American Journal of Earth and Environmental Sciences
2018; 1(2): 55-62
http://www.aascit.org/journal/ees

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# Pharmacological and Chemical Constituents of Termite Nest

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## Citation

Ibikunle Gabriel Femi, Daniel Augustine Innalegwu, Olowodalu Oluwaseyi Mary. Pharmacological and Chemical Constituents of Termite Nest. *American Journal of Earth and Environmental Sciences.* Vol. 1, No. 2, 2018, pp. 55-62.

**Received**: February 5, 2018; **Accepted**: March 5, 2018; **Published**: April 10, 2018

**Abstract:** Phytochemical, antimicrobial and GC-MS analysis of termite nest was studied using standard methods. Phytochemical screening of the crude extract ($TN_0$) revealed the presence of alkaloids, flavonoids, phenols, terpenes, steroids and cardiac glycoside while saponins, tannins and anthraquinones are absent. Antibacterial activity of the crude extract and fractions against *Streptococcus mutans* ATCC 25175*, Streptococcus pneumoniae* ATCC 33400, *Streptococcus pyogenes* JCM 5674, *Pseudomonas aeruginosa* ATCC 10145*, Klebsiella pneumoniae* BYK-9 and *Micrococcus luteus* ATC 10240 at 40 mg/mL revealed a narrow spectrum of activity in $TN_0$ and Hexane fraction ($TN_1$) in two bacterial isolate each with zone of inhibition ranging from 5.33±1.25 to 8.02±0.13 and 8.50±1.25 mm, chloroform fraction ($TN_2$) was susceptible against all the bacterial isolates except *P. aeruginosa* with inhibition zone ranging from 7.33±2.50 to 18.33±1.25 mm while ethyl acetate fraction ($TN_3$) and aqueous fraction ($TN_4$) was susceptible against all the test bacterial isolates with zone of inhibition ranging from 7.33±0.67 to 14.00±1.50 mm in $TN_3$ and 15.00±0.58 to 19.67±0.30mm in $TN_4$ where significantly different ($p<0.05$) with the standard antibiotics (Amoxicillin and Ampiclox) used. MIC and MBC of $TN_2$, $TN_3$ and $TN_4$ also ranged from 5 to 20 mg/mL and MBC 5 to 40 mg/mL in all the test bacterial isolates respectively. GC-MS analysis of $TN_3$ and $TN_4$ gave the presence of 21 compounds in $TN_3$ and 16 in $TN_4$ with different % compositions. The study revealed that termite nest contains phytochemicals that can be a good source therapeutic agent.

**Keywords:** Termite Nest, Phytochemicals, Antibacterial, Amoxicillin, Ampiclox, GC-MS

## 1. Introduction

Recently, insects have been discovered to be a good raw material for antibiotics and anticancer drugs [1]. Since the evolution of the insects over 500 million years ago, they have flourished in all sorts of habitat, and as a result they may contain some important chemicals that aid them in the fight against pathogens and predators that attack them [1]. Insects have been observed to be exposed to a large number of nasty bacteria and fungi, therefore they have developed a strong immune system, which enable them to fight against these pathogens. In spite of all the potentials in these creature, there is little or no attention given to the medicinal uses of insect especially in Africa and some part of Europe [2]. In view of recent discoveries, insects will soon graduate from been a pest to survivors who have prevailed under good and bad conditions [1].

Termite nest or mounds are known by many different names such as "Ngete" in Ngankikurungkurr, "Bilaya" in Jingulu, "Bellar" in Mudburra 1, “Ole-Eha” in Idoma and “Gidan gara” in Hausa. They are popularly known as "ant"-bed, "ant"-hill and termite hill [2]. They are used medicinally throughout the Northern Territory from Bathurst Island to the Central Northern region in Australia, Africa, Asia and some part of Europe [2]. Different Aboriginal communities use termite nest for different purposes and in many different ways. It is used either internally or externally. Their usage, as with other traditional medicine, was more widespread prior to European contact. For example, termite nest is crushed, wet with water and rob around any swollen part of the body to ease pain and ultimately reduce the swelling.

Increase in human population and the rapid rise in multi drug resistance by microorganisms have necessitate the need for a continuous search into nature’s wealth for an alternative medicine to tackle these challenges. Hence, this research is aimed at evaluating the pharmacological and chemical

component of termite nest.

