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Phytochemistry and Acute Toxicity Study of Aqueous and Methanol Stem Bark Extract of *Terminalia catappa*

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ABSTRACT

Terminalia catappa is a folklore plant used to treat infectious diseases such as dermatosis, hepatitis, scabies and leprosy. This study investigated the phytochemistry and toxicological activities of stem bark aqueous and methanol extracts of *Terminalia catappa*. The study was carried out in three phases; extraction, phytochemical profiling, and toxicity evaluation. Pulverized stem bark was mixed with water and methanol. The two mixtures were subjected to cold marceration procedure for 48 and 72 hours and concentrated with a Buchi rotary evaporator and freeze dryer. Extracts obtained were subjected to Gas chromatography-mass spectrometry (GC-MS). GC-MS single phase ion mode spectra of detected compounds were matched with spectra of known compounds of the National Institute of Standards and Technology (NIST). The acute toxicity test was carried out using Lorke's method to ascertain the median lethal dose (LD_{50}) of the extract. Thirty-nine (39) phytochemical compounds were detected by GC-MS single phase ion mode including 2-Methoxy-4-vinylphenol, Diethyl Phthalate, 3,4,5-Trimethoxyphenol, 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol, Neocurdione and Octadecanoic acid as the most predominant. Acute toxicity test revealed that the LD_{50} of both extracts was greater than 5000 mg/kg body weight. The study revealed that extract of *Terminalia catappa* contained various phytochemical compounds with reported medicinal properties. This study has shown that the plant extract is non-toxic and relatively safe. The plant can thus be used as a potential candidate for drug development.

Keywords: *Terminalia catappa*, Phytochemistry, Gas chromatography, Acute toxicity, Lethal dose.

Introduction

In many developing countries, a large proportion of the population relies on traditional practitioners and their knowledge of medicinal plants to meet healthcare needs. In the continent of Africa, the practice of traditional healing is much older than some of the other medical sciences and seem to be much more prevalent compared to conventional medicine.[1] Over the years, a variety of medicinal plants have been popular for the cure of a number of both human and animal diseases.[2] *Terminalia catappa* is a large tree that is widely planted throughout the tropics. Studies from literatures have shown that this plant is known to possess antioxidant, antimicrobial, antidiabetic, anticancer, anti-inflammatory, anti-ageing, wound healing and hepatoprotective activities.[3-6] It is also used in traditional medicine as a remedy for many ailments such as leprosy, headache, dermatitis, diarrhea, pyrosis, gastritis and urinary tract infection.[7,8] Although it is generally agreed that medicinal plants and their products are naturally safer than synthetic drugs,[9] a general assumption of this safety should not be made without carrying out a proper investigation.

In this view, this study investigated the acute toxicity effect of aqueous and methanol stem bark extract of *Terminalia catappa* on wistar rats. A phytochemistry study of the methanol extract was also carried out using GC-MS single phase ion mode. The various structures of detected bioactive compounds were discussed.

Materials and Methods

Collection of plant materials

The stem barks of *Terminalia catappa* were gotten from the trees of *Terminalia catappa* cut down at the open field of the Students Affairs Division, University of Benin, Benin City, Nigeria. The tree well fell on the 15th of November, 2019 and the logs of wood were collected on the 12th of December, 2019 and identified by a recognized taxonomist of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Edo State. The specimen with Voucher number UBHS260 was deposited in the same Department, while the stem barks were removed and dried in the sun for 14 days.

Preparation of the extracts

The stem barks of *Terminalia catappa* gotten were dried and pulverized using a saw machine and pulveriser. Using a dilution factor of 7 mL to 1 g of the pulverized sample, 2000 g of the powder were weighed into 14 L of distilled water, and 1100 g of powder were weighed into 7.5 L of methanol in a glass jar and stirred using a spatula. The aqueous and methanol solution were left for 48 and 72 hours to allow percolation for efficient extraction. The aqueous and methanol solution were filtered thrice using several folded clean pieces of cheesecloth during each filtration process to remove debris. High extract yields were obtained from cold maceration method. Throughout the study, the extracts were kept in an airtight container and stored in the refrigerator (2-8°C) to avoid deterioration due to microbial growth.

