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Bioactivity of Chromatographic Fractions from *Eucalyptus citriodora* Leaf Against Some Bacterial Pathogens

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Abstract

Considering the application of *Eucalyptus citriodora* in Folklore medicine in the treatment of Typhoid fever and respiratory tract infections, fractions were obtained from n-hexane extract by reflux extraction and chromatography techniques to determine its antibacterial activity and phytochemical properties. A total of 25.85g (5.17%) was obtained from 500gms of dry plant material while the phytochemical analysis result revealed the presence of 10 different bioactive constituents viz; alkaloids, flavonoids, saponins, cardiac glycosides, tannins, steroids, terpenes, resins, phenols and volatile oils with the absence of anthraquinones. The crude extract exhibited antibacterial activity against all the test organisms at 20mg/mL with mean inhibition zone (MZI) ranging from 15.00 ± 0.57^{a} mm to 20.66 ± 0.33^{a} mm, which were effective than the fractions. A total of 8 fractions namely ECO1, ECO2, ECO3, ECO4, ECO5, ECO6, ECO7 and ECO8 were obtained from the crude extract by column chromatography, 5 (ECO2, ECO4, ECO6, ECO7 and ECO8) were active at 40mg/mL against all the test organisms with MZI range of 8.00±0.00mm to 21.66±0.88mm while ECO2 and ECO4 exhibited intermediate activity with MZI range of 15.00±0.00mm 18.66±0.33mm and 9.33±0.33mm to 21.66±0.88mm respectively. The minimum inhibitory concentration (MIC) and corresponding minimum bactericidal concentration (MBC) of the crude extract and fractions were 7.5mg/mL & 120mg/mL and 3.25mg/mL & 120mg/mL respectively. Thirty nine (39) and fifteen (15) compounds were identified in ECO2 and ECO4 respectively by column chromatography with gamma.-Tocopherol (10.25%), Hemimellitene (8.64%), n-Decane (6.82%), 11-Octadecenoic acid, methyl ester (6.56%) & Decane, 2-methyl- (5.13%) and delta. (Sup9)-cis-Oleic acid (21.02%), Stigmasterol, 22,23-dihydro- (14.35%), 6.beta.Bicyclo[4.3.0]nonane, 5.beta.-iodomethyl-1.beta.-isopropenyl-4.alpha.,5.alpha.-dimethyl- (14.13%), n-Hexadecanoic acid (8.62%), Hydrofol Acid 150 (7.64%) and Ricinoleic acid methyl ester (7.61%) as the most abundant respectively. All the identified compounds have been reported to possessing antibacterial activity, therefore, based on the results obtained in this research study and considering the toxicity level of the plant extract with a safe dose (LD_{50}) of

1369mg/kgbw, fractions from *Eucalyptus citriodora* can be used to develop drug for the treatment of infections caused by the test organisms.

1. Introduction

Medicinal plants contain biologically active components which have been employed in traditional medical practice for the treatment of human infections (Adebanjo *et al.,* 1985). Plant-based medicines have been used for decades especially in rural areas to prevent or even eliminate diseases worldwide and have proven to be promising in their actions (Bonjar and Farrokhi, 2004). Herbal medicines otherwise called herbal drugs are generally of natural plant parts such as stem, leaves, roots, flowers, stem bark, seeds, bulb (Robinson, 2006). In addition to providing the animal kingdom it's food, fuel and shelter, plants accumulate other phytochemical constituents - the secondary metabolites which are produced as by-products and are sometimes not directly useful to them. These secondary metabolites give plants their medicinal value. Some of these include alkaloids, tannins, saponins, flavonoids, antraquinones, glycosides, terpenes, essential oils, resins (Robinson, 2006). Medicinal plants have therefore been described as one in which one or more of its organs contain substances that can be used for therapeutic purposes (Rios and Recio, 2005). It may be in the form of vegetable drugs which may either be organized (material which possess a cellular structure e.g. Leaf, bark petal, flower, stem, root, etc) or unorganized drugs (a cellular structural medicinal agent such as gums, balsams and Latex). Such plant materials may be utilized in the form of decoctions in cold water or warm water, concoctions, preparations of soups, drinks etc made fully from many ingredients.