## 2. Materials and Methods

### 2.1. Materials

#### 2.1.2. Collection of Termite Nest Material

The termite nest was collected from a farm in Kakao village in Chikun Local Government Area, of Kaduna State, Nigeria on 16th August 2016. It was dried and at a room temperature and milled with mortar and pestle and sieved to obtain a fine powdered material.

#### 2.1.3. Bacterial Strains

The bacteria used for the biological test were obtained from Federal Institute for Industrial Research Oshodi (FIIRO), Lagos, Nigeria. They include Gram positive *Streptococcus mutans* ATCC 25175*, Streptococcus pneumoniae* ATCC 33400, *Streptococcus pyogenes* JCM 5674 and Gram negative *Pseudomonas aeruginosa* ATCC 10145*, Klebsiella pneumoniae* BYK-9 and *Micrococcus luteus* ATC 10240.

### 2.2. Methods

#### 2.2.1. Processing and Extraction of Termite Nest

Eight hundred and five grams (805 g) of the milled sample was macerated with 50% methanol for 24 hours. The extract was filtered using a glass funnel and a cotton wool and the filtrate was concentrated using RE-6000 rotary evaporator rotary evaporator at 50°C and the concentrated extract was further dried in a water bath at 40°C into a fine brown paste.

#### 2.2.2. Qualitative Phytochemical Screening of Extracts

Preliminary qualitative phytochemical screening which involved performing simple chemical tests to detect the presence of secondary metabolites such as tannins, flavonoids, phenols, phenolic compounds, saponins, and glycosides, was carried out according to Trease and Evans and Sofowora [3], [4].

### 2.3. Determination of Antibacterial Activity of the Extract

#### 2.3.1. Assay for Antibacterial Activity

Agar well diffusion method was used to evaluate the antibacterial activity of the Crude extracts and fractions [4]. Eighteen-hour culture of bacteria adjusted to 0.5 McFarland standard was used as inoculum on sterile Mueller Hinton agar. The plate was kept on flat bench for 30 minutes to solidify. Five wells (4mm) deep were made on the agar using a sterile 6mm diameter cork borer. Then 0.5 mL of the reconstituted extract at a concentration of 40 mg/mL was pipetted into the wells using micro pipette. Zero point five milliliter (0.5mL) each of 5mg/mL of Ampiclox and Amoxicillin solution were used as positive controls and 0.5mL of Di-Methyl Sulphoxide (DMSO) as a negative control. The plates were allowed to stand on a flat bench for 30 min to allow diffusion of the extract into the agar before incubation at 37°C for 24 h. Each test was carried out in triplicates and mean zone diameter of inhibition was recorded.

#### 2.3.2. Determination of the Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of the extract was determined using the double fold dilution. An aliquot of 1mL of the reconstituted extract with 50% DMSO at a concentration of 40 mg/mL was diluted serially to give concentrations of 20, 10, 5, 2.5, 1.25, 0.625 and 0.3125 mg/mL in eight test tubes. One milliliter (1mL) of 18h culture of bacteria previously adjusted to 0.5 McFarland standard ($1.0X10^6$cfu/mL) was added to each of the test tubes and the content was mixed thoroughly. The tubes were incubated at $37^oC$ for 4 h. The 9th test tube contained 1mL of 50% DMSO with no extract served as negative control. The 10th and 11th test tubes containing a solution of 5 mg/mL of Ampiclox and Amoxicillin served as positive control. The procedure was repeated for the test and the control. The test tube with the lowest concentration of the extract without visible turbidity of growth was taken as the MIC [5].

#### 2.3.3. Determination of the Minimum Bactericidal Concentration (MBC)

From each of the test tubes without any visible growth, a loopfull of the broth was aseptically inoculated on a sterile Mueller Hinton agar. The inoculated plates were incubated for 24hr at a temperature of 37°C. After incubation, the MBC was determined as the lowest concentration with no visible growth on the plate [6].

### 2.4. Statistical Analysis

Data obtained in this study were analysed using the IBM Statistical Package for Social Science (SPSS) 20.0, 2011 version (SPSS Inc., Chicago, Illinois, USA). Numerical data were presented as mean ± standard error of mean (SEM) of the triplicate.