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Animals

Male and female Albino rats weighing between 140-200 g (Average weight is 160 g), bred in the animal house, Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Nigeria, were used for the study. Ethical approval was obtained from Life Sciences ethical committee, University of Benin and registered with the ethical number: LS19304. The rats were kept in a well-ventilated room in wooden cages having wire mesh floor and top and allowed free access to food (growers mash) and water. The animals were exposed to 14 h light and 10 h dark cycle. They were allowed two weeks to acclimatize to the environment. Sanitation of the cages and the environment were carried out daily to prevent the outbreak of infectious diseases.

Acute toxicity study

The acute toxicity study was conducted in accordance with Lorke's method (Lorke, 1983).[10] This method was modified during the study. The study was conducted in two phases using a total of thirty-two male and female rats. In the first phase, nine rats were divided into 3 groups of 3 rats each. Groups 1, 2 and 3 animals were given 10,100 and 1000 mg/kg body weight (b.w.) of both extracts, respectively, to possibly establish the range of doses producing any toxic effect, in addition, a group of three rats was setup as control group and animals in the group were not given the extract. In the second phase, further specific doses (1600, 2900 and 5000 mg/kg b.w.) of both extracts were administered to three rats (one rat per dose) to further determine the correct LD_{50} value. The extract was dissolved in 1 mL of distilled water and administered orally with a steel gavage to experimental animals. All animals were observed frequently within duration of treatment and surviving animals were monitored daily for 72 h for signs of acute toxicity and death.

The lethal dose (LD_{50}) of extracts was calculated thus:

$$LD_{50} = \sqrt{(D0\ x\ D100)}$$

where D_0 = Highest dose that gave no mortality, D_{100} = Lowest dose that produced mortality

GC-MS analysis

GC-MS analysis of the leaf methanol extract was performed using a Perkin-Elmer GC Clarus 500 system and Gas chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with an Elite-I, fused silica capillary column (30 mm x 0.25 mm 1D x 1 µMdf, composed of 100% Dimethylpolysiloxane). For GC-MS detection, an electron ionization system with ionizing energy in a single-phase ion mode of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at a constant flow rate of 1 mL/min and an injection volume of 2 µL was employed (split ratio of 10:1); Injector temperature 250°C; Ion-source temperature 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min.), with an increase of 10°C/min, to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan-interval of 0.5 seconds and fragments from 45 to 450 Da. The total GC running time was 36 minutes. The relative % amount of each component was calculated by comparing its average peak area to the total areas. Software adapted to handle mass spectra and chromatograms was a Turbo mass.

Identification of components

Interpretation of mass spectrum from GC-MS was conducted using the database of National Institute Standard and Technology (NIST) and having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known and authentic samples stored in the NIST library. Computer searches in an HP Mass Spectral Library NIST98 were also applied. The name, molecular weight and structure of the components of the test materials were ascertained.

Results and Discussion

Gas chromatography in tandem with mass spectrometry (GC-MS) phytochemical profiling of methanol stem bark extract detected thirty-nine compounds (39) whose spectra were identified and matched with spectra of known compounds of the National Institute What will of Standards and Technology (NIST) library. The chromatogram and mass spectra of some detected compounds are presented in Figures 1-7, while the chemical constituents, structures, retention time (RT), molecular formular, molecular weight and concentration (area %) are presented in Table 1.

Nineteen peaks were captured, each peak represents a distinct compound. The area covered by each spectrum corresponds to the relative abundance of that compound. The identified compound corresponding to each peak is presented in Table 1.

