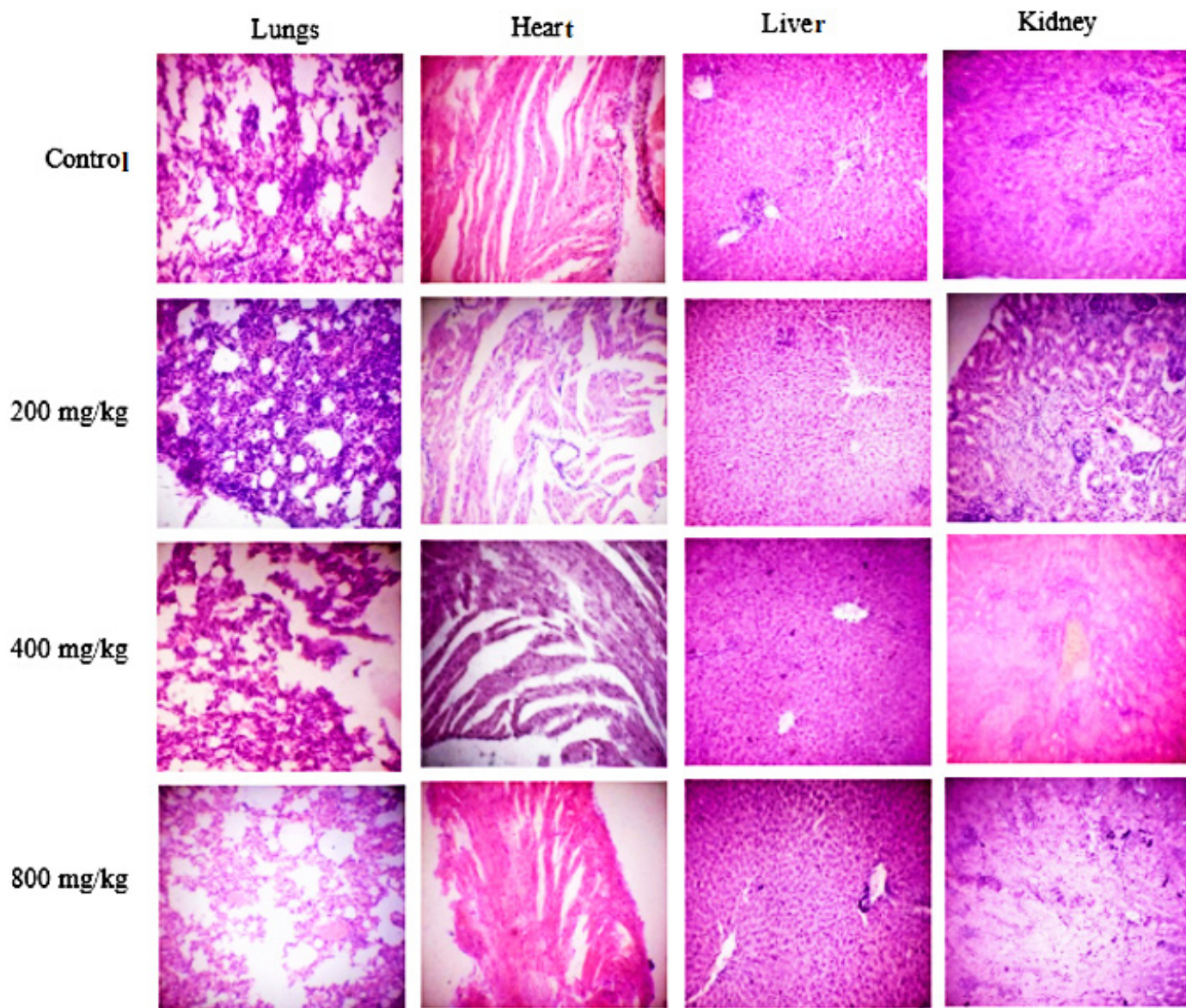


Figure 3: Effect of *T. catappa* seed oil on relative body weights of mice treated for 28 consecutive days.

Values are shown as percentage of mean \pm SD; Significant in relation to control at * $p < 0.05$, two-way Analysis of variance (ANOVA) followed by Bonferroni *post hoc* test.