### 2.5. Gas Chromatography (GC)–Mass Spectrometer (MS) Analysis

GC-MS analysis of ethyl acetate and aqueous fractions ($TN_3$ and $TN_4$ respectively) was carried out on a GC-MS-QP 2010 Plus Shimadzu system (SHIMADZU, JAPAN). Gas chromatograph interfaced to a mass spectrometer (GC-MS) instrument was used; Column elite-1 fused silica capillary column (30m×0.25mm 1D × µ1 df, composed of 100% dimethyl polysiloxane). For GC-MS detection, an electronic ionization system with ionization energy of 70eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1ml/min and an injection volume of 2µl 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 mins isothermal at 280°C. Mass spectra were taken at 70eV; a scan interval of 0.5s and fragments from 40 to 550Da. 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 adopted to handle mass spectra and chromatogram was a turbomass and detection of compounds employed the database of National Institute of Science and Technology (NIST) NIST Ver. 2.0 year 2009 library.

### 2.6. Identification and Quantification of Constituents

The identification of the constituents of the fraction was conducted based on the GC retention times on HP-5MS capillary column and by matching their corresponding names, molecular weight and the acquired mass spectra (and the fragmentation patterns) with those of similar compounds stored on commercial libraries, in the NIST 05L Mass Spectral Library. The relative quantification (percent composition) of the fractions constituent were determined by computerized peak area measurements using the internal normalization methods. Automated Mass Spectral Deconvolution and Identification System (AMDIS) software was used to collect and compare the chromatographic profiles (fingerprint) of each fraction with those stored in the libraries. Match Factors above 80% (very good to perfect agreement/match) of the spectra were considered for identification of individual components of the fractions [7]. The structural elucidation of the compounds was carried out using ChemDraw Professional version 15.0

## 3. Results

***Table 1.*** *Percentage yield of crude extract and fractions of termite nest.*

| Extract | Yield (%) |
|---|---|
| $TN_0$ | 11.090 |
| $TN_1$ | 0.026 |
| $TN_2$ | 3.588 |
| $TN_3$ | 1.081 |
| $TN_4$ | 95.314 |

Keys: $TN_0$ = Crude extract, $TN_1$ = n-Hexane fraction, $TN_2$ = Chloroform fraction, $TN_3$ = Ethyl acetate fraction, $TN_4$ = Aqueous fraction

***Table 2.*** *Qualitative phytochemical screening of crude extract of termite nest.*

| Phytochemicals | Test | Observation | Indication |
|---|---|---|---|
| Saponins | Frothing | No froth | - |
| Alkaloids | Dragendorff's | Orange red | + |
| Tannins | Ferric chloride | Absent | - |
| Flavonoids | Ferric chloride | Yellow solution disappearing on addition of dilute HCl | + |
| Phenols | Ferric chloride | Yellow solution disappearing on addition of dilute HCl | + |
| Anthraquinone | | Absent | - |
| Terpenes | Shinodal's | Reddish color | + |
| Steroids | Salkowskii's | Reddish brown ring | + |
| Cardiac glycosides | Keller-killani | Violet ring | + |

Keys: + = Present, - = Absent

***Table 3.*** *Mean zone of inhibition of crude and fractions of termite nest.*

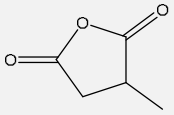
| Bacterial isolates | $TN_0$ | $TN_1$ | $TN_2$ | $TN_3$ | $TN_4$ | Amoxicillin* 5mg/mL | Ampiclox* 5mg/mL |
|---|---|---|---|---|---|---|---|
| *P. aeruginosa* | - | - | - | $8.67\pm0.67^{a}$ | $15.67\pm0.33^{b}$ | $26.00\pm0.58^{cd}$ | $27.67\pm0.33^{d}$ |
| *S. pneumoniae* | - | $8.02\pm0.13^{a}$ | $16.25\pm1.5^{cd}$ | $11.33\pm0.67^{b}$ | $18.33\pm1.20^{d}$ | $13.67\pm0.33^{c}$ | $20.67\pm0.33^{e}$ |
| *S. pyogenes* | - | $8.50\pm1.25^{a}$ | $18.33\pm1.25^{c}$ | $13.33\pm0.67^{b}$ | $19.67\pm0.33^{cd}$ | $19.00\pm1.00^{cd}$ | $24.33\pm0.33^{d}$ |
| *K. pneumonaie* | - | - | $13.01\pm0.50^{b}$ | $7.33\pm0.67^{a}$ | $14.33\pm0.67^{c}$ | $26.33\pm0.33^{e}$ | $22.33\pm0.33^{d}$ |
| *S. mutans* | $5.33\pm1.25^{a}$ | - | $7.33\pm2.50^{b}$ | $11.33\pm0.67^{c}$ | $18.33\pm1.20^{d}$ | $15.67\pm0.33^{cd}$ | $24.33\pm0.67^{e}$ |
| *M. luteus* | $6.20\pm1.33^{a}$ | - | $10.00\pm0.25^{b}$ | $14.00\pm1.15^{c}$ | $15.00\pm0.58^{c}$ | $25.67\pm0.33^{d}$ | $28.00\pm1.00^{e}$ |

