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**Toxicological, Bioassay-Guided Fractionation and Antibacterial Activity of Methanol Stem Extract of *Terminalia microptera* on Oral Bacteria Pathogens**Daniel A. Innalegwu<sup>1,2\*</sup>, Amuda Oladunni<sup>3,4</sup>, Gara T. Yebo<sup>1</sup>, Muhammad F. Mahmoud<sup>1</sup>, Saidu T. Bosso<sup>3,5</sup>, Atolagbe S. Oluwafemi<sup>6</sup><sup>1</sup>Department of Biochemistry, School of Life Sciences, Federal University of Technology Minna, Nigeria<sup>2</sup>Drug Discovery Unit, Centre for Genetic Engineering and Biotechnology, Federal University of Technology Minna, Nigeria<sup>3</sup>Department of Microbiology, School of Life Sciences, Federal University of Technology Minna, Nigeria<sup>4</sup>Dental Department, Niger State General Hospital Minna, Nigeria<sup>5</sup>Department of Biological Sciences, Niger State Polytechnic Zungeru, Nigeria<sup>6</sup>Forestry Research Institute of Nigeria, Ibadan, Ibadan, Nigeria

## ARTICLE INFO

## ABSTRACT

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The search for new lead compounds with improved therapeutic activity is ongoing due to microbial resistance to the currently available drugs. This study was aimed at evaluating the toxicological, bioassay-guided fractionation and antibacterial activity of methanol stem extract of *Terminalia microptera* on oral bacterial pathogens. Quantitative phytochemical determination was carried out using spectrophotometric method, acute and sub-chronic toxicity study was carried out following Lorke's and Organization for Economic Co-operation and Development (OECD) guidelines. Fractionation and antibacterial activity of the extract (20, 30 and 40 mg/mL) against *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Micrococcus luteus*, *Streptococcus mutans*, *Streptococcus pneumoniae* and *Streptococcus pyogenes* were carried out following standard protocol. The extract contains phenols, flavonoids, tannins, alkaloids and saponins at varying concentration range. Acute toxicity study of the extract gave an LD<sub>50</sub> of 1386 mg/kg. Sub-chronic toxicity study of the extracts revealed that the extracts have no significant effect ( $p > 0.05$ ) on the body weight, haematological, biochemical and renal parameters of the rats. The mean zones of inhibition of the extract and fractions revealed a significant ( $p < 0.05$ ) spectrum of activity ranging between 13.33–25.33mm and 15.50–24.00mm, respectively. Column chromatography of ethyl acetate sub-fraction of the extract yielded 4 fractions (TME<sub>1-4</sub>) and their antibacterial sensitivity test showed a spectrum of activity that ranged between 6.50–25.50mm against the tested bacterial isolates. GC-MS analysis of the fractions revealed 13 compounds in *T. microptera*. The current study revealed the pharmacological significance and safety of *T. microptera*. However, uncontrollable use of the extract without adequate dose regulation should be discouraged.

**Keywords:** Acute toxicity, Antibacterial sensitivity test, Bioassay-guided fractionation, Column chromatography, *Terminalia microptera*.

**Introduction**

Medicinal plants from ancient times play a key role in the quest for lead compounds for the synthesis and production of drugs<sup>1</sup>. Because of the issue of resistance, cost, inaccessibility, and bioaccumulation of harmful substances from the persistent or over utilization of the conventional drugs, consideration is currently turning towards nature to look for lead compounds with better therapeutic potentials, modest, less harmfulness and promptly accessible for use.<sup>3-</sup>

<sup>5</sup> This supports the report of the World Health Organization, 2008 that more than 80 % of the total populace of the world especially those of the developing nations depend majorly on medicinal plants for their day-to-day essential medical needs.<sup>3</sup> At present, over 20% of the

currently available drugs contain phytochemicals as their active components.<sup>1</sup> The greater part of the human ailments resulting from bacterial infections, protozoans, and parasitic diseases, disorder from metabolic complications and sickness related with oxidative stress have been overseen appropriately using medicinal plants.<sup>6-10</sup>

The development of multi-drug resistance in pathogenic microorganisms and parasites to different anti-infection agents and non-accessibility of safe antimicrobial agents for treatment has prompted a search for other antimicrobial agents from different sources with special interest on plants that might be utilized for the production of new drugs.<sup>11-12</sup> The vast majority of the natural products, basically of plant origin, have been screened to assess their antimicrobial activities on oral microorganisms.<sup>13-19</sup>

Different oral hygiene methods have been used to overcome widely endemic diseases such as dental caries and oral infections. Due to increasing awareness and expected evolving population; the use of safe, effective and economical products such as medicinal plant has expanded drastically.<sup>20</sup> Studies have shown that plant and herbal extracts possess significant antimicrobial potential against oral bacteria. As a result, several toothpaste and mouth rinses contain these herbal components to aid the conventional toothpaste. However only a limited amount of published data exists regarding the efficacy of these products on the initial plaque forming bacteria.<sup>21</sup> There exists a unique balance between the microbiota and the host in the oral cavity. Any

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alteration in these symbiotic relationships might result to an increase in the numbers of microorganism that colonize the oral cavity which may subsequently allow the cohabitation of other pathogenic microorganisms resulting in conditions such as dental caries, endodontic injuries, periodontitis, and even cancer.<sup>23,24</sup> Factors responsible for these changes in the oral cavity among others includes poor/improper diet, unhealthy lifestyles such as the use of tobacco, alcohol, poor oral hygiene, and social lifestyle.<sup>25</sup> It is important to reestablish this symbiotic relationship in the oral cavity when these changes occur. Likewise, the use of antimicrobial agents couple with mechanical treatment is a prerequisite in the avoidance and control of oral maaicrobes.<sup>23</sup>

Literature review shows that bioassay-guided fractionation is one of the methods utilized by researchers to examine and isolate pharmacologically active compounds from their natural source.<sup>26-32</sup> This is a stepwise and thorough process that guides the researcher to screen and isolate bioactive compound from natural source and also ensure that the activity of the compound is not lost in the process due to contamination as the compounds or extracts is intermittently screened in the process for their bioactivity.

*Terminalia microptera* belong to the family *Combretaceae* also known as "Orin idi odan" among the Yoruba people in the South Western part of Nigeria. It is a typical tropical African plant found in the guinea savanna. The leaves, fruits, and stems are used for the treatment of different ailments such tuberculosis and hepatitis.<sup>32,33</sup> Phytochemical analysis of the stem bark extract was reported to contain around 9-11 bioactive compounds.<sup>34</sup>

Several herbal toothpastes are now readily available to the public. Many are found in retail stores and are available without prescription.<sup>21</sup> The greatest challenge confronting the use of medicinal plant products or extracts and its subsequent rejection by many is dose quantification and safety. This is on the grounds that the traditional herbal practitioners find it difficult to determine the actual or effective safe dose and delayed or adverse effects resulting from prolong use of these extracts.<sup>35</sup> This study was therefore aimed at evaluating the toxicological, bioassay-guided fractionation and antibacterial activity of methanol stem extract of *Terminalia microptera* on oral bacterial pathogens.

## Materials and Methods

### Plant material

Stems of *Terminalia microptera* was collected from Kaba-bonu Local Government Area of Kogi State, North Central Nigeria in the month of August, 2015. The plant was identified and authenticated at the Herbarium Department of the National Institute of Pharmaceutical Research and Development, Idu, Abuja, Nigeria, and a voucher specimen was deposited with voucher numbers: NIPRD/H/6797.

### Experimental animals

Healthy female albino rats weighing between 170-200g were procured from the Animal Breeding unit of the National Institute of Pharmaceutical Research and Development, Idu, Abuja, Nigeria. The animals were maintained under standard laboratory conditions of 25°C ± 2 room temperature and a relative humidity of 40-50 % with access to commercial feed pellets (growers) and water ad libitum. Animals handling and experimentations complied with international standards (NIH Publication No. 85-23, 1985). The animals were fasted for 12 h before the commencement of any study so as to prevent any reaction or side effect that may result from the food consumed.

### Source of bacterial isolates

The bacterial isolates (*Klebsiella pneumoniae* strain BYK-9, *Streptococcus pneumoniae* ATCC 33400, *Streptococcus pyogenes* strain JCM 5674, *Micrococcus luteus* ATCC 10240, *Pseudomonas aeruginosa* ATCC 10145, and *Streptococcus mutans* ATCC 25175) were obtained from the Dental Department of General Hospital Minna, Niger State, Nigeria and were preserved in a nutrient agar slant bottle in a refrigerator at 4°C until required for use.

### Chemicals and reagents

The media, chemical and reagent are of analytical grade.

### Sample preparation and extraction

The stem of *Terminalia microptera* was washed, cut into pieces, and air-dried at room temperature (37°C) for 3 weeks. The dried stem was crushed using mortar and pestle and finally grounded using a grinder mill. Five hundred grams (500 g) of the plant materials were extracted with methanol at 40°C using the reflux apparatus and the resulting extract was concentrated using a rotary evaporator.