Abotsi WKM, *et al.*^[19] as repeated exposure of pharmaceuticals may accumulate in the body and may gradually effect the body tissues.^[29,30]

The sub-acute toxicity studies revealed that the weight of the mice reduced significantly ($p < 0.05$) at 200 and 400 mg/kg and a significant ($p < 0.05$) increase at 800 mg/kg (Figure 3). The sub-acute studies have associated the body weight of experimental animals with the toxicity of pharmaceutical compounds.^[31,32] Weight increase of an organ may suggest the development of hypertrophy and the other hand, a decrease may suggest necrosis in the target organ.^[33] *T. catappa* oil showed reduction in weight at a lower dose but increase at a higher dose. The variability seen in the weights may be due to the chemical constituents of the oil causing weight loss at a lower dose but having a negative feedback

mechanism which may result in weight gain. Further studies are needed to ascertain this. However, this report is in contrast with a similar study on *T. catappa* leaves which indicated that the extract has no statistical significant effect on the body weight of animals.^[34] *T. catappa* seed oil may possess different constituents from that of its leaves.

The evaluation of biochemical parameters showed that, *T. catappa* seed oil did not cause significant ($p > 0.05$) changes in the serum liver and kidney functions. Similar reports have been documented from *T. catappa* leaf extract.^[30,35] Previous researches have shown that when the liver is injured, additional serum aminotransferases (AST, AP and ALT) escaped into the bloodstream and raise the serum enzymes levels.^[36] As it is evident in our study, the oil appears to be nontoxic to the liver. The kidney's functional

integrity is to maintain total body homeostasis through its role in the excretion of metabolic wastes and in regulation of intracellular fluid volume, electrolyte composition, and acid-base balance.^[37] Our result showed that *T. catappa* seed oil did not induce any biochemical changes in the kidneys compared to control. The histological studies showed no changes in the liver, kidney, heart and lung tissues in the mice when compared to that of the control. This implies that the weight changes observed in the course of the 28 days study may not be due to the toxicity of the seed oil.

CONCLUSION

The findings from the research based on the physicochemical parameters of the powder kernel showed that the kernel is of good quality. The oil had acid value of 10.66 mgKOH/g, saponification value of 10.94 mgKOH/g and iodine value of 63.45 gI₂/100 g of oil which indicated that the oil is of good quality. The oil demonstrated antioxidant activity with IC₅₀ = 1.425 mg/mL which showed that it had better antioxidant activity when compared with tannic acid (standard) with IC₅₀ = 5.005 mg/mL. The oral acute toxicity study revealed that the oil is relatively safe with LD₅₀ greater than 5000 mg/kg in mice. The sub-chronic toxicity study affirmed that the oil is nontoxic to the liver, kidney, lungs and heart. The reduction in weight caused by the oil suggest that the oil may be used in weight loss therapy.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

DPPH: 2,2-diphenyl-1-picrylhydrazyl; **GC-MS:** Gas Chromatography Mass Spectrometry.

SUMMARY

The findings from the research showed that the oil is relatively safe and the sub-chronic toxicity study affirmed that the oil is not toxic to the liver, kidney, lungs and heart. The antioxidant activity demonstrated by the oil was better when compared with tannic acid.