The detected compounds include 2-Methoxy-4-vinylphenol, Diethyl Phthalate, 3,4,5-Trimethoxyphenol, 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol, Neocurdione and Octadecanoic acid as the most predominant. GC-MS is a highly sensitive, effective and versatile analytical technique. It is a hyphenated analytical technique that combines the separation properties of gas-liquid chromatography with the detection feature of mass spectrometry to identify different substances within a test sample[11]. Gas chromatography (GC) is used to separate and identify the volatile and thermally stable substitutes in a sample whereas GC-MS fragments the analyte to be identified based on its mass. Plants of vegetable origin, rich in fibre, minerals and vitamins, contains substances that display potent anti-carcinogenic and curative effects on various diseases and illnesses. These substances, known as phytochemicals are currently the subject of intense study and represent the new frontier in the nutritional and pharmaceutical investigation.

Phytochemical compounds are only found in plants and possess important health and healing properties. Isoflavones which are primarily found in plants of soy (*Glycine max*), red clover (*Trifolium pretens*) and Kudz *(Pueraria lobata)* are hormone-like compounds that are often used in remedies to reduce menopausal and post-menopausal symptoms. They are even associated with a low breast cancer rate in Asia and the retarded progression of Alzheimer disease.[12] The various phytochemical constituents present in the plant is suggestive of the medicinal potency as reported by early studies.[13] Medicinal plants are known to play a very important role in diseased conditions by exhibiting anti-oxidative properties. The GC-MS results obtained from this study revealed that *Terminalia catappa* possess bio-medicinal constituents which have been reported to have antioxidant, antiviral, antimicrobial and anti-inflammatory activities. The antioxidant activity as reported by Chyau *et al*. could be related to the presence of hydroxyl groups, double bonds conjugation and resonance effect in the structure of the compounds. 3,4,5-Trimethoxyphenol detected in *Terminalia catappa* has been shown to have antioxidant and antidiabetic potential.[5] The antiviral activity of octadecanoic acid has been reported to inhibit the enzyme required for viral replication and cells invasion.[14] Studies have also shown that it has an effect against the measles virus, and can inhibit the growth of the virus in *in vivo* studies.[15] 2-Methoxy-4-vinylphenol another phenolic compound detected in *Terminalia catappa* act as an anti-inflammatory agent, preventing excess production of inflammatory mediators that are involved in many diseases such as rheumatoid arthritis, atherosclerosis, asthma and pulmonary fibrosis. They act by the suppression of NF-kB, MAPK activation and histone acetylation.[16,17] Phenolic compounds can act as hydrogen donors to chelate metal ions such as iron and copper, by inhibiting the oxidation of low-density lipoproteins (LDL). These characteristics in the phenolic compounds are associated with a decrease in risks of neurodegenerative diseases, such as cardiovascular diseases, gastrointestinal, colon, breast and ovarian cancers, and leukemia.[18] Phenolic compounds also have vasorelaxation and anti-allergenic activity.[18]