### Quantitative phytochemical contents determination

The methanol extract of *T. microptera* was analyzed quantitatively for the presence of phenols, flavonoids, alkaloids, tannins, and saponins using spectrophotometric method.<sup>36-38</sup>

### Acute Toxicity Study

The rats were randomized into 8 groups comprising 4 animals each. Predetermined test doses (100, 200, 400, 800, 1200, 1600, and 2000 mg/kg body weight) were administered orally in a single dose to the rats orally according to Lorke method<sup>13</sup>. Thereafter, animals were observed for the manifestation of toxicity and mortality for 72 hours.<sup>33</sup>

### Chronic Toxicity Study

A repeated-dose toxicity study was employed following OECD guidelines to assess the toxic effects resulting from the accumulation of compounds in an organism. The selected safe dose for the plant extract was used. A total of 40 rats (20 rats for the test and 20 rats for the control) was used. The study was conducted for ten (10) weeks and at the end of week 1, 5, and 10, four rats are randomly selected, anesthetized and their blood samples collected through the cardiac puncture into sample bottles containing EDTA for hematological analyses while the remaining blood was kept in the plain bottle from which serum was collected and stored for biochemical analysis. At the end of the experiment, the remaining rats were sacrificed, organs harvested, weighed, and preserved in 10 % formalin for histopathology study of the liver and kidney.<sup>33</sup>

### Determination of the antibacterial activity of the extracts and fractions

#### Standardization of the bacterial culture

Zero-point two milliliters (0.2 mL) of overnight cultures of the test organism were transferred into 20 mL of sterile nutrient broth and the culture was incubated for 3 hours at 37°C to standardize the culture to 10<sup>6</sup> CFU/mL McFarland. A loopful of the standardized inoculum was used for the antibacterial assay.<sup>39</sup>

#### Susceptibility testing of plant crude extracts

Thirty-nine gram (39 g) of the Nutrient agar was dissolved into 1L of distilled water in a conical flask. The mixture was sterilized in the autoclave at 121°C for 15minutes and the medium was allowed to cool to 45°C. Sterile molten Muller Hinton agar (20 mL) at 45°C was dispensed into sterile Petri dishes and allowed to set. A sterile cork borer of diameter 6 mm was used to bore equidistant wells onto the agar plates. One drop of the molten agar was used to seal the bottom of the bored wells to prevent the extract from sipping beneath the agar. Sterile cotton swab sticks were used to streak on the surface of the agar plates with the standardized test organisms and 100 µL of the extract (20, 30, and 40 mg/mL) of methanol extract of the plant were added separately to the bored wells and 5 mg/mL of the standard drug (Amoxicillin and Ampiclox) were used as positive control while dimethyl sulfoxide (DMSO) served as the negative control. Thirty minutes pre-diffusion time was allowed after which the plates were incubated at 37°C for 24 hours. The zones of inhibition were then measured in millimeters. The above method was carried out in triplicates and the mean of the result was taken. Test with ten millimeters (10 mm) zones of inhibition was considered sensitive to the plant extract. The performed agar well diffusion susceptibility test was based on the modified methods of the Clinical Laboratory Standards Institute.<sup>40</sup>

#### Determination of minimum inhibitory concentration (MIC)

The tube dilution method as described by Kabir *et al.*<sup>41</sup> and Akinyemi *et al.*<sup>42</sup> with slight modification using spectrophotometer were used to determine the minimum inhibitory concentration. A series of two-fold dilutions of each extract ranging from 40 mg/mL to

0.039 mg/mL was made in nutrient broth. Zero-point one milliliter (0.1 mL) of each of the standardized test organisms (0.5 McFarland turbidity standard) was added to each dilution. Two controls were maintained for each batch. These included tubes containing extract and growth medium without inoculum and tube containing the growth medium and inoculum (organism control). The tubes were incubated at 37°C for 24 hours. At the end of the incubation period, the optical density of the cultures in the test tubes was read using a spectrophotometer at a wavelength of 600 nm (this wavelength was used because absorbance of light by other molecules in the microbial cells such as flavin's and carotenoids is minimal at this wavelength, also spectrophotometer was used because the absorbance is directly proportional to the number of cells in the cultures and the color intensity of the extract will not allow an effective visual observation while the spectrophotometer was adjusted to zero using sterile nutrient broth void of extracts and test organism. The MIC was determined by subtracting the absorbance of the negative control from the absorbance of the test and comparing the result with the absorbance of the positive control using the formula:

Absorbance of Test (T) – Absorbance of Control (C0) = Absorbance of positive control (C1)

The concentration/test tube where a significant reduction in absorbance was observed was recorded as the MIC.

*Determination of minimum bactericidal concentration (MBC)*

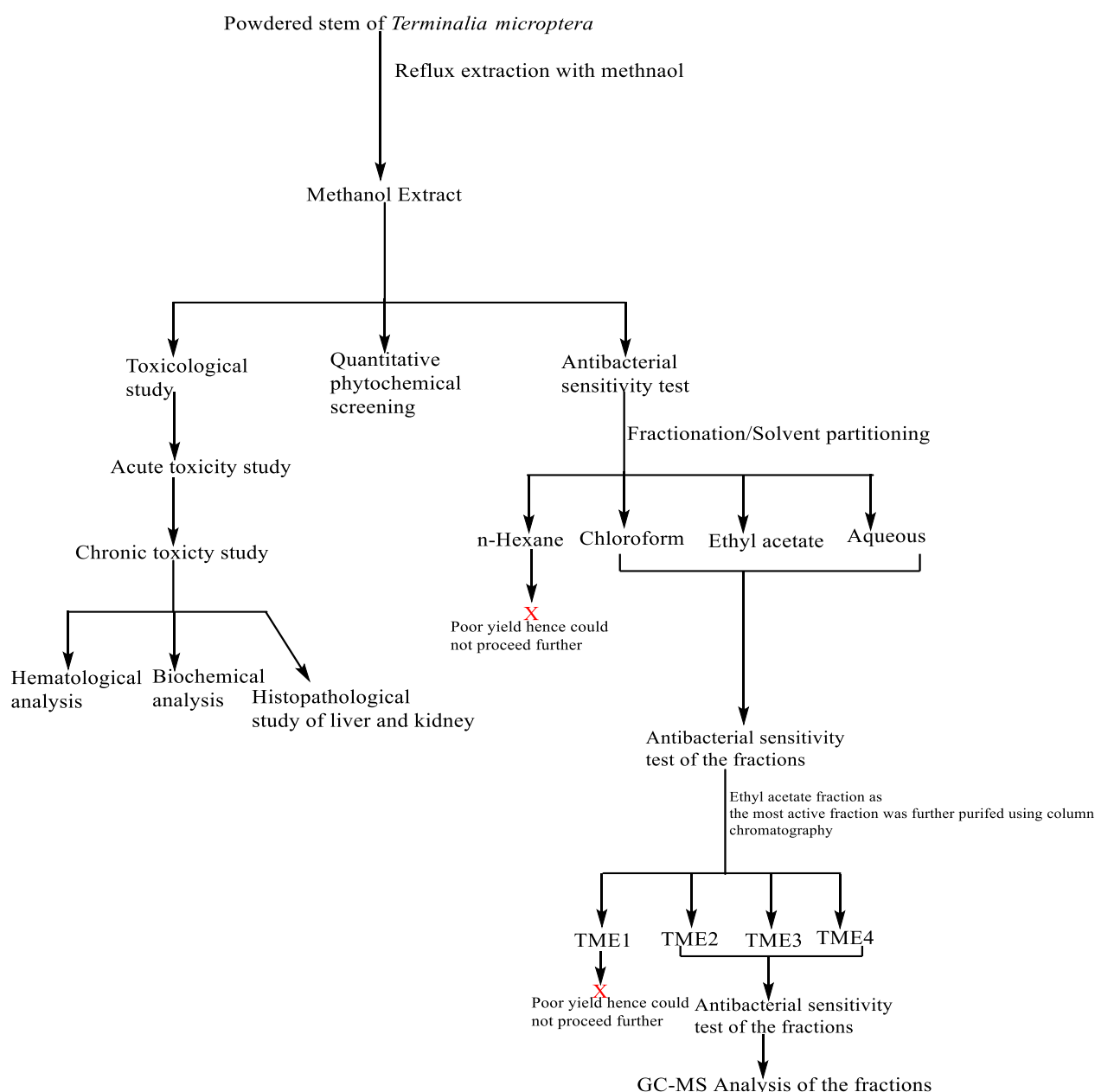
The minimum bactericidal concentration (MBC) was determined by subculturing the cultures with the lowest optical density beginning with the test tube containing the minimum inhibitory concentration and above onto a freshly prepared nutrient agar medium. The cultures were incubated for 24 hours at 37°C, after incubation, the culture concentration without visible growth was regarded as the minimum bactericidal concentration.<sup>41,42</sup>

*Fractionation of the crude extracts*

*Partitioning of the crude extract*

Methanol extract (25 g) was dissolved in 100 mL of distilled water and was partitioned into n-hexane, chloroform, and ethyl acetate in increasing order of the solvent polarity (n-hexane < chloroform < ethyl acetate < distilled water) using separating funnel. The resultant fractions were evaporated to dryness at a reduced temperature of 40°C in the water bath and the weight of each of the fractions was taken.