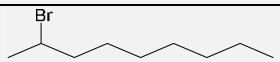
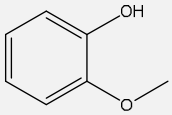
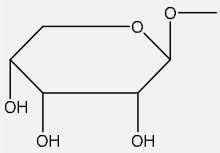
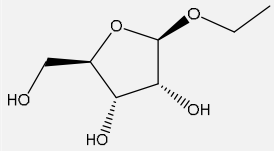
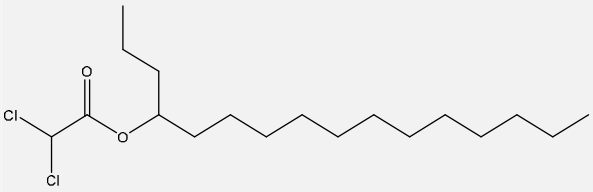
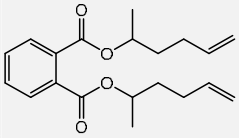
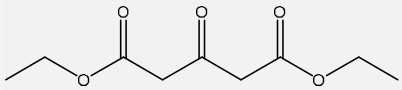
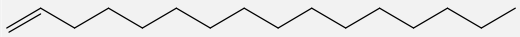
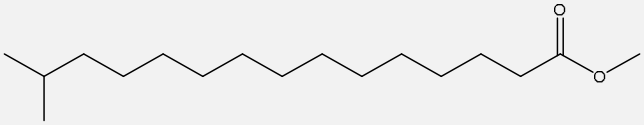
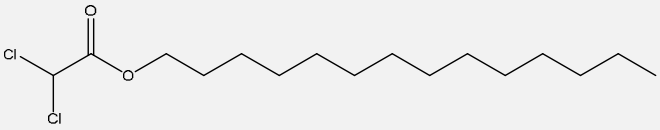
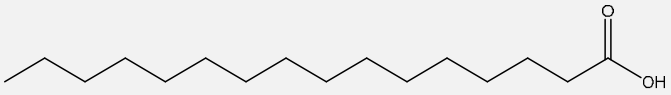
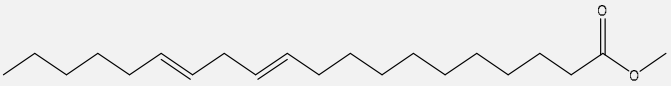
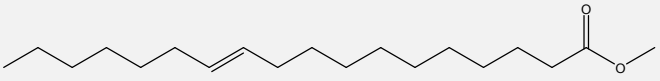
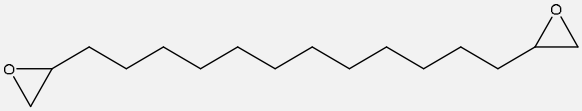
Values are expressed in mean ± standard error of mean of duplicate determination. Values with the same superscript on the same row have no significance difference ($p<0.05$).

* Specification for Amoxicillin and Ampiclox are: ≤19 (resistance) and ≥20 (susceptible) (CLSI, 2012).