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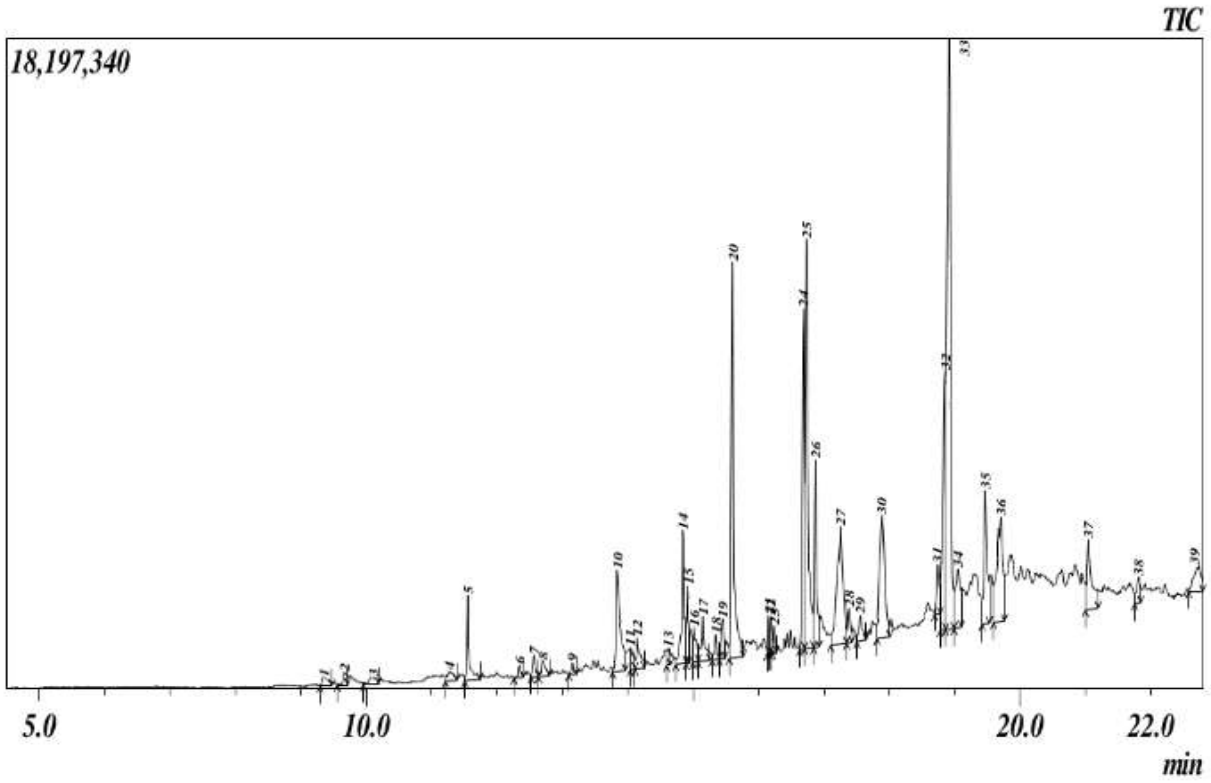

Figure 1: GC chromatogram of stem bark methanol extract of *Terminalia catappa*.

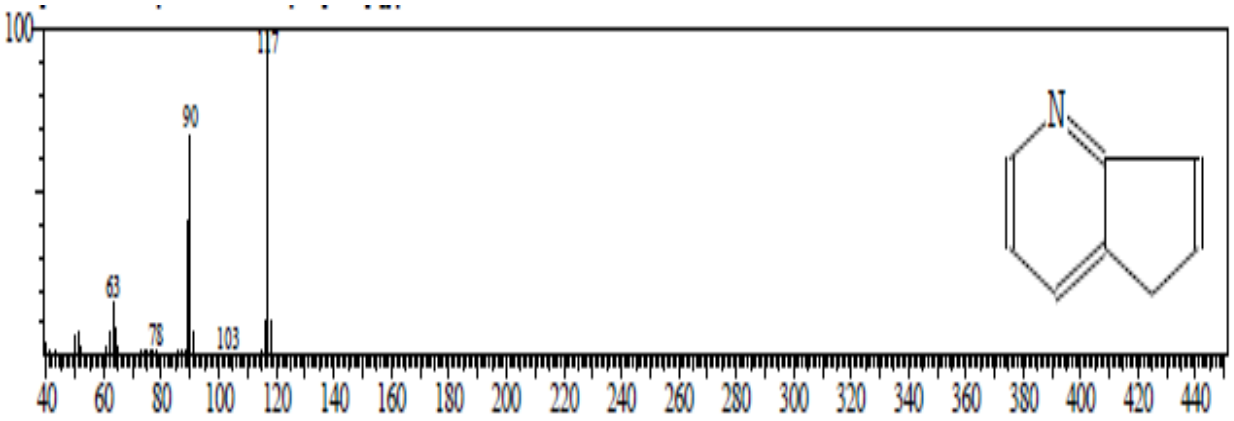

Figure 2: Mass spectrum of 5-H-1-pyridine (with x-axis *m/z* ratio and y-axis as intensity).