The fractions were then subjected to antibacterial sensitivity test. MIC and MBC were also carried out to detect the active fraction (s) using the methods described Kabir *et al.*<sup>41</sup> and Akinyemi *et al.*<sup>42</sup>



**Figure 1:** Flow chart showing the stepwise Bioassay-Guided Fractionation of methanol extract of *Terminalia microptera*

### Column chromatography of active fraction

Three grams (3 g) of the sample was adsorbed in 10 mL of distilled water with 2g of silica gel and allowed to dry. The dried sample was properly crushed into powder to give it a good surface area for the penetration of the solvent. The column which was made of a sintered glass at the base was packed to two-third of its height with the slurry made of silica gel and n-hexane. The solvent was eluted gradually to allow good compact silica gel in the column. The sample was applied at the top of the silica gel and eluted with different solvents mixture in increasing order of polarity (100% n-hexane, 9:1 n-hexane/chloroform, 4:1 n-hexane/chloroform, 1:1 n-hexane/chloroform, 1:4 n-hexane/chloroform, 1:9 n-hexane/chloroform, 100% chloroform, 1:1 chloroform/ethyl acetate, 100% ethyl acetate, 1:1 ethyl acetate/methanol and 100% methanol). The different fractions collected were spotted on a Thin Layer Chromatographic (TLC) Plate, developed in an appropriate solvent system, and viewed under the UV-lamp and iodine tank to detect fractions with similar components. All fractions with similar Retardation Factor (Rf) were bulk together and properly labeled.<sup>43</sup> The resulting fractions were subjected to an antibacterial susceptibility test and their MIC and MBC also determined.

### Gas Chromatography (GC)-Mass Spectrometer (MS) Analysis

The GC-MS analysis of the purified fractions from aqueous sub-fraction of *Terminalia microptera* methanol extract (TME<sub>1</sub>, TME<sub>2</sub>, and TME<sub>3</sub>) was carried out on a GC-MS-QP 2010 Plus Shimadzu system (SHIMADZU, JAPAN). Gas chromatograph interfaced with a mass spectrometer (GC-MS) instrument was used; Column elite-1 fused silica capillary column (30m x0.25mm ID x  $\mu$ L df, composed of 100 % dimethyl polysiloxane). For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1ml/min and an injection volume of 2 $\mu$ L 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/min, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scanning interval of 0.5s and fragments from 40 to 550 Da. The total GC running time was 27 minutes. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adapted to handle mass spectra and chromatogram was a turbo mass and the detection of compounds employed the database of the National Institute of Science and Technology (NIST) NIST Ver. 2.0 year 2009 library.

### Statistical analysis

The analysis was performed using the SPSS statistical package for WINDOWS (version 21.0; SPSS Inc., Chicago). Data were expressed as the Mean $\pm$ Standard Error of Mean of three determination. Results were subjected to one ANOVA followed by the Duncan Multiple Range Test (DMRT). Values were considered statistically significant at  $p < 0.05$ .

## Results and Discussion

The quantitative phytochemical contents of the extract revealed varying amounts of phytochemicals (Table 1). These phytochemicals in plants are the major determinant for their medicinal uses since they are responsible for various metabolic processes and therapeutic efficacies<sup>52</sup>. Phenols and flavonoids are a natural antioxidant that helps in ameliorating the problem of reactive oxygen species (ROS) generated by some physiological conditions.<sup>54,55</sup> Saponins have been reported to have harmful effects by haemolyzing circulating erythrocytes<sup>56</sup>. The pharmacological significance of alkaloids includes antihypertensive, antiarrhythmic, antimalarial and anticancer while tannins have been reported to play a major role as anti-inflammatory, antiseptic, antioxidant and hemostatic.<sup>54</sup>

One of the toxicological parameters used for the assessment of plant extracts safety is the Median Lethal Dose (LD<sub>50</sub>), which is the amount of a drug required to cause mortality in half of the test population.<sup>4, 49,50</sup> In this study, an acute toxicity study of methanol stem extract of *T. microptera* revealed that the extract is safe at an LD<sub>50</sub> of 1386

mg/kg bodyweight with mortality of 3 animals at 1600 mg/kg body weight of the extract (Table 2).

**Table 1:** Quantitative phytochemical contents of methanol stem extract of *Terminalia microptera*

Phytochemicals	Amount (mg/100g)
Total Phenols	880.393 $\pm$ 6.05
Total Flavonoids	305.667 $\pm$ 222.94
Alkaloids	133.863 $\pm$ 8.50
Tannins	145.009 $\pm$ 1.74
Saponins	7.338 $\pm$ 0.84

Values are expressed in mean $\pm$ standard error of mean of duplicate determination.

These recorded deaths maybe as a result of delayed drug effects according to the Organization for Economic Co-operation and Development (OECD) guidelines 2002.<sup>51</sup> Akponova *et al.*,<sup>4</sup> and Etuk *et al.*<sup>57</sup> reported LD<sub>50</sub> of 5000 mg/kg and 2000 mg/kg bodyweight ethanol stem extract of *T. microptera*. These variations may occur due to differences in the type of solvents used for extraction, the developmental stage of the plant, place and season of collection of the sample. The behavioral profiles of all the tested animals revealed response to pain and touch, with no signs of depression or restlessness. Monitoring of body weight during treatment usually provides a fair index on the reactions of drugs or supplements in the animals.<sup>53</sup> Extract of *T. microptera* initiated a significant weight loss in the treated groups compared to the control group (Figure 3). This observed weight loss may be influenced by the loss of appetite or weakness resulting from the intake of the extract as seen in Table 2 above<sup>34</sup>.

There was no significant difference ( $p > 0.05$ ) in the hematological, biochemical, liver and renal parameters of the control and the treated groups throughout the 10 weeks of treatment (Table 3-5). However, there exists a significant difference ( $p < 0.05$ ) between the serum enzymes of the control and treated groups throughout the period of treatment with the extracts (Figure 2). The serum enzyme level shows there was no significant difference ( $p > 0.05$ ) between the treated and the control group (Figure 2) suggesting that the extract may not have adverse effect on the integrity of the liver. This observation was further supported by the significant decrease ( $p < 0.05$ ) in the concentration of the total and conjugated bilirubin (Table 4) an index that shows a stable and good performance of the liver in the presence of the extract.<sup>34</sup> The result of the liver and kidney histology of the treated and control groups (Plate I-IV) revealed normal histoarchitecture, confirming the observation in the serum enzymes level which may not be due to the damage on the liver and kidney but an indication of the health status of the experimental animals before usage.

The antibacterial potentials of the extracts and fractions were assessed based on the zones of inhibition of bacterial growth of the test organisms. The mean zones of inhibition of the isolates are a function of the relative antibacterial activity of the extracts. The zone of inhibition is the area that remains free of bacterial growth and the sizes of the zones of inhibition are usually related to the susceptibility of the extract.<sup>58</sup> The methanol extract of *T. microptera* showed a broad spectrum of activity ranging from 13.33 – 25.33 mm in a dose-dependent manner and a significant difference ( $p < 0.05$ ) with the standard antibiotics (Amoxicillin and Ampiclox) (Table 6). The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the methanol extract further confirm the activity of the extract on the test bacterial isolates with *Streptococcus pneumoniae* as the most susceptible organisms with an MIC and MBC of 2.5 mg/mL (Figure 5). Partitioning of the methanol extract (Table 7) shows that ethyl acetate fraction has the highest yield (50.24%) while the n-hexane fraction has the lowest (0.92 %). This shows that the methanol extract of *T. microptera* contains less amount of non-polar substance with much of secondary polar materials. Subsequent antibacterial susceptibility test of the fraction also showed an improvement in the zone of inhibition in the ethyl acetate sub-fraction

(TME) with zones of inhibition ranging between 15.50 – 24.00 mm at 20, 30 and 40 mg/mL compared to chloroform sub-fraction (TMC) and aqueous sub-fraction (TMA) which shows a decrease in their zones of inhibition compared to the crude methanol extract (Table 8). The essence of the bioassay-guided fraction is to screen for the fractions or compounds responsible for the observed activity in the crude extract. It also aids in isolation or identification of the component present in the extract that may be responsible for the observed level of activity<sup>43</sup>. Ethyl acetate is a secondary polar solvent and its extract has been reported to contain phytochemicals majorly alkaloids, saponins and some classes of phenols that play important roles as antibacterial agents and maybe the rationale behind the observed level of activity in TME fraction. This observation supports the findings of Tata *et al.*<sup>59</sup> and Sakar *et al.*<sup>43</sup> who reported similar

observation in their respective studies. The variation in the zones of inhibition between the crude (Table 6), partitioned fraction (Table 8), and fractions obtained from the column (Table 10) may be due to a decrease in the concentration of the phytochemical constituents present in these fractions as the purification processes progress. It is worthy of note to remember that fractionation does not in all instances increase the level of activity as some of these phytochemicals act in synergy to produce better activity than when acting independently<sup>43,60</sup>. Result from this study revealed that high MIC and MBC values obtained against the test organisms (Figure 8, 9 and 10) is an indication of low concentration of the active constituent in the fractions while low MIC and MBC values (Figure 7, 9 and 10) indicate that the extract contain active constituents with high potency against bacterial growth.