Keys: $TN_0$ = Crude extract, $TN_1$ = n-Hexane fraction, $TN_2$ = Chloroform fraction, $TN_3$ = Ethyl acetate fraction, $TN_4$ = Aqueous fraction

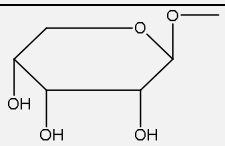
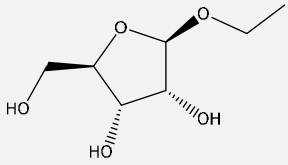
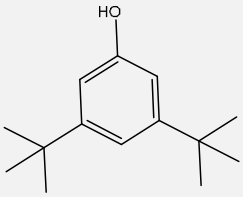
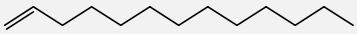
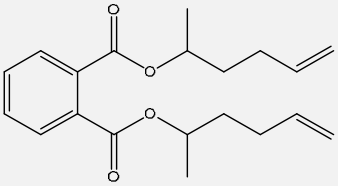
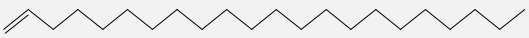
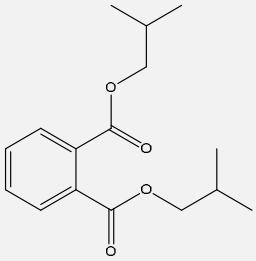
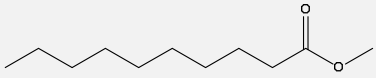
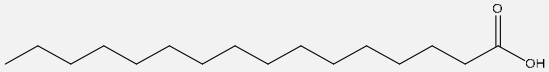
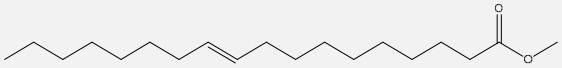
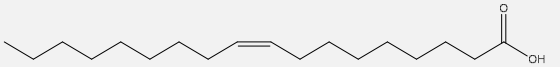
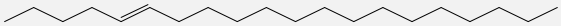
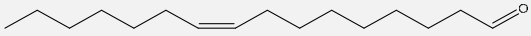
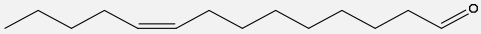
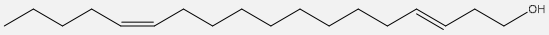
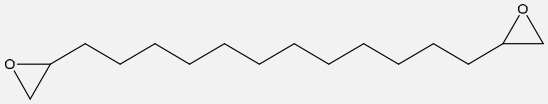
***Table 4.*** *Chemical composition of ethyl acetate fraction $TN_3$ of termite nest.*

| Peak No | $M_w$ | $R_t$ | % composition | Name | Structure |
|---|---|---|---|---|---|
| 1 | 144 | 4.848 | 0.332 | 2,5-Furandione, dihydro-3-methyl- |  |

| Peak No | $M_w$ | $R_t$ | % composition | Name | Structure |
|---|---|---|---|---|---|
| 2 | 206 | 5.141 | 0.220 | 2-Bromononane |  |
| 3 | 124 | 5.532 | 0.152 | Phenol, 2-methoxy- |  |
| 4 | 964 | 9.104 | 13.43 | beta. -D-Ribopyranoside, methyl |  |
| 5 | 178 | 9.819 | 1.76 | Ethyl. beta. -D-riboside |  |
| 6 | 352 | 11.416 | 0.23 | Dichloroacetic acid, 4-hexadecyl ester |  |
| 7 | 330 | 11.818 | 0.31 | Phthalic acid, di-(1-hexen-5-yl) ester |  |
| 8 | 202 | 12.461 | 0.76 | Pentanedioic acid, 3-oxo-, diethyl ester |  |
| 9 | 224 | 13.692 | 0.31 | 1-Hexadecene |  |
| 10 | 270 | 15.727 | 0.68 | Pentadecanoic acid, 14-methyl-, methyl ester |  |
| 11 | 324 | 16.848 | 0.34 | Dichloroacetic acid, tetradecyl ester |  |
| 12 | 256 | 17.070 | 2.47 | n-Hexadecanoic acid |  |
| 13 | 322 | 18.937 | 1.27 | 11,14-Eicosadienoic acid, methyl ester |  |
| 14 | 296 | 18.997 | 1.88 | 11-Octadecenoic acid, methyl ester |  |
| 21 | 254 | 23.592 | 9.97 | 1,2-15,16-Diepoxyhexadecane |  |