REFERENCES

- Damude HG, Kinney AJ. Enhancing plant seed oils for human nutrition. *Plant Physiol.* 2008;147(3):962-8. doi: 10.1104/pp.108.121681, PMID 18612073.
- Savva SC, Kafatos A. Vegetable oils: dietary importance. *Encyclopedia of food and health* 2016; Pp. 365-72. doi: 10.1016/B978-0-12-384947-2.00709-1.
- Halilu EM, Muhammad B. Phytochemical and antioxidant studies of *Hibiscus cannabinus* seed oil. *Phys Sci Rev.* 2021. doi: 10.1515/psr-2021-0184.
- Nurham TD. Edible oil quality. Oklahoma State University; 2016/Id. FAPC-197. Available from: <https://extension.okstate.edu/fact-sheets/edible-oil-quality.html> [cited 14/11/2022].
- Wealleans AL, Bierinckx K, Witters E, di Benedetto M, Wiseman J. Assessment of the quality, oxidative status and dietary energy value of lipids used in non-ruminant animal nutrition. *J Sci Food Agric.* 2021;101(10):4266-77. doi: 10.1002/jsfa.11066, PMID 33417265.
- Schwingshackl L, Bogensberger B, Benčić A, Knüppel S, Boeing H, Hoffmann G. Effects of oils and solid fats on blood lipids: a systematic review and network meta-analysis. *J Lipid Res.* 2018;59(9):1771-82. doi: 10.1194/jlr.P085522, PMID 30006369.
- Samira AG, Jinous A. Essential and fixed oil chemical compositions of the Seeds from the endemic *Species salvia* Sharifii Rech. F. and Esfand. *J Chil Chem Soc.* 2015;60(4):2695-7.
- Gulçin AP, Zehra A. Chemical composition of the fixed and essential oils of *Nigella sativa* L. from Turkey. *Curr. Perspect. on med. and aromatic plants.* 2018;1:19-27.
- Solomon MG, Amali AM, Mohemmed U, Abdulraman M, Halilu EM, Aliyu SI. Histological effects of aqueous stem bark extract of *Cadaba farinosa* on gastrointestinal tract of Wistar rats, *Global journal of medical research: B pharma, drug Discov. Toxicol. Med.* 2019;19(3):41-7.
- Chatatikun M, Chiabchalard A. Phytochemical screening and free radical scavenging activities of orange baby carrot and carrot (*Daucus carota* Linn.) root crude extracts. *J Chem Pharm Res.* 2013;5(4):97-102.
- Ali EAS. Oils and fats contents of medicinal plants, as natural ingredients for many therapeutic purposes- A review, *IOSR J. Pharm.* 2020; 10 (7);II:01-41.
- Janporn S, Ho C, Chavasit V, Pan M, Chittrakorn S, Ruttarattanamongkol K, et al. Physicochemical properties of *Terminalia catappa* seed oil as a novel dietary lipid source. *J Food Drug Anal.* 2015;23(2):201-9. doi: 10.1016/j.jfda.2014.06.007.
- Mohale DS, Dewani AP, Chandewar AV, Khadewar CD, Tripathi AS, Agrawal SS. Brief review on medicinal potential of *Terminalia catappa*, *J. Herbal Med. and Toxicol.* 2009;3(1):7-11.
- Laisa PS, Célio DA, Flavia B, Hélio K, Francisco JM, Lourdes CS, et al. *Terminalia catappa* L.: A medicinal plant from the Caribbean pharmacopeia with anti-Helicobacter pylori and antiulcer action in experimental rodent models. *J Ethnopharmacol.* 2015;159:85-295.
- Halilu ME, Sylvestre NM, Millicent LU, Adam BA. Phytochemical and antioxidant evaluation of *Cassia sieberiana* D.C. stem bark extracts. *The Proc Nig Acad Sci.* 2020;13(2):97-110.
- Halilu ME, Yabalu ZA, Cynthia S, Sani SB, Sa'adatu JA. Evaluation of physicochemical and antioxidant potential of fixed oil from *Curcuma longa* Linn. *Trends in nat. Prod Res.* 2021;2(2):66-74.
- Olivia NU, Goodness UC, Obinna OM. Phytochemical profiling and GC-MS analysis of aqueous methanol fraction of *Hibiscus asper* leaves. *Futur J Pharm Sci.* 2021;7(1):1-5. doi: 10.1186/s43094-021-00208-4.
- OECD Organization for Economic Development. Guideline for testing of chemicals. Guidance no. 425. Up Down Proc Adopted: 17th December 2001. 2001.
- Ugwah-Oguejiofor CJ, Okoli CO, Ugwah MO, Umaru ML, Ogbulie CS, Mshelia HE, et al. Acute and sub-acute toxicity of aqueous extract of aerial parts of *Caralluma dalzielii* N. E. Brown in mice and rats. *Heliyon.* 2019;5(1). doi: 10.1016/j.heliyon.2019.e01179.
- WHO. Quality control methods for medicinal plant materials. Geneva: World Health Organization; 2011. p. 5-43.
- Seyed MTG, Seyed MM, Mohammad G. A survey on moisture-dependent physical properties of castor seed (*Ricinus communis* L.). *Aust J Crop Sci.* 2011;5(1):1-7.
- Santos OV, Soares SD, Dias PCS, Duarte SPA, Santos MPL, Nascimento FCA et al. Chemical-functional composition of *Terminalia catappa* oils from different varieties. *Grasas Aceites.* 2022;73(2):1-11. doi: 10.3989/gya.0102211.
- Abbas O, Baeten V. Advances in the identification of adulterated vegetable oils. *Advances in food authenticity testing; food Sci. Technol. Nutr.* 2016; 519-42. doi: 10.1016/B978-0-08-100220-9.00019-9.
- Ramalingan K, Chari KS. Solubilities of vegetable oils in aqueous ethanol and ethanol-hexane mixtures. Presented at fall meeting. Cincinnati: American Oil Chemists' Society; 1959; 37(2):77-80. doi: 10.1007/BF02540248.
- Ichu CD, Nwakanma HO. Comparative Study of the physicochemical characterization and quality of edible vegetable oils; 2019. *Inter J. Res. Informative Sci Appl Techniq;*3(2):19321-9.
- Verleyen T, Forcades M, Verhe R, Dewettinck K, Huyghebaert A, De Greyt W. Analysis of free and esterified sterols in vegetable oils. *J Am Oil Chem Soc.* 2002;79(2):117-22. doi: 10.1007/s11746-002-0444-3.
- Konyalioğlu S, Sağlam H, Kivçak B. α-Tocopherol, Flavonoid, and Phenol Contents and Antioxidant Activity of *Ficus carica*. *Leaves. Pharm Biol.* 2005;43(8):683-6. doi: 10.1080/13880200500383538.
- Yunus K, Mardhiah Jaafar AM, John A. Acute-lethal toxicity (LC₅₀) effect of *Terminalia catappa* Linn. Leaves Extract on *Oreochromis niloticus* (red Nile tilapia) Juveniles under static toxicity exposure. *Orient J Chem.* 2019;35(1):270-4. doi: 10.13005/ojc/350132.
- Abotsi WM, Woode E, Ainooson GK, Amo-Barimah AK, Boakye-Gyasi E. Antiarthritic and antioxidant effects of the leaf extract of *Ficus exasperata* P. Beauv. (Moraceae). *Pharmacognosy Res.* 2010;2(2):89-97. doi: 10.4103/0974-8490.62958, PMID 21808547.