Eucalyptus (also called *Corymbia*) is a diverse genus of trees in the family Myrtaceae. Out of the more than 700 species that comprise this genus, most are endemic to Australia. A smaller number are also native to New Guinea, Indonesia and the Phillipines. *Eucalyptus* can be found in almost every region of the Australian continent. They have also been widely introduced into the subtropical and tropical regions in areas as diverse as Africa, the Middle East, India, USA and South America. In many of these areas these trees are considered invasive, whilst in other areas they are prized for their commercial applications. *Eucalyptus* are valued for their wood and some are also valuable sources of proteins, tannins, gum, and dyes although their most valuable product is the eucalyptus oil that is readily distilled from their leaves (Trivedi and Hotchandani, 2004). Plant oils from some *Eucalyptus* species (e.g *Eucalyptus pulverulenta*) comprise up to 90% cineol (Brophy *et al*., 1985). Plant oils from other plants containing cineol have previously demonstrated antimicrobial properties (Gundidza *et al*., 1993). *Eucalyptus* oil is used extensively in cleaning and deodourising products as well as in cough drops and decongestants (Sartorelli *et al*., 2007). *Eucalyptus* oil has insect pest repellent properties and is a component in many commercial pesticides Fradin and Day, (2002). Their bioactivity is yet to be fully harnessed and therefore, the aim of this research study is to determine the antibacterial activity of column chromatography fractions from n-hexane extract of the leaf of *Eucalyptus citriodora*.

2. Materials and Methods

2.1. Sample Collection

The plant samples, Lemon scented gum (*Eucalyptus citriodora*) Figure 1, were collected from house-hold gardens in Bosso Local Government, Minna, Niger State Nigeria in the month of August. The plant materials were identified by local herbal practitioners in Minna, Niger State while authentication of the plant sample was done by Dr. Ugbabe Grace E and Mr. John Atogwe in the Herbarium Department of the National Institute of Pharmaceutical Research, and Development, Idu, Abuja where voucher specimens were deposited with voucher numbers: NIPRD/H/6787. The plant materials (leaves) were dried under shade until a constant weight was obtained. The plant samples were pulverized into powdered form with a milling machine (Lab world NAVBHART, with serial No. R66902 by MOTOR MFG. CO. Mumbai-India), and sieved with a 150µm pore size filter to obtain a fine powdered-like texture. This was done to enhance the penetration of the extraction solvents into the plant cells, thus facilitating the release of the active principles (Sukhdev *et al*., 2008).

Figure 1. *Lemon scented gum (Eucalyptus citriodora).*

2.2. Source of Test Organisms

The test organisms, Salmonella enterica subs. enterica serotype typhi, Salmonella enterica serotype paratyphi A, B & C, Klebsiella pneumoniae, Streptococcus pneumoniae and Streptococcus pyogenes were obtained from stock cultures in the Microbiology laboratory Federal University of Technology, Minna.

2.3. Molecular Identification

The test organisms include species of *Salmonella enterica*, *Klebsiella pneumonia*, *Streptococcus pneumonia* and *Streptococcus pyogenes*. They were all obtained from stock cultures in the Microbiology Laboratory of the Federal University of Technology, Minna, Nigeria. The organism's identity were molecularly authenticated according to Promega Protocol (Technical Manual #TM050)

(www.promega.com) and their identity and accession numbers were determined by BLAST (comparison of the extracted GENE sequence with the known sequence from the GENE bank) (*www.ncbi.nlm.nih.gov*).

Extraction

The pulverized plant samples were subjected to reflux extraction method according to Galhiane *et al*. (2006). One hundred grams (100gms) of the plant sample transferred into a round-bottum flask of 1000ml capacity. The extraction solvent (n-hexane) was gradually added until a ratio of 1:4 of the pulverized plant sample to the extraction solvent was attained. The flask containing the mixture was then placed on the heating mantle and the extracting apparatus was set. The mixture was allowed to reflux for 2 hours at 30°C after which it was then filtered through a whatman filter paper with pore size of 20µm. The solvent was then evaporated out in a rotary evaporator leaving the unevaporated plant extract. The semi-solid extract was then freeze-dried in a lyophilizer to a powdered form and the weight was measured and recorded. The extraction process continues until a total of 500g of dry plant material was extracted and the percentage yield of the crude extract was calculated using the formula below:

Equation 1:

$$Percentage\ Yield\ (g) = \frac{Weight\ of\ Extract\ or\ Oil\ (g)}{Weight\ of\ Dry\ Plant\ Material\ (g)}\ x100/1$$

Phytochemical Screening of Crude Extract

The plant extracts were analysed for phytochemical properties using the methods of Hajir *et al.,* (2016).

Phenols

Two ml of extract was added to one ml of distilled water and warmed at 45°C - 50°C. Then 2 ml of 3% FeCl3 was added. Appearance of green or blue colour indicate the presence of phenols.

Flavonoids

One ml of extract was added to one ml of 10% KOH. It was gently shaken. Appearance of yellow color indicated the presence of flavonoids.

Tannins

One ml of extract was added to one ml of 3% FeCl3. A greenish black precipitate indicated the presence of tannins.