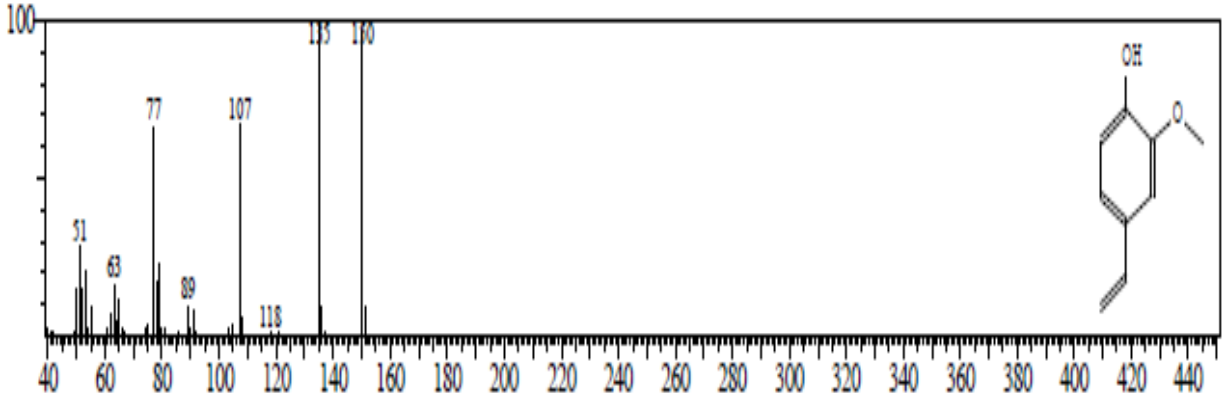

Figure 3: Mass spectrum of 2-Methoxy-4-vinylphenol (with x-axis *m/z* ratio and y-axis as intensity).

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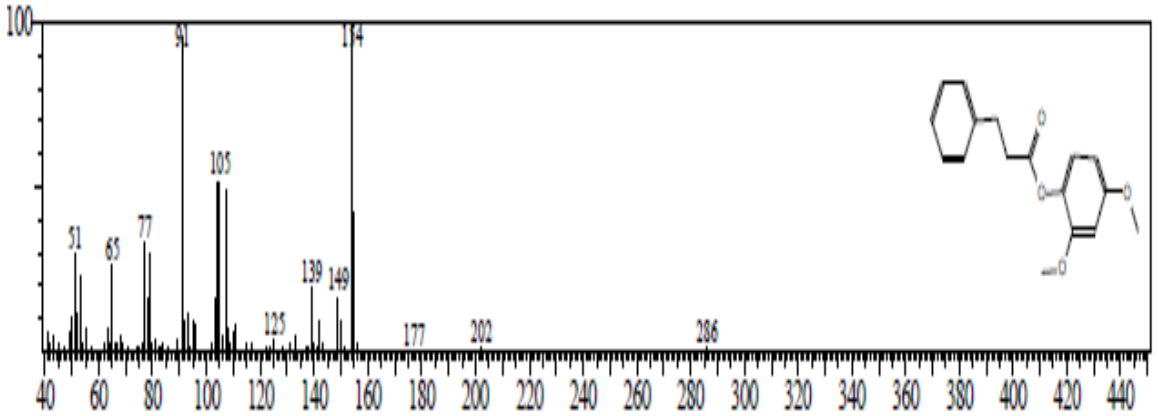

Figure 4: Mass spectrum of 2,4-dimethoxyphenyl betaphenpropionic acid (with x-axis *m/z* ratio and y-axis as intensity).