**Table 2:** Effect of different doses of crude extract of *T. microptera* in rats

Doses (mg/kg bw)	Observations	Mortality
100	The animal appeared healthy with normal physical activity and feed uptake. No mortality was obtained within 72 hours.	0/4
200	The animals were generally devoid of any impairment. Cardiac, respiratory output and other physical activities were normal. There was no mortality in 72 hours.	0/4
400	Minor alterations in physical activity were observed. Animals were initially sluggish but regained full physical output consequently. None of the animals died in 72 hours.	0/4
800	Initial poor response to external stimuli, poor uptake of feed, and generally diminished physical activity. The animals however become more alert after 48 hours. None died within the 72 hours of observation.	0/4
1200	Animals were generally healthy with no indications of any distress. Absence of mortality in 72 hours.	0/4
1600	Marked acute toxicity symptoms in the four rats including tachycardia, erythema of the nose, paws and ears shock, and the death of three out of four experimental animals.	3/4

Selected save dose = 1200 mg/kgbw

$$LD_{50} = \sqrt{(Maximum\ tolerated\ dose)(Minimum\ lethal\ dose)} = \sqrt{(1200)(1600)} = 1386\text{ mg/kg b.w.}$$

**Table 3:** Effect of methanol stem extract of *T. microptera* on the haematological parameters of rat

Parameters	Treatments	Week 1	Week 5	Week 10
Hb (g/L)	Test	14.41 ± 0.25 <sup>c</sup>	15.56 ± 0.24 <sup>c</sup>	15.03 ± 0.13 <sup>c</sup>
	Control	13.37 ± 0.29 <sup>c</sup>	14.54 ± 0.20 <sup>c</sup>	14.71 ± 0.19 <sup>c</sup>
PCV (%)	Test	35.28 ± 0.41 <sup>e</sup>	32.63 ± 0.21 <sup>e</sup>	32.58 ± 0.28 <sup>e</sup>
	Control	33.29 ± 0.75 <sup>e</sup>	32.47 ± 0.22 <sup>e</sup>	32.11 ± 0.46 <sup>e</sup>
MCHC (g/dL)	Test	40.96 ± 0.82 <sup>f</sup>	48.57 ± 0.78 <sup>f</sup>	46.14 ± 0.73 <sup>f</sup>
	Control	41.34 ± 1.03 <sup>f</sup>	44.81 ± 0.76 <sup>f</sup>	45.83 ± 0.51 <sup>f</sup>
MCH (pg)	Test	23.27 ± 0.18 <sup>d</sup>	21.80 ± 0.11 <sup>d</sup>	25.87 ± 0.11 <sup>d</sup>
	Control	22.27 ± 0.36 <sup>d</sup>	25.09 ± 0.07 <sup>d</sup>	22.84 ± 0.19 <sup>d</sup>
MCV (fL)	Test	59.81 ± 0.19 <sup>g</sup>	60.88 ± 0.29 <sup>h</sup>	61.85 ± 0.13 <sup>h</sup>
	Control	59.52 ± 0.25 <sup>g</sup>	58.01 ± 0.35 <sup>g</sup>	58.10 ± 0.32 <sup>g</sup>
WBC (x 10 <sup>9</sup> /L)	Test	9.13 ± 0.26 <sup>b</sup>	9.86 ± 0.14 <sup>b</sup>	9.28 ± 0.39 <sup>b</sup>
	Control	8.48 ± 0.22 <sup>b</sup>	8.38 ± 0.20 <sup>b</sup>	8.86 ± 0.10 <sup>b</sup>
Neutrophils (%)	Test	58.65 ± 0.25 <sup>g</sup>	59.30 ± 0.14 <sup>g</sup>	59.07 ± 0.32 <sup>g</sup>
	Control	60.68 ± 0.35 <sup>g</sup>	59.38 ± 0.22 <sup>g</sup>	59.16 ± 0.86 <sup>g</sup>
Lymphocytes (%)	Test	67.59 ± 0.19 <sup>h</sup>	68.28 ± 0.08 <sup>i</sup>	67.94 ± 0.41 <sup>i</sup>
	Control	65.80 ± 0.45 <sup>h</sup>	66.97 ± 0.41 <sup>i</sup>	67.53 ± 0.13 <sup>h</sup>
Eosinophiles (%)	Test	0.88 ± 0.01 <sup>a</sup>	0.93 ± 0.01 <sup>a</sup>	0.97 ± 0.03 <sup>a</sup>
	Control	0.88 ± 0.01 <sup>a</sup>	0.89 ± 0.01 <sup>a</sup>	0.94 ± 0.03 <sup>a</sup>
Platelet (x 10 <sup>9</sup> /L)	Test	97.84 ± 0.92 <sup>i</sup>	97.20 ± 0.42 <sup>j</sup>	97.12 ± 0.93 <sup>j</sup>
	Control	97.52 ± 0.24 <sup>i</sup>	98.52 ± 0.25 <sup>j</sup>	96.83 ± 0.51 <sup>i</sup>

Key: Hb: Haemoglobin, PCV: Packed Cell Volume, MCHC: Mean Corpuscular Haemoglobin Concentration, MCV: Mean Corpuscular Volume, WBC: White Blood Corpuscles. Values on the same row with different superscript are significantly different at p ≤ 0.05.

**Table 4:** Effect of methanol stem extract of *T. microptera* on the liver and biochemical parameter of rat

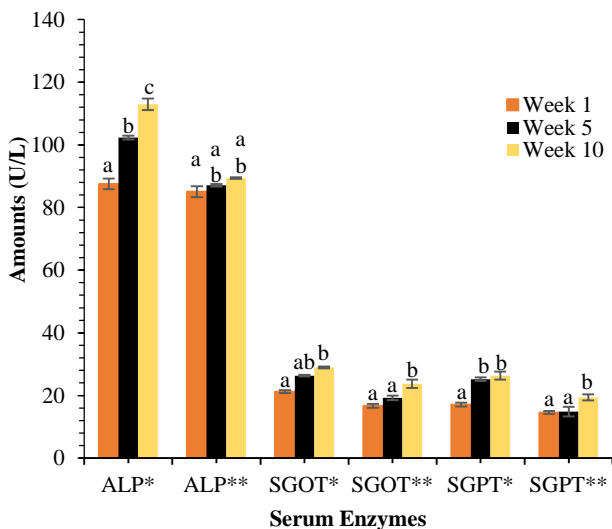
Parameters	Treatments	Week 1	Week 5	Week 10
Glucose (mg/dL)	Test	106.13 ± 0.77 <sup>d</sup>	105.38 ± 0.67 <sup>d</sup>	100.88 ± 0.44 <sup>c</sup>
	Control	105.98 ± 0.53 <sup>d</sup>	102.66 ± 0.35 <sup>d</sup>	103.52 ± 0.25 <sup>d</sup>
Triglycerides(mg/dL)	Test	146.09 ± 1.08 <sup>e</sup>	147.51 ± 0.51 <sup>e</sup>	148.88 ± 0.21 <sup>d</sup>
	Control	141.10 ± 1.20 <sup>e</sup>	144.00 ± 0.74 <sup>e</sup>	145.36 ± 0.26 <sup>e</sup>
Total protein (g/L)	Test	6.32 ± 0.20 <sup>a</sup>	6.99 ± 0.48 <sup>b</sup>	4.67 ± 0.25 <sup>a</sup>
	Control	6.19 ± 0.08 <sup>a</sup>	6.91 ± 0.06 <sup>b</sup>	7.04 ± 0.07 <sup>b</sup>
Albumin (g/L)	Test	4.92 ± 0.06 <sup>a</sup>	3.72 ± 0.29 <sup>a</sup>	3.04 ± 0.15 <sup>a</sup>
	Control	5.44 ± 0.17 <sup>a</sup>	5.61 ± 0.19 <sup>b</sup>	6.37 ± 0.20 <sup>b</sup>
Total Bilirubin (mg/L)	Test	22.85 ± 0.12 <sup>b</sup>	26.88 ± 0.42 <sup>c</sup>	19.67 ± 0.46 <sup>ab</sup>
	Control	21.57 ± 0.26 <sup>c</sup>	21.89 ± 0.55 <sup>b</sup>	22.45 ± 0.21 <sup>c</sup>
Conjugated Bilirubin (mg/dL)	Test	8.80 ± 0.11 <sup>b</sup>	5.23 ± 0.17 <sup>ab</sup>	4.59 ± 0.17 <sup>a</sup>
	Control	8.32 ± 0.14 <sup>b</sup>	9.00 ± 0.10 <sup>b</sup>	9.40 ± 0.21 <sup>b</sup>

Values on the same row with different superscript are significantly different at  $p \leq 0.05$ .