Alkaloids

One ml of Dragendorff reagent was added to 1 ml of filtrate. The formation of cloudy orange was observed.

Terpenoids and Steroids

Five ml of extract was mixed in two ml of chloroform. Then 3 ml concentrated sulphuric acid was carefully added to observe a reddish brown coloration between upper and lower layer was observed.

Saponins

Approximately 0.2 ml of extract was mixed with 5 ml of distilled water. Mixture was shaken vigorously for 5min. Persistence of foams indicated the presence of saponins.

Test for Resins

Solutions of 5ml petroleum ether was made using 0.1g of powdered leaf extract and was labelled appropriately. An equal volume of copper acetate solution was next added and shaken vigorously then allowed to separate. A green colour was indicative of the presence of resins.

Test for Volatile Oils

Volatile oils are characterized by their odour, oil-like appearance and ability to volatilize at room temperature. The plant materials was distilled with water by steam distillation and the distillates were collected in a graduated tube. The aqueous portion which separates automatically was returned to the distillation flask. The formation of emulsion which floats on top of the aqueous phase owing to its low density is indicative of the presence of plant oils.

Test for Anthraquinones

One millilitres of chloroform was added to 0.1 g of plant extract and shaken thoroughly for 5 min; it was filtered and the filtrate was mixed with 100% ammonia solution. Pink, violet or red colours in the ammoniac layer (lower layer) indicate the presence of free anthraquinones.

Test for Cardiac Glycosides

Borntrager's test – To show the presence of free Anthraquinones, 0.5g of the pulverized leaf extract was taken in dry test tubes. Ten millilitres of Chloroform was added and the mixtures shaken for 5 minutes. The extracts was next filtered and an equal volume of ammonia was added to the filtrate and thoroughly shaken. A bright pink colour in the upper aqueous layer indicates the presence Cardiac Glycosides.

TLC Studies of Crude Extract

The Analytical thin layer chromatographic techniques using the TLC silica gel 60 F_{254} Aluminium sheet (0.015-0.04mm mesh size) by Merck KGaA, Millipore Corporation Germany was adopted to spot, separate and determine the presumable number of fraction, Rf (Retention factors) values and a suitable solvent systems for fractionation of the phytochemical components by column chromatography on the crude extracts. This was achieved by spotting a small amount of the dilute extract solution (about 1% of the crude extract in a volatile solvent) onto the origin line (1.5cm from one end of the TLC Figure). The spotted Figure was placed with the bottom downward into a developing chamber containing a shallow pool of a development solvent and allowed travel up the Figure by capillary action after which the developed spots/Figure was brought out, allowed to dry for 5sec and the spots were visualized under UV light/iodine vapour. The distance moved by the solvent and the spots were measured and was used to determine the Rf with the application of the equation below.

Equation 2:

$$Rf = \frac{Distance\ moved\ by\ substance\ cm}{Distance\ moved\ by\ solvent\ (cm)}$$

Fractionation by Column Chromatography (partial purification)

The micro scale column chromatographic method according to Fair and Kormos (2008) was used to separate the fractions of the crude n-hexane extracts. The column

(40mm diameter width and 150mm length) was prepared by packing it with 150g silica gel (0.015-0.04mm mesh size), dissolved in 500ml n-hexane to make a slurry using the wet method. After filling the column with the prepared silica gel, it was allowed to pack for about 1 hour. The extract was prepared by dissolving 3g in 5ml of volatilizable solvent (chloroform) with the addition of 5g of dried silica gel powder to aid adsorption and drying of the extract. The column was next loaded with the dried sample by the wet method. The fractionation process was monitored by changing the polarity of the mobile phase, collection of eluents, TLC analysis, bulking of fractions based on Rf values and finally evaporation. This process continues until a total of 18gms was completely fractionated.

Antimicrobial Sensitivity Test

Standardization of Inoculum

The test organisms were standardized according to the method of Chessbrough, (2002) by transferring 0.2ml of overnight culture of the test organism into a freshly prepared nutrient broth (20ml) and the culture was incubated for 3 to 5 minutes to give a turbidity equivalent of 10^6cfu/ml. This was used to inoculate the media for the determination of antimicrobial activity and minimum inhibitory concentration.

Preparation of Extract

Concentrations of 20mg/mL and 40mg/mL was prepared for the crude extract and pure fractions respectively by dissolving 100mg and 200mg of the extract in 5ml of 10% DMSO separately while for the standard antibiotics, 0.1g was weighed and dissolved in 100ml of distilled water to give 1mg/ml.