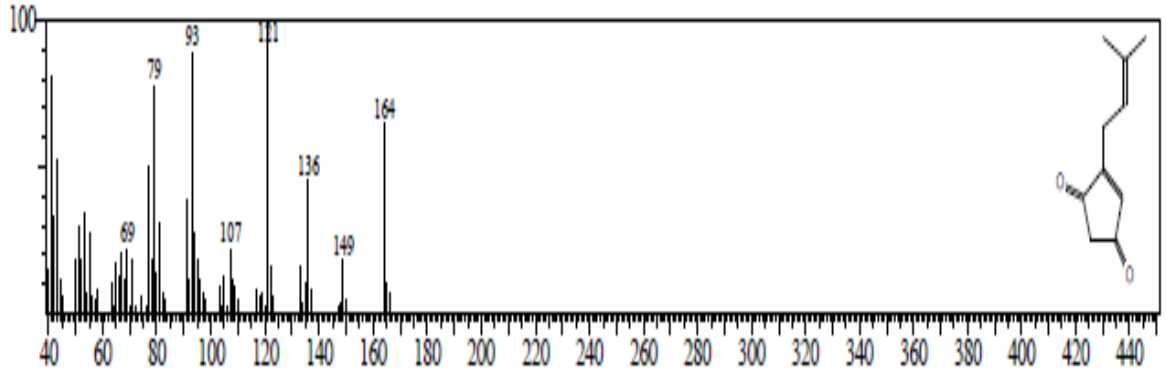

Figure 5: Mass spectrum of Methyl-2-butenyl-4-cyclopentene-1,3-dione; (with x-axis *m/z* ratio and y-axis as intensity).

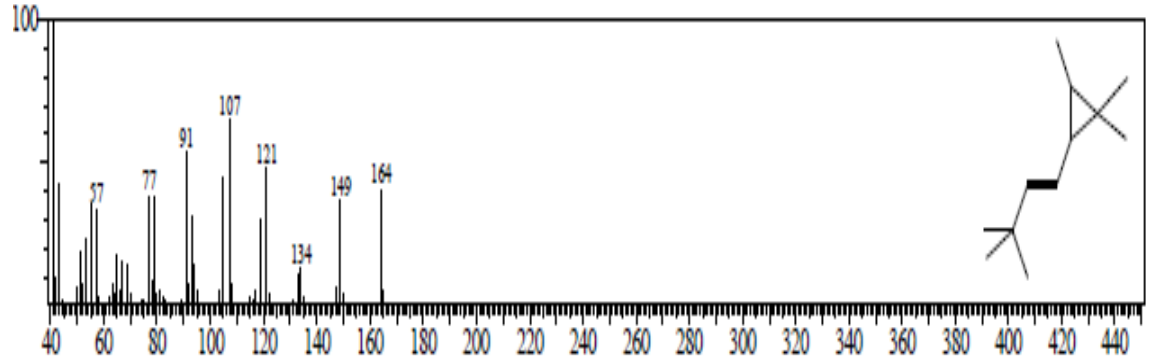

Figure 6: Mass spectrum of 2-(3,3 Dimethyl cyclopropane (with x-axis *m/z* ratio and y-axis as intensity).