30. Bariweni PA, Tawari CC, Abowei JFN. Some environmental effects of flooding in the Niger Delta Region of Nigeria. *Int J Fish Aquat Sci.* 2012;1(1):35-46.
31. Michael B, Yano B, Sellers RS, Perry R, Morton D, Roome N, *et al.* Evaluation of organ weights for rodent and non-rodent toxicity studies: a review of regulatory guidelines and a survey of current practices. *Toxicol Pathol.* 2007;35(5):742-50. doi: 10.1080/01926230701595292. PMID 17849357.
32. Sireeratawong S, Lertprasertsuke N, Srisawat U, Thupia A, Ngamjariyawat A, Suwanlikhid N, *et al.* Acute and subchronic toxicity study of the water extract from *Tiliacora triandra* (Colebr.) Diels in rats. *Sonklanakar J. Sci Technol.* 2008;30(5):611.
33. Teo S, Stirling D, Thomas S, Hoberman A, Kiorpes A, Khetani V. A 90-day oral gavage toxicity study of d-methylphenidate and d,l-methylphenidate in Sprague–Dawley rats. *Toxicology.* 2002;179(3):183-96. doi: 10.1016/S0300-483X(02)00338-4.
34. Ahmed SM, Vrushabendra SBM, Dhanapal PG, Chandrashekara VM. Antidiabetic activity of *Terminalia catappa* Linn. Leaf extracts in alloxan-induced diabetic rats. *Iran J Pharmacol Ther.* 2005;4:36-9.
35. Ikele CB, Elijah O, Anya N. I. Further studies on evaluation of the toxicity potential of *Terminalia catappa* Linn. Combretaceae leaf extract: effects on the histology, liver enzymes, and haematology profile of albino rats. *Jordan J Biol Sci.* 2021;14(5):889-97.
36. Lee CY, Yang SF, Wang PH, Su CW, Hsu HF, Tsai HT, *et al.* Antimetastatic effects of *Terminalia catappa* leaf extracts on cervical cancer through the inhibition of matrix metalloprotein-9 and MAPK pathway. *Environ Toxicol.* 2019;34(1):60-6. doi: 10.1002/tox.22657. PMID 30259628.
37. Orisakwe OE, Husaini DC, Afonne OJ. Testicular effects of sub-chronic administration of *Hibiscus sabdariffa* calyx aqueous extract in rats. *Reprod Toxicol.* 2004;18(2):295-8. doi: 10.1016/j.reprotox.2003.11.001, PMID 15019726.