Mueller Hinton agar was prepared according to manufacturer's manual and 20 ml of the prepared medium was poured into petri dishes and allowed to solidify. The media was then inoculated (using the spread Figure method) with the standardized test organisms and labelled appropriately according to the test organisms and concentrations used. Wells were then made on the media

Screening of Extracts and Fractions for Antibacterial Activity

Antimicrobial susceptibility screening was done using the agar-diffusion method. Mueller Hinton agar a with the aid of a sterile cork borer of 6 mm diameter after which 100µl of the prepared extracts and fractions was transferred into the wells. In the same vein, 100µl of the standard antibiotics (ciprofloxacin 1mg/ml) was also transferred into the well as the positive control while 100µl of 10% DMSO was used as the negative control. The antimicrobial sensitivity test screening was done in triplicate and all the culture Figures were next incubated at 37°C for 18-24 h. The susceptibility of the test organisms to the plant extracts was indicated by clear zone of inhibition (IZ) around the wells containing the plant extracts and the diameter of the clear zones were taken as an index of the degree of sensitivity. The experiment was carried out in triplicate and the mean inhibition zone was statistically extrapolated using statistical package for social science (SPSS version 20).

Minimum Inhibitory Concentration (MIC) Test

The MIC of Crude extracts and the most active fractions against the test organisms was determined using the standardized inoculum. The fraction concentrations was first prepared using a twofold serial dilution method. 480mg of the extract/fraction was dissolved in a test tube labelled A containing 2ml Mueller Hinton broth (this gives 120mg/ml), from test tube A, 2ml was transferred into a second test tube labelled B containing 2ml sterile Mueller Hinton broth (to obtain 60mg/ml). This process continued to obtain concentrations of 30mg/ml, 15mg/ml, 7.5mg/ml, 3.75mg/ml, 1.875mg/ml until the last concentration was prepared in a test tube labelled H (to give 0.938mg/ml) and 0.1ml of the standardized organism was added to each test tubes. Positive and negative controls were also maintained for each test batch of extract concentrations and test organisms (Akinyemi *et al*., 2006 and Kabir *et al*., 2005) i.e. sterile broth plus fraction but without inoculation of the test organism for the negative control while for the positive control, sterile broth was inoculated with the test organisms but without the addition of the plant extracts. The test tubes were incubated at 37°C for 24 h in a water bath with shaker, and the optical density (OD) of each test tubes were read using Spectrophotometer at 600nm wavelength while spectrophotometer (Koch, 1970) was blanked using sterile Mueller Hinton broth void of extract and text organism. The MIC was determined by using the formula below in relation to the absorbance of the controls.

$$T - C_0 = C_1$$

The MIC is equal to the absorbance of the test concentration (T) with a significant reduction in absorbance after the subtraction of the absorbance of the negative control (C_0) and when compared with the absorbance of the positive control (C_1).

Minimum Bactericidal Concentration (MBC) Test

The tube with no turbidity as compared to the control, which was regarded as the MIC together with other tubes higher in extract/fraction concentration but lower in optical density (OD) than that of the MIC tube were sub cultured on to freshly prepared nutrient agar and the cultures were incubated at 37°C for 24-48 h. The culture concentration without any visible growth after incubation was recorded as the MBC.

Quantitative Analysis and Identification of Compounds by GC-MS

The determination of the identity of active components in the fractions (ECO2 & ECO4) was done by GC-MS analysis using GC-MS-QP 2010 Plus Shimadzu system (SHIMADZU, JAPAN). The gas chromatograph interface to a mass spectrometer (GC-MS) instrument was used while the Column elite-1 was fused with silica capillary column (30m x0.25mm 1D x µ1 df, composed of 100% dimethyl polysiloxane). An electronic ionization system with ionization energy of 60eV was used for the GC-MS detection while Helium gas (99.99%) was used as the carrier gas at a flow rate of 1ml/min and injection size of the fraction was 2µl (0.002ml with split ratio of 1:40 and film thickness of

0.20µm). The GC oven temperature was set at 70°C for 3.00min and then programmed to rise from 70 to 250°C at a rate of 3°C min^{-1} and held isothermally for 3.00min at 200°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 70eV; a scan interval of 0.5s and fragments from 40 to 550Da. Total GC running time was 28.00minutes. Relative percentages and amount of each components were deduced by comparing individual average peaks area to the total areas. Turbomass was used for the mass spectra and chromatogram while the detection of compounds was done using the database from the library of National Institute of Standard and Technology (NIST) NIST Ver. 2.0 year 2009 (Sarswati *et al*., 2013).