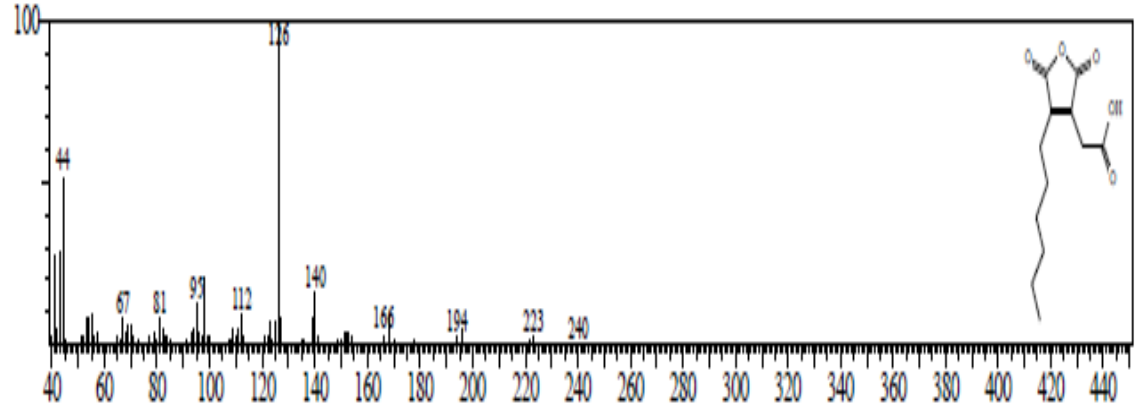

Figure 7: Mass spectrum of 3-furanacetic acid (with x-axis *m/z* ratio and y-axis as intensity).

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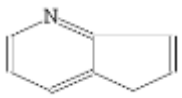
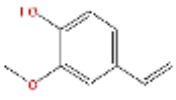
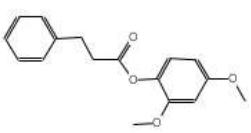
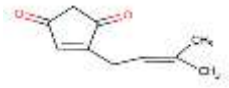
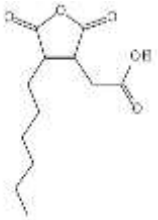
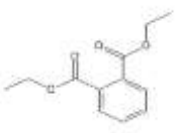
Table 1: Phytocomponents identified in stem bark methanol extract of *Terminalia catappa*

Compound name; chemical formula	**Retention time (min)**	**Area (%)**	**Chemical structure**	**Molecular weight (g/mol)**	**Compound type**	**Biological activity*** **
5H-1-Pyrindine; C_8H_7N	9.48	0.47		117	Pyridine	No activity
2-Methoxy-4-vinylphenol; $C_9H_{10}O_2$	9.666	0.44		150	Phenol	Anti-inflammatory activity (Jeong*et al.*, 2011).
2,4-Dimethoxyphenyl-beta phenpropionic acid; $C_{17}H_{18}O_4$	10.100	0.45		286	Benzene	No activity.
4-(3-Methyl-2-butenyl)-4-cyclopentene-1,3-dione; $C_{10}H_{12}O_2$	11.272	0.51		164	Cyclopentene	No activity.
(4-Hexyl-2,5-dioxo-2,5-dihydro-3-furanyl) acetic acid; $C_{12}H_{16}O_5$	11.551	1.64		240	Furan	No activity.
3-Butyl -1,2,4-cyclopentanetrione; $C_9H_{12}O_3$	12.340	0.29		168	Cyclic tricarboxylic anhydride	No activity.
Diethyl Phthalate; $C_{12}H_{14}O_4$	12.564	0.46		222	Benzene-1,2-dicarboxylic acid	Antimicrobial activity (Janu, 2014).