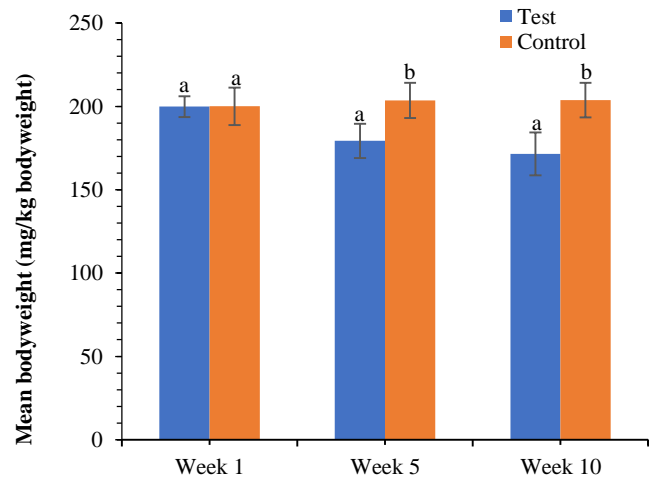
**Table 5:** Effect of methanol stem extract of *T. microptera* on the renal parameter of rat

Parameters	Treatments	Week 1	Week 5	Week 10
Urea (mg/dL)	Test	35.36 ± 0.24 <sup>a</sup>	35.01 ± 0.14 <sup>a</sup>	36.20 ± 0.39 <sup>a</sup>
	Control	34.70 ± 0.18 <sup>a</sup>	35.18 ± 0.70 <sup>a</sup>	34.79 ± 0.35 <sup>a</sup>
Creatinine(mg/dL)	Test	7.59 ± 0.23 <sup>a</sup>	9.06 ± 0.06 <sup>ab</sup>	7.02 ± 0.08 <sup>a</sup>
	Control	7.18 ± 0.18 <sup>a</sup>	8.47 ± 0.22 <sup>a</sup>	8.48 ± 0.32 <sup>a</sup>
Uric acid (mg/dL)	Test	6.02 ± 0.04 <sup>a</sup>	6.02 ± 0.06 <sup>a</sup>	5.94 ± 0.10 <sup>a</sup>
	Control	5.09 ± 0.06 <sup>a</sup>	5.98 ± 0.03 <sup>a</sup>	6.01 ± 0.02 <sup>a</sup>
Na <sup>+</sup> (mmol/L)	Test	135.08 ± 0.40 <sup>b</sup>	143.44 ± 1.44 <sup>c</sup>	146.17 ± 0.46 <sup>c</sup>
	Control	131.42 ± 0.56 <sup>a</sup>	137.83 ± 0.98 <sup>bc</sup>	137.59 ± 0.28 <sup>bc</sup>
K <sup>+</sup> (mmol/L)	Test	5.87 ± 0.05 <sup>a</sup>	6.75 ± 0.11 <sup>a</sup>	8.26 ± 0.06 <sup>b</sup>
	Control	5.82 ± 0.04 <sup>a</sup>	6.61 ± 0.11 <sup>a</sup>	7.18 ± 0.24 <sup>a</sup>
Cl <sup>-</sup> (mmol/L)	Test	96.70 ± 0.93 <sup>a</sup>	100.70 ± 0.69 <sup>b</sup>	102.77 ± 0.03 <sup>b</sup>
	Control	99.23 ± 0.63 <sup>a</sup>	98.50 ± 0.22 <sup>a</sup>	99.87 ± 0.83 <sup>a</sup>
HCO <sub>3</sub> <sup>-</sup> (mmol/L)	Test	25.61 ± 0.27 <sup>a</sup>	27.82 ± 0.26 <sup>a</sup>	27.69 ± 0.54 <sup>a</sup>
	Control	25.33 ± 0.28 <sup>a</sup>	25.29 ± 1.08 <sup>a</sup>	26.33 ± 0.26 <sup>a</sup>

Values on the same row with different superscript are significantly different at  $p \leq 0.05$



**Figure 2:** Effect of crude extract of *T. microptera* on the serum enzymes of rat. Bars of same color with the same alphabet have no significant different at  $p < 0.05$  \* = Test group, \*\* = Control group



**Figure 3:** Effect of methanol stem extract of *T. microptera* on the bodyweight of rats. Bars with same alphabet on the same week have no significant difference ( $p > 0.05$ ).

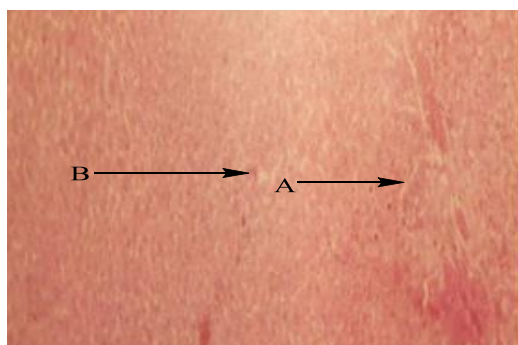


Plate I: Liver section of control showing normal hepatocyte architecture  
Keys: A = Sinusoid, B = Hepatocyte

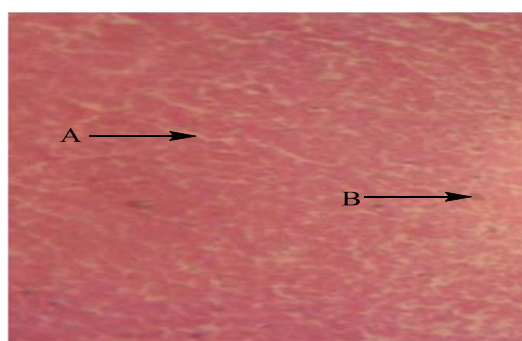


Plate II: Liver section of test showing normal hepatocyte architecture  
Keys: A = Sinusoid, B = Hepatocyte

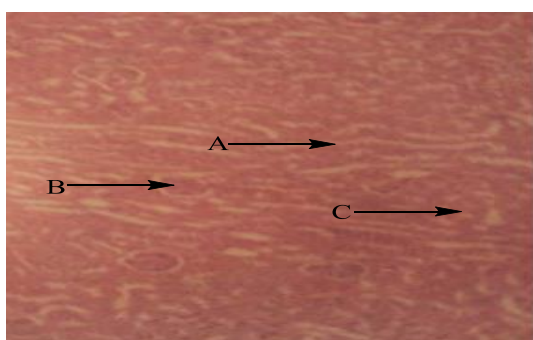


Plate III: Kidney section of control with normal glomeruli and collecting duct

Keys: A = Collecting duct, B = Capsule, C = Glomerulus

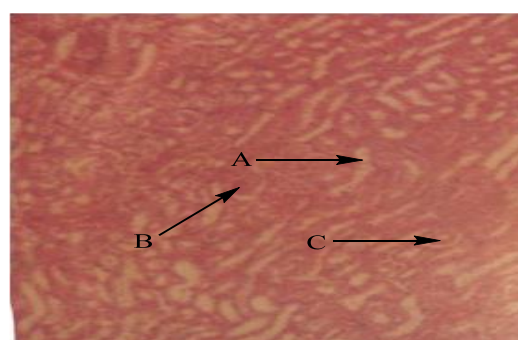


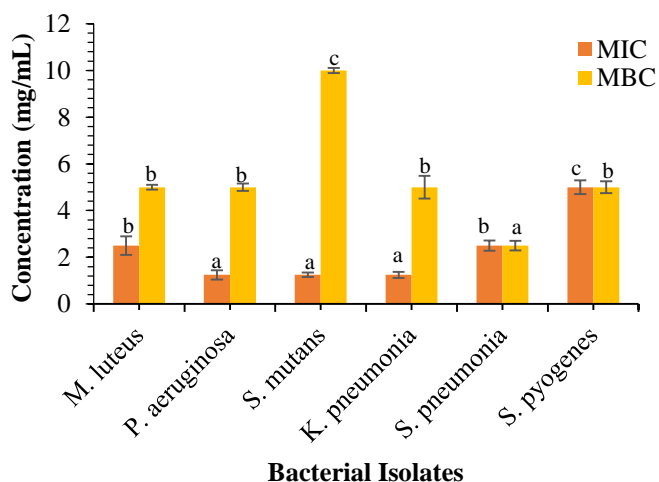
Plate IV: Kidney section of test with normal glomeruli and collecting duct

Keys: A = Collecting duct, B = Capsule, C = Glomerulus

**Table 6:** Mean zones of inhibition (mm) of methanol extracts of *Terminalia microptera* against test organisms

Isolates	20 mg/mL	30 mg/mL	40 mg/mL	Amoxicillin* 5 mg/mL	Ampiclox* 5 mg/mL
<i>M. luteus</i>	0.00 ± 0.00 <sup>a</sup>	14.67 ± 0.67 <sup>b</sup>	20.33 ± 0.67 <sup>c</sup>	26.33 ± 0.33 <sup>c</sup>	22.33 ± 0.33 <sup>cd</sup>
<i>P. aeruginosa</i>	17.33 ± 0.88 <sup>bc</sup>	16.00 ± 0.5 <sup>b</sup>	23.67 ± 1.86 <sup>d</sup>	13.67 ± 0.33 <sup>a</sup>	20.67 ± 0.33 <sup>c</sup>
<i>S. mutans</i>	20.67 ± 1.20 <sup>ab</sup>	20.33 ± 0.67 <sup>ab</sup>	23.67 ± 0.33 <sup>bc</sup>	25.67 ± 0.33 <sup>c</sup>	28.00 ± 1.00 <sup>d</sup>
<i>K. pneumoniae</i>	18.67 ± 0.33 <sup>a</sup>	20.33 ± 0.33 <sup>b</sup>	25.33 ± 0.33 <sup>cd</sup>	19.00 ± 1.00 <sup>ab</sup>	24.33 ± 0.33 <sup>c</sup>
<i>S. pneumoniae</i>	19.00 ± 0.58 <sup>d</sup>	19.67 ± 0.33 <sup>d</sup>	21.33 ± 0.6 <sup>de</sup>	15.67 ± 0.33 <sup>c</sup>	24.33 ± 0.67 <sup>e</sup>
<i>S. pyogenes</i>	17.00 ± 1.00 <sup>b</sup>	13.33 ± 0.33 <sup>a</sup>	17.00 ± 0.67 <sup>b</sup>	26.00 ± 0.58 <sup>c</sup>	27.67 ± 0.33 <sup>f</sup>

Values are expressed in mean ± standard error of mean, values with the same superscript on the same row have no significance difference ( $p > 0.05$ ),  $n = 3$  \* Specification for Amoxicillin and Ampiclox are:  $\leq 19$  (resistance) and  $\geq 20$  (susceptible) Clinical Laboratory Standard Institute (CLSI, 2012).