Acute Oral Toxicity Study

This test measures relative toxicological response of an experimental organism to single or brief exposure to a test substance (OECD, 2001). The Lorke's method of acute toxicity was used in this experiment and this test was also used to calculate the median lethal dose (LD_{50}) of the crude extracts using the oral gavage rout of exposure (Lorke, 1983; OECD 2001). The toxicity study was carried out using 18 Swiss albino mice (20g to 25g body weight) of either sex. The extracts were administered in two phases (Phase I and Phase II), while the animals were divided into two major groups. The first groups received the plant extract while the second group which served as the control group received normal saline. Dose levels of 10, 100, and 1000mg/kgbw were administered in triplicates for the first phase. The number of deaths in each group within 24 hours was recorded. In the second phase which was extrapolated from the result of the first phase, six rats were grouped into two groups of three rats each and the first group were administered with doses of 2000, 1500 and 1250mg/kgbw of the plant extract and second group was administered with normal saline. Following administration of the test products, the animals were observed individually for signs of toxicity such as paw-licking, stretching, respiratory distress, diarrhoea and death at least once during the first 30 minutes, with special attention given during the first 4 hours and thereafter, for a total of 24 hours. The median lethal dose (LD_{50}) was calculated as the geometric mean of dose that causes 0 % and 100 % mortality according to Lorke's formula as follows:

Equation 1: $LD_{50} = \sqrt{a} \times b$

Where a is the highest dose at which no death occurred and b is the least dosage at which death occurred in the second phase (Oyewole *et al.,* 2013). The dosage in mg/kgbw and mL/kgbw required for each rat in relation to their individual weight was determined using equation 3.4 and 3.5 below respectively.

Equation 2:

$$Required\ Dose\ for\ Rat\ (g) = \frac{\text{Weight of Rat (g)}}{1000\ (\text{g})} \text{ x } Standard\ Dose\ (mg)$$

Equation 3: $Required\ Dose\ for\ Rat\ (mL) = \frac{Weight\ of\ Rat\ (g)}{1000\ (g)} x10mL *$

*Based on 10mL/kg volume selection required for organic solvents (Erhirhie *et al*., 2014).

3. Results and Discussion

The result of the antibacterial activity revealed that the crude extract was more active than the fractions owing to the fact that the crude extract exhibited its activity against the test organisms at 20mg/mL concentration which is significantly ($p<0.05$) different to the fractions (40mg/mL) and control (Table 5). For the crude oil extract, mean inhibition zone (MZI) diameter was 20.66±0.33mm specifically against *Salmonella paratyphi* B, while the least MZI was recorded against *Salmonella* typhi (15.00±0.57mm). Of the 8 fractions eluted from the crude extract, only 5 showed activity against the test organisms while 3 were inactive (Table 5). This could be possible synergy, that is the different phytochemical components could be exhibiting their activity in combination rather than when single. Fraction ECO4 was the most active with the highest MZI of 21.66±0.88mm against *Salmonella paratyphi* B while fraction ECO8 was the least active with MZI of 7.00±0.00mm against *Streptococcus pneumoniae*. Generally, Gram positive organisms were more susceptible to the plant extract and fractions than the Gram negative organisms, which is in contrast to the report of Luqman *et al*. (2008).

The antibacterial activity of plant extracts which is attributed to the abundant presence of phytochemicals WHO, (2012), confirms the claims by local marketers and consumers of the use of the leaf parts of the plants for the treatment of typhoid fever and respiratory tract infections owing to the fact that the test organisms which are responsible for the above mentioned infections are all susceptible to the extract. The phytochemical result of this research work reveals a total of 10 phytocomponents viz: alkaloids, flavonoids, saponins, cardiac glycosides, tannins, steroids, terpenes, resins, phenols and volatile oils except for anthraquinon (Table 2). Several reports have implicated some of the phytochemical constituents as possessing antimicrobial activity. Flavonoids are of three sub-types and they include the bioflavonoids, isoflavonoids and the neoflavonoids (Galeotti *et al.,* 2008). A good example is the existence of different types of tannins such as the hydrolysable and the non-hydrolysable tannins in plant cells (Simon, 1993). Tannins are very important phytochemical components of medicinal plants because of their increasing commercial application in pharmaceutical, nutraceutical and food industries. He *et al*. (2007) reported that tannins are present in some plant-based drugs with therapeutic effects and in some beverages used as supplement to correct cardiovascular disorder. Mateus *et al*. (2004) reported that tannins, being a smaller phenolic compounds may serve as dietary antioxidants and the ability of tannins to precipitate proteins has made their presence in medicinal plant extract detrimental to microbial cells.