Keys: $M_w$ = Molecular weight, $R_t$ = Retention time

***Table 5.*** *Chemical composition of aqueous fraction $TN_4$ of termite nest.*

| Peak No | $M_w$ | $R_t$ | % composition | Name | Structure |
|---|---|---|---|---|---|
| 1 | 164 | 9.312 | 1.33 | Beta. -D-Ribopyranoside, methyl |  |
| 2 | 178 | 9.936 | 0.48 | Ethyl. beta. -d-riboside |  |
| 3 | 206 | 10.911 | 0.95 | 3,5-bis(1,1-dimethylethyl) Phenol |  |
| 4 | 182 | 11.416 | 0.14 | 1-Tridecene |  |
| 5 | 330 | 11.825 | 0.34 | Phthalic acid, di-(1-hexen-5-yl) ester |  |
| 6 | 308 | 13.692 | 0.30 | 1-Docosene |  |
| 7 | 278 | 15.129 | 0.68 | 1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester |  |
| 8 | 186 | 15.731 | 0.35 | Decanoic acid, methyl ester |  |
| 9 | 256 | 17.049 | 12.22 | n-Hexadecanoic acid |  |
| 10 | 296 | 18.994 | 1.14 | 10-Octadecenoic acid, methyl ester |  |
| 11 | 282 | 20.098 | 57.09 | Oleic Acid |  |
| 12 | 280 | 21.067 | 10.81 | (5E)-5-Icosene |  |
| 13 | 238 | 23.134 | 1.21 | cis-9-Hexadecenal |  |
| 14 | 210 | 23.581 | 4.26 | (Z)-9-Tetradecenal |  |
| 15 | 266 | 24.073 | 1.86 | Z,E-3,13-Octadecadien-1-ol |  |
| 16 | 254 | 25.749 | 6.84 | 1,2-15,16-Diepoxyhexadecane |  |