**Figure 4:** MIC and MBC of methanol extract of *T. microptera* against test organisms. Bars of same color with the same alphabet have no significant different at  $p < 0.05$ .

The GC-MS analysis of fraction TME<sub>2-4</sub> revealed 13 compounds in ethyl acetate column fractions of *T. microptera* (Table 11). According to literature survey, the antibacterial activity of the extract and fractions may be due to the presence of compounds such as aliphatic (long-chain alkanes and alkenes) and methyl ester fatty acids in the lipid layer (lipopolysaccharide) of the cell membrane and mitochondria which consequently disturbs the integrity of the cell structure making it permeable<sup>61</sup>. This outcome is comparable to that of Shetonde *et al.*<sup>62</sup> who reported that long-chain unsaturated fatty acids are bactericidal against important pathogenic microorganisms, including methicillin-resistant *Staphylococcus aureus*, *Mycobacterium* and *Helicobacter pylori*. The activity of most of the compounds identified in these fractions may also be attributed to their functional groups such as carboxyl, methyl, hydroxy, and alkene functional groups which have been reported to play a significant role as antibacterial agent.<sup>63</sup>



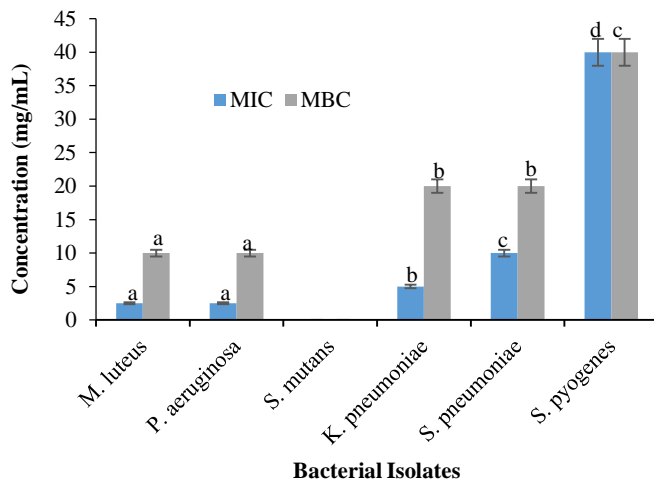
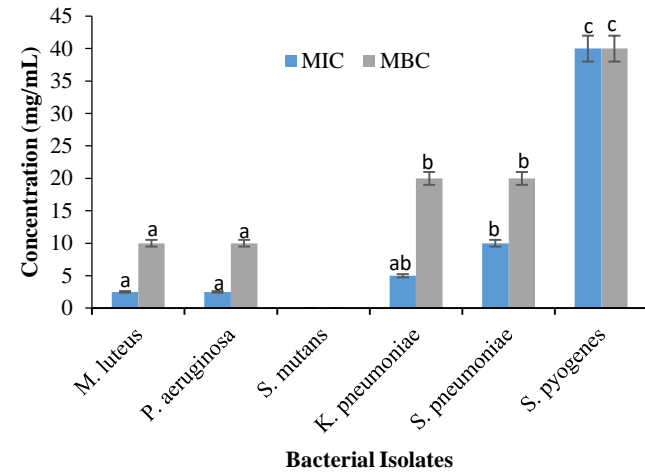
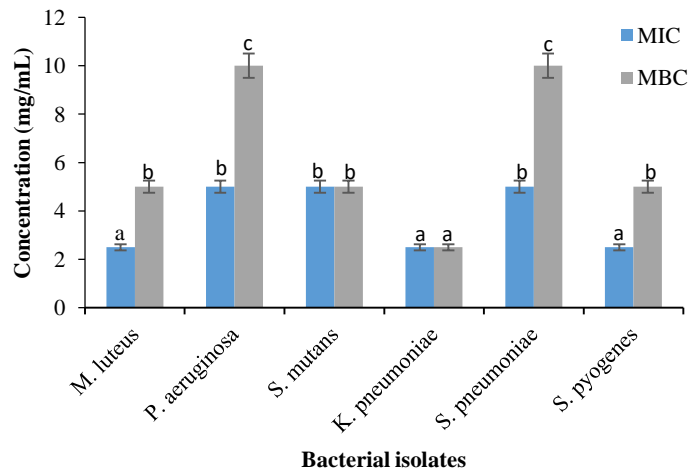
**Table 7:** Yield of partitioned fractions of methanol extract of *T. microptera*

Fractions	Yield, g (%)
Methanol (crude)	35.20 (7.04)
n-hexane	0.23 (0.92)
Chloroform	1.03 (4.12)
Ethyl acetate	12.56 (50.24)
Aqueous	10.25 (41.00)

**Table 8:** Mean zones of inhibition (mm) of sub-fractions of *Terminalia microptera* methanol extract

Isolates	TMC 20mg/mL	TMC 30mg/mL	TMC 40mg/mL	TME 20mg/mL	TME 30mg/mL	TME 40mg/mL	TMA 20mg/mL	TMA 30mg/mL	TMA 40mg/mL
<i>M. luteus</i>	12.00 ± 0.00 <sup>a</sup>	12.00 ± 0.00 <sup>a</sup>	18.50 ± 0.50 <sup>cd</sup>	18.00 ± 1.00 <sup>c</sup>	20.50 ± 0.50 <sup>e</sup>	23.00 ± 1.00 <sup>de</sup>	13.50 ± 0.50 <sup>ab</sup>	14.00 ± 0.00 <sup>b</sup>	14.50 ± 0.50 <sup>b</sup>
<i>P. aeruginosa</i>	12.00 ± 0.00 <sup>bc</sup>	13.50 ± 0.50 <sup>cd</sup>	14.00 ± 0.00 <sup>d</sup>	21.50 ± 0.50 <sup>b</sup>	21.50 ± 0.50 <sup>e</sup>	23.50 ± 0.50 <sup>f</sup>	8.00 ± 1.00 <sup>a</sup>	9.00 ± 0.00 <sup>a</sup>	13.50 ± 0.50 <sup>cd</sup>
<i>S. mutans</i>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	18.00 ± 1.00 <sup>d</sup>	20.50 ± 0.50 <sup>c</sup>	22.00 ± 2.00 <sup>e</sup>	10.50 ± 1.50 <sup>b</sup>	13.50 ± 0.50 <sup>bc</sup>	13.50 ± 1.50 <sup>bc</sup>
<i>K. pneumoniae</i>	7.50 ± 0.50 <sup>a</sup>	10.00 ± 0.00 <sup>b</sup>	13.50 ± 0.50 <sup>c</sup>	15.50 ± 0.50 <sup>d</sup>	18.50 ± 0.50 <sup>e</sup>	24.00 ± 1.00 <sup>f</sup>	8.50 ± 0.50 <sup>a</sup>	9.50 ± 0.50 <sup>a</sup>	14.00 ± 0.00 <sup>c</sup>
<i>S. pneumoniae</i>	11.00 ± 1.00 <sup>b</sup>	12.50 ± 0.50 <sup>bc</sup>	18.00 ± 0.00 <sup>de</sup>	15.50 ± 1.50 <sup>cd</sup>	18.50 ± 0.50 <sup>de</sup>	20.50 ± 0.50 <sup>e</sup>	7.50 ± 1.50 <sup>a</sup>	11.50 ± 1.50 <sup>b</sup>	15.00 ± 1.00 <sup>cd</sup>
<i>S. pyogenes</i>	0.00 ± 0.00 <sup>a</sup>	0.00 ± 0.00 <sup>a</sup>	13.50 ± 0.50 <sup>c</sup>	17.00 ± 1.00 <sup>d</sup>	18.50 ± 0.50 <sup>d</sup>	23.00 ± 1.00 <sup>e</sup>	0.00 ± 0.00 <sup>a</sup>	11.50 ± 0.50 <sup>b</sup>	14.00 ± 0.00 <sup>c</sup>

Values are expressed in mean ± standard error of mean, values with the same superscript on the same row have no significant difference ( $p > 0.05$ ),  $n = 3$   
Keys: TMC= Chloroform sub-fraction, TME = Ethyl acetate sub-fraction, TMA = Aqueous sub-fraction