Thin layer chromatography (TLC) studies reveals the number of possible compounds, solvent system and retention factor (Rf) of the compounds in a mixture such as a plant

crude extract. The Rf is like a finger print that can be used to determine the possible compound in an extract by comparing the Rf with a known standard. The highest number of spot seen in the TLC result of *Eucalyptus citriodora* n-hexane extract was 6 (Table 3). Different solvent composition were used for the mobile phase in order to obtain high resolution and reproducible peak. The *R*f values are between 0 and 1, best between 0.1 and 0.8. If reproducible *R*f values are to be obtained it is, however, essential that several parameters such as chamber saturation, constant composition of solvent mixtures, constant temperature etc. are strictly controlled. The TLC result reveals that there are possibly minimum of 6 compounds in the crude extract but there are situations where some compounds are colourless and are not visible on the TLC Figure (Johnson and Janakiraman, 2013). Therefore, the result of this study agrees with the above claim considering the fact that more than 6 fractions were eluted in the column chromatography. Also the TLC result reveals that majority of the spot were concentrated at the non-polar solvent (mobile phase) system region and this could mean that the compounds in the extract are non-polar.

A total of 18gms of the crude extract was fractionated by column chromatography of which 8 fraction namely ECO1, ECO2, ECO3, ECO4, ECO5, ECO6, ECO7 and ECO8 were eluted (Table 4). The result of the column chromatography was observed to be in agreement with that of the TLC having the compounds concentrated at the non-polar solvent system region. Each of the fractions shows 1 spot except for fraction ECO5 and ECO6 which had 3 spots each and this could indicate that further purification process might be required to separate them.

The antibacterial activity result of the fractions revealed that 5 fractions (ECO2, ECO4, ECO6, ECO7 and ECO8) were active against all the test organisms. However, the activity of ECO2 and ECO4 were most remarkable and significantly ($p<0.05$) higher than the other 3 fractions having mean inhibition zone (MIZ) ranging from 15.00±0.00mm to 18.66±0.33mm and 9.33±0.33mm to 21.66±0.88mm for ECO2 and ECO4 respectively which when compared to the standard drugs, are within the intermediate zone of activity while the highest MIZ of other fractions was 13.00±0.57mm which was why ECO2 and ECO4 fractions were considered for GC-MS analysis (Table 5).

Gas chromatography Mass spectrometry result revealed 39 and 15 compounds in fraction ECO2 and ECO4 respectively (Table 7 and 8). The most abundant compounds identified in fraction ECO2 includes gamma.-Tocopherol (10.25%), Hemimellitene (8.64%), n-Decane (6.82%), 11-Octadecenoic acid, methyl ester (6.56%) and Decane, 2-methyl- (5.13%) while others were in trace amount. Several of the identified compounds belongs to the Terpenes and Terpenoids and Phenol group of compounds in addition to some aliphatic hydrocarbon such as Cycloicosane (0.52%) and non-polar components; this is in agreement with the report of Dagne *et al*. (2000) that the oil of *Eucalyptus citriodora* is mainly composed of citronellol and terpene-4-ol. These compounds are also similar to compounds identified by Akpuaka *et al.* (2013) from oils of medicinal plants using n-hexane which is the same extraction solvent used in this study for oil extraction. Several of these compounds are reported to possess potent antibacterial activity notably is the antibacterial activity of dacane reported by Gholamrez *et al.* (2012) against Gram positive and negative organisms which was also demonstrated in this research work. Rani and Agrawal, (2006) reported on the antibacterial activity of Nonane and its related compounds; a very good example of such compound identified in this research work is 2-Methylnonane reported to possess antibacterial activity against species of *Salmonella enterica* (Pavlović *et al*., 2011). Terpenes and Terpenoids compounds are the most abundant components identified in *Eucalyptus citriodora* fraction ECO2 in this research findings but the antibacterial activity exhibited by this fraction were within the intermediate zones (15.00±0.00mm to 18.66±0.33mm), which could be as a result of the fact that they are only available in trace amount. This result also is in agreement with the report of Hatice and Ayse. (2014) who reported on the limited antibacterial activity of oil compounds from same plant species.