Keys: $M_w$ = Molecular weight, $R_t$ = Retention time

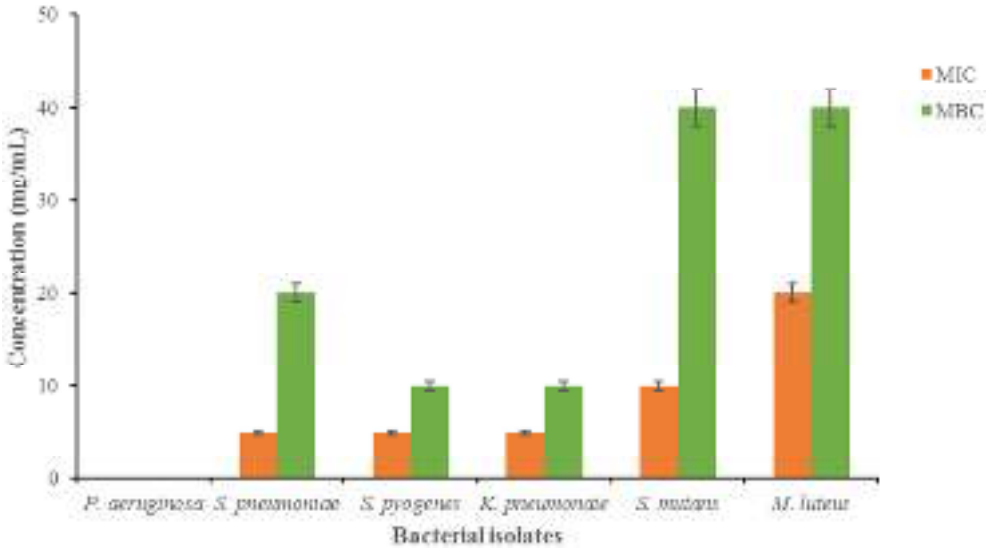



***Figure 1.*** *MIC and MBC of chloroform fraction ($TN_2$) of termite nest.*

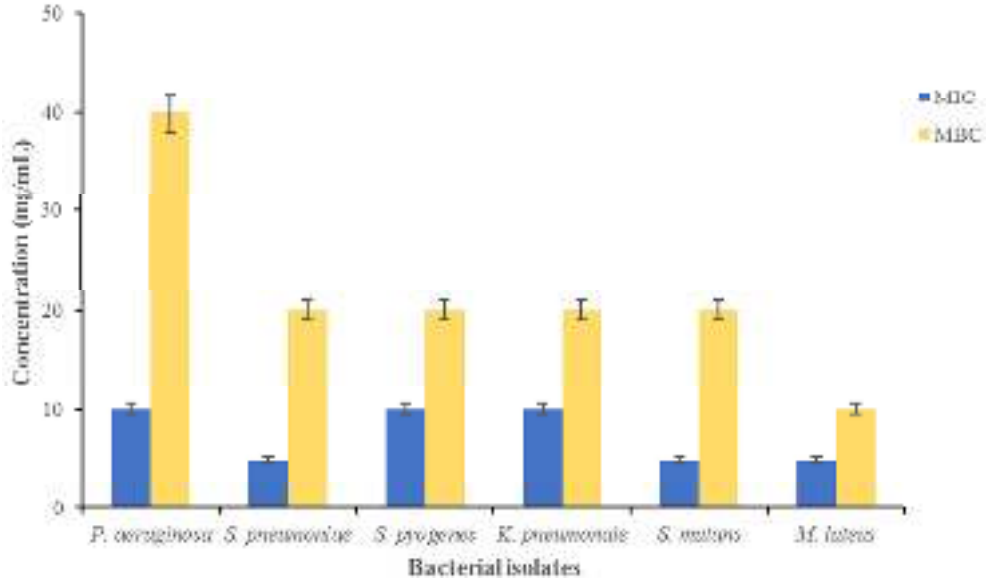



***Figure 2.*** *MIC and MBC of ethyl acetate fraction ($TN_3$) of termite nest.*

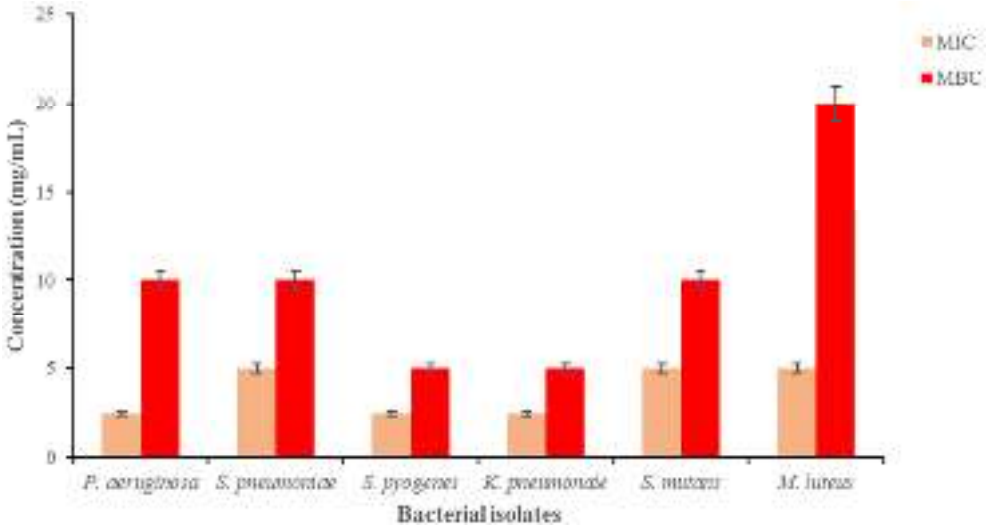



***Figure 3.*** *MIC and MBC of aqueous fraction ($TN_4$) of termite nest.*

## 4. Discussion

Termite activities and their interaction with soil environment have defined and modified ecosystems for ages. Termite nest is a heterogenous mixture of different materials such as vegetables, dead wood and decayed animal materials. Extraction with 50% methanol yielded 11.090% which was partitioned into n-hexane, chloroform, ethyl acetate and distilled water with a resultant yield of 0.026, 3.588, 1.081 and 95.314% respectively (Table 1). Solvent extraction is most frequently used in the isolation of biologically active compounds from plants. However, the extract yields and activities of the plant extract are strongly dependent on the nature of extracting solvent, due to the presence of different compounds of varied chemical characteristics and polarities that may or may not be soluble in a particular solvent [8].