**Figure 5:** MIC and MBC of chloroform sub-fraction of *T. microptera* methanol extract. Bars of same color with the same alphabet have no significant different at  $p < 0.05$ .**Figure 7:** MIC and MBC of aqueous sub-fraction of *T. microptera* methanol extract. Bars of same color with the same alphabet have no significant different at  $p < 0.05$ .**Figure 6:** MIC and MBC of ethyl acetate sub-fraction of *T. microptera* methanol extract. Bars of same color with the same alphabet have no significant different at  $p < 0.05$ .**Table 9:** Yield of column fractions g (%) of ethyl acetate sub-fractions of *T. microptera*

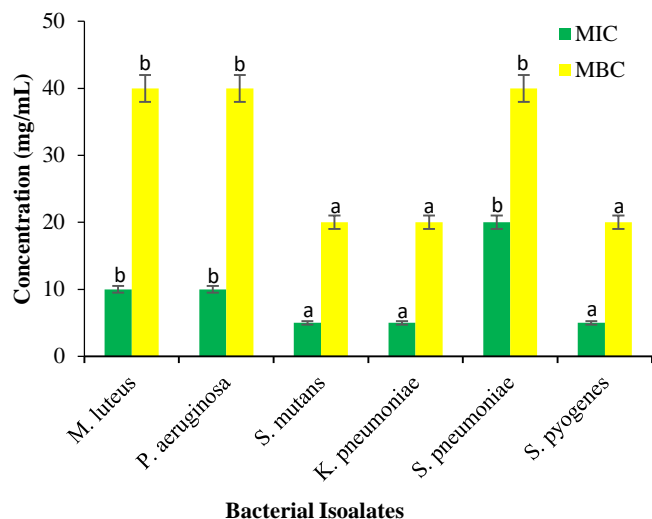
Fractions	Yield g (%)
TME <sub>1</sub>	0.33 (11)
TME <sub>2</sub>	0.45 (15)
TME <sub>3</sub>	0.80(26.67)
TME <sub>4</sub>	1.28(42.67)

Keys: TME<sub>1</sub>: Fraction 1, TME<sub>2</sub>: Fraction 2, TME<sub>3</sub>: Fraction 3, TME<sub>4</sub>: Fraction 4.

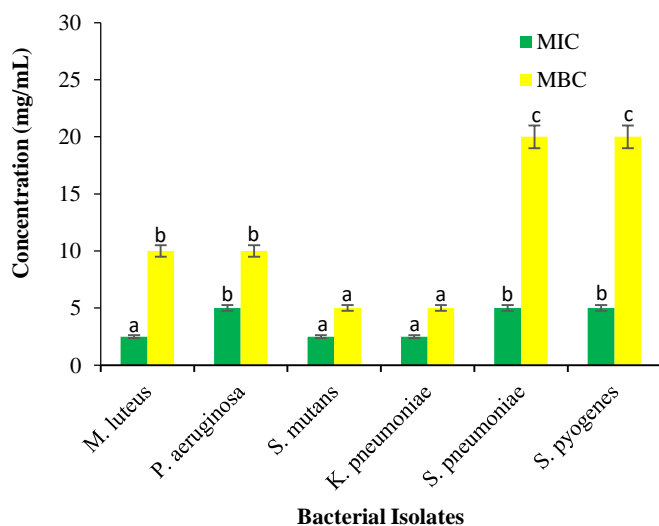
**Table 10:** Antibacterial sensitivity test of column fractions of ethyl acetate sub-fractions of *T. microptera* methanol extract

Isolates	TME <sub>2</sub>	TME <sub>2</sub>	TME <sub>2</sub>	TME <sub>3</sub>	TME <sub>3</sub>	TME <sub>3</sub>	TME <sub>4</sub>	TME <sub>4</sub>	TME <sub>4</sub>
	20mg/mL	30mg/mL	40mg/mL	20mg/mL	30mg/mL	40mg/mL	20mg/mL	30mg/mL	40mg/mL
<i>M. luteus</i>	6.50 ± 0.50 <sup>a</sup>	13.00 ± 1.00 <sup>b</sup>	14.50 ± 0.50 <sup>b</sup>	23.00 ± 0.00 <sup>d</sup>	23.50 ± 0.50 <sup>de</sup>	23.50 ± 0.50 <sup>de</sup>	17.00 ± 1.00 <sup>c</sup>	23.00 ± 1.00 <sup>d</sup>	25.50 ± 0.50 <sup>e</sup>
<i>P. aeruginosa</i>	8.50 ± 0.50 <sup>a</sup>	10.00 ± 1.00 <sup>ab</sup>	12.50 ± 0.50 <sup>b</sup>	19.00 ± 0.00 <sup>cd</sup>	22.50 ± 0.50 <sup>ef</sup>	24.00 ± 1.00 <sup>f</sup>	16.50 ± 0.50 <sup>e</sup>	20.50 ± 1.50 <sup>de</sup>	21.50 ± 1.50 <sup>def</sup>
<i>S. mutans</i>	8.00 ± 1.00 <sup>a</sup>	13.50 ± 0.50 <sup>b</sup>	14.50 ± 0.50 <sup>b</sup>	17.00 ± 1.00 <sup>c</sup>	24.00 ± 0.00 <sup>d</sup>	25.50 ± 0.50 <sup>d</sup>	18.50 ± 0.50 <sup>e</sup>	25.00 ± 1.00 <sup>d</sup>	26.00 ± 1.00 <sup>d</sup>
<i>K. pneumoniae</i>	11.00 ± 1.00 <sup>a</sup>	14.00 ± 0.00 <sup>ab</sup>	15.50 ± 0.50 <sup>b</sup>	17.50 ± 1.50 <sup>bc</sup>	22.00 ± 2.00 <sup>de</sup>	23.50 ± 1.50 <sup>de</sup>	20.00 ± 1.00 <sup>cd</sup>	23.50 ± 0.50 <sup>de</sup>	24.50 ± 0.50 <sup>e</sup>
<i>S. pneumoniae</i>	7.50 ± 0.50 <sup>a</sup>	12.50 ± 0.50 <sup>b</sup>	16.50 ± 0.50 <sup>c</sup>	20.50 ± 0.50 <sup>de</sup>	23.00 ± 1.00 <sup>e</sup>	20.50 ± 0.50 <sup>de</sup>	19.50 ± 0.50 <sup>d</sup>	20.50 ± 1.50 <sup>de</sup>	22.50 ± 0.50 <sup>e</sup>
<i>S. pyogenes</i>	9.50 ± 0.50 <sup>a</sup>	15.00 ± 1.00 <sup>b</sup>	17.00 ± 1.00 <sup>b</sup>	21.00 ± 1.00 <sup>c</sup>	23.00 ± 1.00 <sup>c</sup>	24.00 ± 1.00 <sup>c</sup>	17.50 ± 0.50 <sup>b</sup>	22.00 ± 0.00 <sup>c</sup>	22.00 ± 2.00 <sup>c</sup>

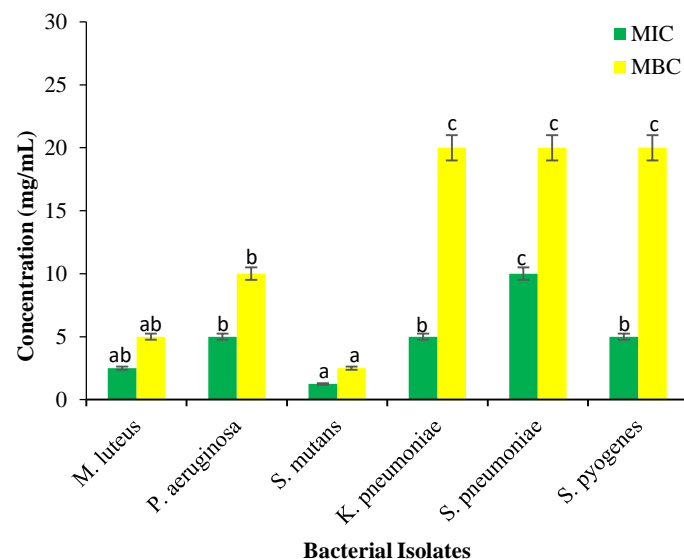
Values are expressed in mean ± standard error of mean, values with the same superscript on the same row have no significant difference (p<0.05), n=3  
Keys: TME<sub>2</sub>, TME<sub>3</sub>, and TME<sub>4</sub>: Fraction 2, 3 and 4 of ethyl acetate sub-fractions of *T. microptera* methanol extract

**Figure 8:** MIC and MBC of TME<sub>2</sub> fraction of ethyl acetate sub-fraction of *T. microptera* methanol stem extract.

Bars of same color with the same alphabet have no significant different at p < 0.05.

**Figure 9:** MIC and MBC of TME<sub>3</sub> fraction of ethyl acetate sub-fraction of *T. microptera* methanol stem extract.

Bars of same color with the same alphabet have no significant different at p < 0.05.

**Figure 10:** MIC and MBC of TME<sub>4</sub> fraction of ethyl acetate sub-fraction of *T. microptera* methanol stem extract.

Bars of same color with the same alphabet have no significant different at p < 0.05.