Fifteen compounds were identified by GC-MS in *Eucalyptus citriodora* oil fraction ECO4 with delta. (Sup9)-cis-Oleic acid (21.02%), Stigmasterol, 22,23-dihydro- (14.35%), 6.beta.Bicyclo[4.3.0]nonane, 5.beta.-iodomethyl-1.beta.-isopropenyl-4.alpha.,5.alpha.-dimethyl- (14.13%), n-Hexadecanoic acid (8.62%), Hydrofol Acid 150 (7.64%) and Ricinoleic acid methyl ester (7.61%) as the most abundant compounds in descending order, while other compounds that makes up the remaining 26.63% are available in trace amounts. Like fraction ECO2, fraction ECO4 was found to be composed mainly of Saturated and unsaturated fatty acids, Terpenes and Terpenoids compounds (α-terpineol, linalool, eucalyptol and α-pinene) and sulfo-containing compound which agrees with the report of Hatice and Ayse. (2014) who reported on the identification and antibacterial activity of Terpenes and Terpenoids compounds in oil of *Eucalyptus citriodora* against species of *Salmonella enterica* and *Klebsiella pneumoniae* but in contrast, this research result revealed higher number of compounds compare to their research findings and the reason for this disparity could be as a result of geographical location. Other compouds identified are alkanes, phenols and aliphatic sulfuric compounds with functional groups of C-H, O-H and C-S respectively. The result reveals that both saturated and unsaturated fatty acid constitute the majority compounds identified in fraction ECO4 and this could the reason why ECO4 exhibited more activity than fraction ECO2 which also is in support of the report of Desbois and Smith, (2010); Desbois, (2012) that the potentials of fatty acids as therapeutic antimicrobial agents owing to their potency, broad spectrum of activity and absence of resistance mechanisms by microorganisms against the actions of these compounds is becoming well acknowledged as a viable means of drug development. Andrew *et al.* (2013) reported on the antibacterial activity of six long-chain poly unsaturated fatty acids namely dihomo-γ-

linolenic acid, docosahexaenoic acid, eicosapentaenoic acid, γ-linolenic acid, 15-hydroxyeicosatrienoic acid and 15-hydroxyeicosapentaenoic acid against some Gram negative and positive organisms. Three notable fatty acid compounds identified in this research work are 11-Octadecenoic acid, methyl ester, Octadecenoic acid, methyl ester and Ricinoleic acid methyl ester with antibacterial activity against species of *Salmonella* and *Klebsiella* as reported by Abayomi *et al.* (2012). It has been reported that fatty acids are part of the components responsible for the first line of defence in breast milk for suckling infant against invading pathogenic microorganisms notably *Streptococcus* species as reported by Isaac, (2005). Research findings by Petshow *et al.* (1996) and Ruzin and Novick, (2000) shows that fatty acids exert their antibacterial activity by supressing antibiotic resistance genes in bacteria and provoke a relatively low frequency of spontaneous development of resistance in bacteria while other reports describes its activity to direct penetration of the cell walls and cytoplasmic membranes of bacteria, where it gain access to enter, and disrupt the cytoplasm while Bergsson *et al*. (2001) reported that Terpenes and Terpenoids compounds on the other hand act by increasing membrane permeability.

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of a drug reveals the potency level of the drug i.e. the lower the MIC the more potent the drug. The MIC can be a guide on the choice of antimicrobial drugs used in treatment by predicting efficacy (Mouton and Vinks, 2007). If pharmacokinetic and pharmacodynamics (PKPD) principles are observed by careful selection of a particular antimicrobial drug administered at an appropriate dosage, it will lead to clinical cure, eradication of carrier status of a system, and prevention of selection of resistance. In this study result, the least MIC for the crude extract was 7.5mg/mL against *Salmonella paratyphi* B and the highest MIC was 15mg/mL against the other 6 organisms while for the MBC the least and highest was 60mg/mL and 120mg/mL respectively (Fig 1). For the fractions, the MIC was as low as 3.25mg/mL while the MBC was as high as 120mg/mL (Table 6). The result of the MIC suggest that the fractions could be more active than the crude extract against some of the test organism. Generally, the antibacterial activity result revealed that *Salmonella paratyphi* B and *Streptococcus pyogenes* were the most susceptible organisms while *Salmonella typhi* and *Salmonella paratyphi* C were the least susceptible. Also, the Gram positive organisms were more susceptible than the Gram negative.

The application of medicinal plants for the treatment of infections by traditional medicine practitioners is not always a reliable approach in terms of safety since it is difficult for the traditional practitioners to detect or monitor delayed effects, adverse effects, and rare adverse effects such as mutagenicity which could arise from long-term administration. The acute toxicity result of the crude extracts in this research work revealed a safe oral dose of 1369mg/kgbw, which is within the standard range of 500mg/kgbw to 5000mg/kgbw as proposed by Lorke, (1983) without any adverse effect (Table 9). However, some acute toxicity signs and reactions such as shivering, loss of sensitivity, reduced activity, motionlessness and maybe weakness could be experienced within the first few hours of administration but will naturally fades away. These experiences are not strange due to the fact that the use of drug for the treatment of any ailment is not completely free from some adverse effect and plant extract is not left out but they can only be useful after careful measurement of the advantages and disadvantages associated with their use. It has been reported that natural plant products are relatively safe and could be applied after thorough toxicological evaluations using modern scientific methods (Aniagu *et al*., 2005). It is therefore recommended that the chronic toxicity studies be done on the extract to determine its long term effect.