Phytochemical screening of the crude extract revealed the presence of alkaloids, flavonoids, phenols, terpenes, steroids and cardiac glycoside while saponins, tannins and anthraquinones are absent (Table 2). Different mechanisms of action of phytochemicals have been proposed. They may inhibit microorganisms, interfere with some metabolic processes or may modulate gene expression and signal transduction pathways [9]; [10]; and [11]. Phytochemicals may either be used as chemotherapeutic or chemo preventive agents. In general, the mechanism of action is considered to be the disturbance of the cytoplasmic membrane, disrupting the proton motive force, electron flow, active transport, and coagulation of cell contents [12].

The antibacterial activity of the crude extract and fractions against Gram positive *Streptococcus mutans* ATCC 25175*, Streptococcus pneumoniae* ATCC 33400, *Streptococcus pyogenes* JCM 5674 and Gram negative *Pseudomonas aeruginosa* ATCC 10145*, Klebsiella pneumoniae* BYK-9 and *Micrococcus luteus* ATC 10240 revealed a narrow spectrum of activity in $TN_0$ and $TN_1$. $TN_0$ was only susceptible to two organisms; *S. mutans* and *M. luteus* with 5.33±1.25 and 6.20±1.33 mm zone of inhibition respectively while $TN_1$ was susceptible to *S. pneumoniae* and *S. pyogenes* with an inhibition zone of 8.02±0.13 and 8.50±1.25 mm respectively. $TN_2$ was susceptible against all the bacterial isolates except *P. aeruginosa* with zone of inhibition ranging between 7.33±2.50 to 18.33±1.25 mm while $TN_3$ and $TN_4$ was susceptible against all the test bacterial isolates with zone of inhibition ranging between 7.33±0.67 to 14.00±1.50 mm in $TN_3$ and 15.00±0.58 to 19.67±0.30mm in $TN_4$ which is significantly different ($p<0.05$) with the standard antibiotics (Amoxicillin and Ampiclox) at 40 mg/mL (Table 3). Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of $TN_2$, $TN_3$ and $TN_4$ also confirmed the potency of the extract with the MIC ranging between 5 to 20 mg/mL and MBC 5 to 40 mg/mL in all the test bacterial isolates (Figure 1-3).

The GC-MS analysis of $TN_3$ and $TN_4$ revealed the presence of 21 compounds in $TN_3$ and 16 in $TN_4$ (Table 4 and 5). The antibacterial activity of the fractions may be due to the accumulation of aliphatic (long chain alkanes and alkenes), and methylester fatty acids in the lipid layer (lipopolysaccharide) of the cell membrane and mitochondria and consequently disturb the intergrity of the cell structure which becomes permeable [13]. The activity may also be attributed to the presence of long chain unsaturated fatty acids such as 11-Octadecenoic acid, methyl ester, (Z)-9-Tetradecenal, Decanoic acid, methyl ester and (5E)-5-Icosene. [7] and [14] reported that long chain unsaturated fatty acids are bactericidal against important pathogenic microorganisms, including methicillin resistant *Staphylococcus aureus, Mycobacterium* and *Helicobater pylori.* The findings in this study are thus comparable to the fingings of [7] and [14]. The activity of most of the compounds identified from the two fractions could be attributed to their functional groups such as carboxyl, methyl, hydroxy and alkene functional groups [15]. The functional groups, electronic, solubility and stearic effects play a key role in the activity of these compounds against bacteria cells and thus inhibiting their growth [14]. $TN_4$ show a better zone of inhibition when compared to $TN_1$, $TN_2$, and $TN_3$ and this may be due to the presence of the phenolic compound [3,5-bis(1,1-dimethylethyl) Phenol)] and more of the terpenoidal compounds.

Studies on other plant species have established their antimicrobial activity as being related to the presence of Decanoic acid, methyl ester [13], [16] and 1-Heptacosanol, 1-Dodecanol, n-Hexadecanoic acid [7]. Oliec acid and linolenic acid are used as food additives to prevent growth of microorganisms in food industries. Thus, these compounds seem most likely to be responsible for the observed antibacterial activity and can serve as an analogue for the synthesis of new drugs.

## 5. Conclusion

The study revealed that termite nest is a good source of medicine not only for the developing world but also for the developed world as the GC-MS analysis of the fractions revealed an important compounds that have numerous biological uses tnot only against the studied microorganisms but may be use against other pathogenic microorganisms.

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