## Conclusion

The study revealed that extract of *Terminalia microptera* is safe at 1386 mg/kg b.w. dose. Prolong use of this extract without adequate prescription should also be discourage. The study also shows that the extract possesses good antibacterial potential that maybe utilized in the fight against multidrug resistance bacteria pathogens. The bioassay guided fractionation also revealed that the ethyl acetate sub-fraction shows an improve level activity in the zone of inhibition compare to the crude methanol extract. GC-MS analysis of the fractions predicted pharmacologically active compounds present in the ethyl acetate sub-fractions that may serve as lead compounds in drug formulation.

## Conflict of interest

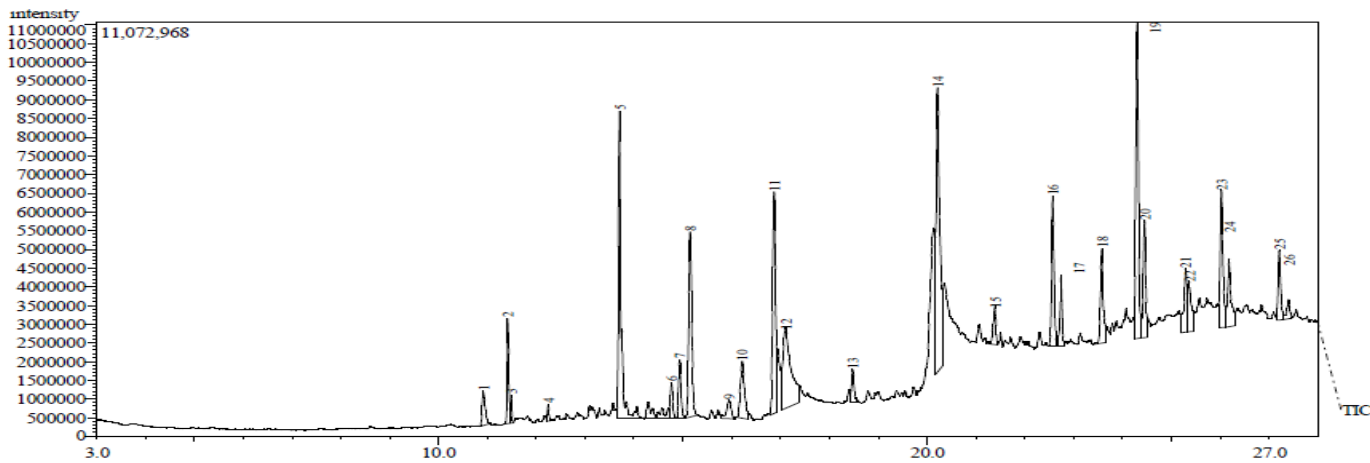
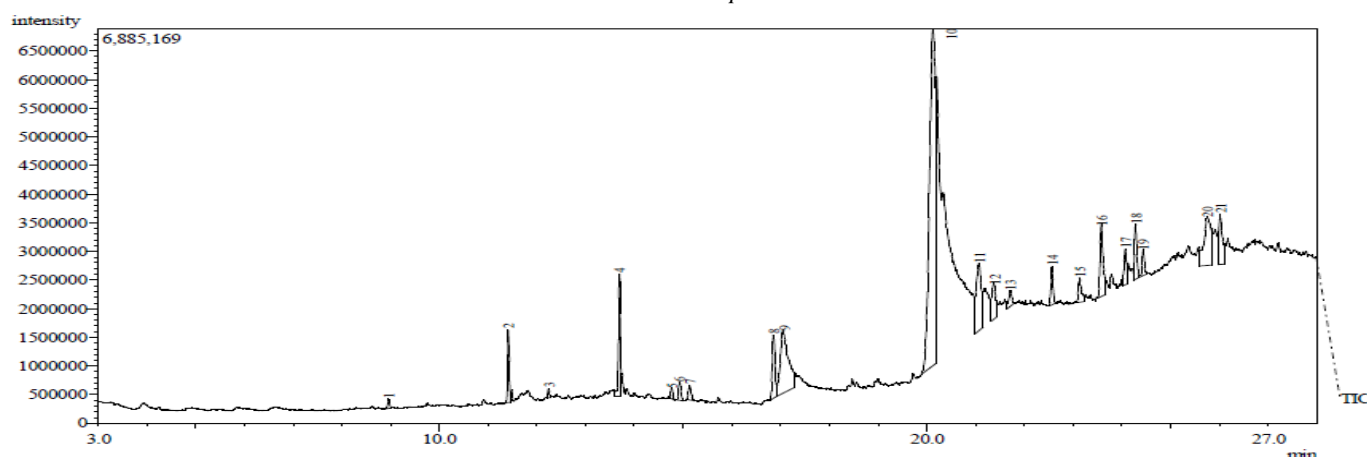
The authors declare no conflict of interest.

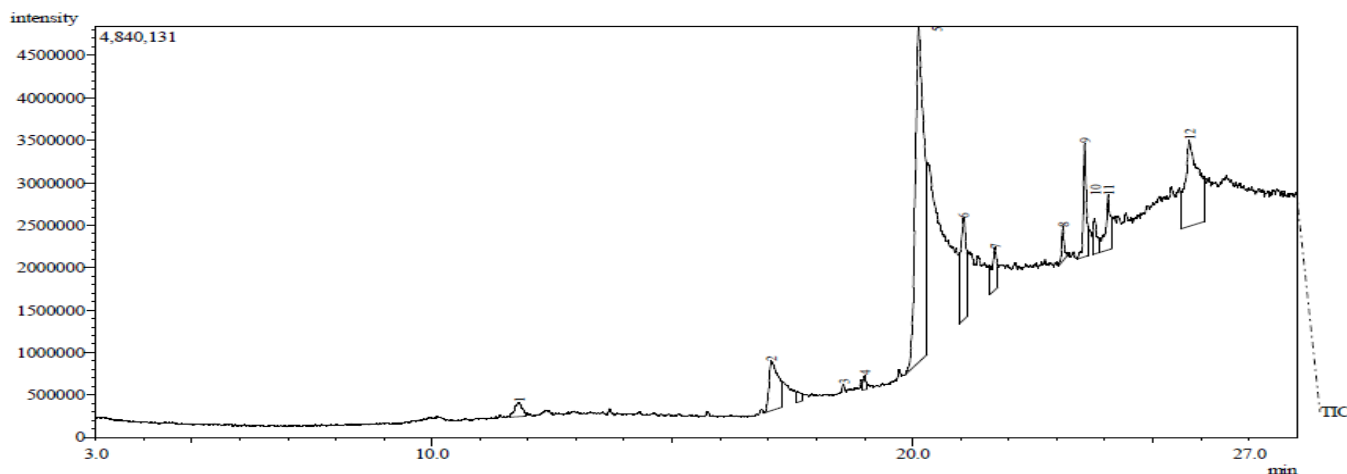
## Authors' Declaration

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.

**Table 11:** GC-MS profiles of aqueous sub-fractions from *Terminalia microptera* methanol extract

Peak No.	Retention time	Compound names	Molecular formula	Molecular weight	% Concentration
<b>Fraction TME<sub>2</sub></b>					
1	10.917	3,5-bis(1,1-dimethyl) phenol	C <sub>14</sub> H <sub>22</sub> O	206	1.34
8	15.150	Bis(2-methylpropyl)-1,2-benzenedicarboxylic acid	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	278	4.38
9	15.940	Butylcarboxymethylphthalate	C <sub>18</sub> H <sub>24</sub> O <sub>6</sub>	336	6.29
10	16.208	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	C <sub>17</sub> H <sub>24</sub> O <sub>3</sub>	276	5.96
11	17.102	5-Eicosene	C <sub>20</sub> H <sub>40</sub>	280	11.17
<b>Fraction TME<sub>3</sub></b>					
7	15.133	Bis(2-methylpropyl)-1,2-benzenedicarboxylic acid	C <sub>16</sub> H <sub>22</sub> O <sub>4</sub>	278	1.27
9	17.053	Hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256	8.41
10	16.208	Oleic acid	C <sub>18</sub> H <sub>34</sub> O <sub>2</sub>	282	8.41
11	21.070	1,19-Eicosadiene	C <sub>20</sub> H <sub>38</sub>	278	7.66
21	26.013	Cyclobutyl-4-nitrobenzoic acid	C <sub>11</sub> H <sub>11</sub> NO <sub>4</sub>	221	5.70
<b>Fraction TME<sub>4</sub></b>					
1.	11.807	Di-(1-hexen-5-yl)phthalic acid	C <sub>20</sub> H <sub>26</sub> O <sub>4</sub>	330	10.89
2	17.068	n-hexadecanoic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256	13.30
3	17.603	2-propenyldecanoic acid	C <sub>16</sub> H <sub>30</sub> O <sub>2</sub>	212	6.79

**Figure 11:** GC-MS Chromatogram of fraction TM<sub>2</sub> from column chromatography of ethyl acetate sub fraction of methanol stem extract of *T. microptera***Figure 12:** GC-MS Chromatogram of fraction TM<sub>3</sub> from column chromatography of ethyl acetate sub fraction of methanol stem extract of *T. microptera*



**Figure 13:** GC-MS Chromatogram of fraction  $TM_4$  from column chromatography of ethyl acetate sub fraction of methanol stem extract of *T. microptera*

### Acknowledgements

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