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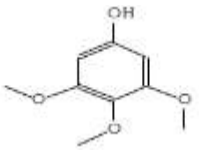
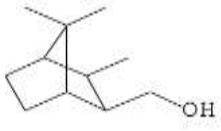
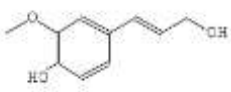
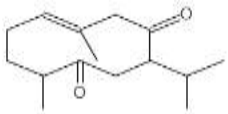
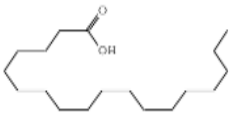
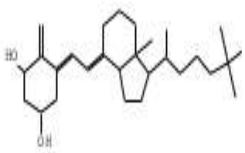
3,4,5-Trimethoxyphenol; $C_9H_{12}O_4$	12.703	0.55		184	Methoxy benzene	Antioxidant activity (Matos *et al.,* 2008).
(3,7,7-Trimethyl-bicyclo [2.2.1] hept-2-yl)-methanol; $C_{11}H_{20}O$	13.150	0.25		168	Monoterpenoid	No activity.
4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol; $C_{10}H_{12}O_3$	13.837	3.52		180	Phenylpropanoid	Antimicrobial agent (Ravikumar *et al.,* 2012).
Neocurdione; $C_{15}H_{24}O_2$	14.050	0.55		236	Germacrane sesquiterpenoid	Anti-tumour agent (Syed AbdulRahman*et al.,* 2013).
Octadecanoic acid; $C_{18}H_{36}O_2$	14.142	0.99		284	Fatty acid	Antiviral agent (Reagan *et al.,* 2013)
9,10-Secocholesta-5,7,10(19)-triene-1,3-diol, 25-[(trimethylsilyl)oxy]-, (3.beta.,5Z,7E); $C_{30}H_{52}O_3Si$	14.625	0.38		488	Secosteroid	No activity

NIST LIBRARY (Open chemistry database) ***Activity source: Dr Duke's phytochemical and Ethnobotanical Database

Table 2: The acute toxicity induced by oral administration of the stem bark aqueous and methanol extracts of *Terminalia catappa*.

Dose (mg/kg)	Number of animals	Number of deaths	Survival	Mortality ratio
Control	3	0	3	0/3
10	3	0	3	0/3
100	3	0	3	0/3
1000	3	0	3	0/3
1500	1	0	1	0/1
2500	1	0	1	0/1
2900	1	0	1	0/1
5000	1	0	1	0/1

Number of deaths recorded = 0
Number of animals that survived = 16
Mortality ratio = Number of deaths / Number of survival
LD_{50} > 5000mg/kg body weight

In recent studies, neocurdione have been shown to affect abnormal tissue growth which may be benign or malignant and against D-GalN / tumour necrosis factor-alpha-induced liver injury in mice.[18]
Phenylpropanoid is a biogenic compound reported to help plants overcome biotic and abiotic stress, and effective for plant defense. It has been shown to have promising potential anthelmintic in livestock.[19] Another study reported that phthalates and cyclopentanol can inhibit the growth of several pathogenic bacteria including Bacillus cereus, Helicobacter pylori, Salmonella spp, and Staphylococcus spp.[18] Bello among others have reported that some herbal bioactive agents have negative side effects contributed by their secondary metabolites, therefore toxicity evaluation of herbal medicines is not a misplaced priority.[20-22]
Results in Table 2 show Acute oral toxicity study of stem bark aqueous and ethanol extracts of *Terminalia catappa.* The study was carried out to determine dose(s) lethal to 50% of the experimental animals and establish its median lethal dose (LD_{50}). It may also serve as an initial assessment of toxic symtoms likely to arise from exposure to a test compound and can also provide a therapeutic index showing the margin of safety of a test compound[23].The results of both stem bark aqueous and methanol extract administered at the doses of 10, 100, 1,000, 1,600, 2,900 and 5000mg/kg showed no significant behavioural changes and mortality in the rats within 72 hours of exposure. The results of the acute toxicity studies carried out in this

present research are in sharp contrast to the reports of previous studies that reported the plant extract to be toxic at dose 5000mg/kg body weight. This study showed that the median lethal dose LD_{50} of both aqueous and methanol stem bark extracts are relatively > than 5 g/kg body weight and may be considered non-toxic and relatively safe. This is in agreement with the report of Clarke and Clarke (1975) that reported that any substance with LD_{50} above 5000mg/kg should be regarded as safe.[24]

Conclusion

The study provides evidence that the stem bark methanol extract of *Terminalia catappa* contain various volatile phytochemical compounds which may be of medicinal value. The structure-activity relationship of these detected bioactive compounds may be responsible for the various reported biological, therapeutic and pharmacological potential of the plant. The plant extract is nontoxic and considered to be relatively safe.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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