Total and Percentage yield of Crude Extract 25.85g (5.17%) of 500g

Table 1. *Identity and Accession Number of Test Organisms.*

Test Organisms	Total Score	Query Cover	E	Identity	Accession
S.enterica subsp.enterica serovar paratyphi A strain SPA2 16s Ribosomal RNA gene partial sequence. (1460bp)	2697	100%	0.0	100%	*KM977902.1*
S.enterica subsp.enterica serovar paratyphi B strain 374 16s Ribosomal RNA gene partial sequence.(1467bp)	2676	100%	0.0	100%	*JQ694526.1*
S.enterica subsp.enterica serovar paratyphi C strain DT4 16s Ribosomal RNA gene partial sequence. (1503bp)	2776	100%	0.0	100%	*JF951185.1*
S.enterica subsp.enterica serovar typhi strain T4 16s Ribosomal RNA gene partial sequence. (1546)	2856	100%	0.0	100%	*EU118111.1*
Klebsiella pneumoniae strain BYK-9 16s ribosomal RNA gene partial sequence (1504bp)	2778	100%	0.0	100%	*KP255917.1*
Sreptococcus pneumoniae strain ATCC 33400. 16s ribosomal RNA gene partial sequence (1515bp)	2795	100%	0.0	100%	*NR028665.1*
Sreptococcus pneumoniae strain JCM 5674. 16s ribosomal RNA gene partial sequence (1480bp).	2734	100%	0.0	100%	*LC071824.1*

Table 2. *Phytochemical Properties of Eucalyptus citriodora n-hexane Crude Extract.*

	Phytochemical Properties										
Plant Extracts	Flavonoid	Phenols	Alkaloids	Tannins	Steroids	Cardiac gylcosides	Saponins	Terpenes	Volatile oil	anthraquinon	Resins
EC (n-hex)	+	+	+	+	+	+	+	+	+	-	+

Key: +: Present, EC: *Eucalyptus citriodora,* n-hex: n-hexane

Table 3. *Result of TLC Studies of Eucalyptus citriodora n-hexane Crude Extract.*

Solvent System	NS	DMSp (cm)	DMS (cm)	Rf (cm)
100% n-hexane	2	0.6, 0.9	5.0	0.12, 0.18
9:1 hexane: chloroform	3	0.6, 2.3, 3.3	5.0	0.12 - 0.66
4:1 hexane: chloroform	4	0.8, 2.1, 2.8, 3.5	5.0	0.16 – 0.70
1:1 hexane: chloroform	5	0.6, 1.8, 3.4, 4.0, 4.6	5.0	0.12 – 0.92
1:4 hexane: chloroform	6	0.7, 1.0, 1.3, 2.4, 3.1, 4.6	5.0	0.14 – 0.92
1:9 hexane: chloroform	6	0.6, 1.0, 1.4, 2.6, 3.2, 4.6	5.0	0.12 – 0.92
100% chloroform	5	0.7, 1.7, 2.9, 4.2, 4.6	5.0	0.14 – 0.92
9:1 chloroform: ethyl acetate	2	3.1, 4.9	5.0	0.36, 0.98
4:1 chloroform: ethyl acetate	2	1.0, 4.9	5.0	0.2, 0.98
1:1 chloroform: ethyl acetate	4	0.6, 1.2, 1.9, 4.5	5.0	0.12 – 0.90
1:4 chloroform: ethyl acetate	3	0.5, 2.8, 4.9	5.0	0.1 – 0.98
1:9 chloroform: ethyl acetate	1	3.2	5.0	0.64
100% ethyl acetate	1	4.1	5.0	0.82
9:1 ethyl acetate: methanol	1	3.8	5.0	0.76
4:1 ethyl acetate: methanol	1	4.9	5.0	0.98
1:1 ethyl acetate: methanol	1	4.9	5.0	0.98
1:4 ethyl acetate: methanol	1	4.9	5.0	0.98
1:9 ethyl acetate: methanol	-	-	5.0	-

Key: DMS= distance moved by the solvent (mobile phase), DMSp= distance moved by spot, Rf= Retention factor, NS: Number of spot.

Table 4. *Percentage Yield of Fractions of Eucalyptus citriodora Oil extract (18g).*

Fractions	Solvent system & Volume(ml)	Description	Percentage Yield (%)	NS	Rf (cm)
ECO1	100% n-hexane (500)	Yellow and oily	0.9 (5)	1	0.68
ECO2	1:4 n-hex: $CHCl_3$(600)	Red and oily	2.75 (15.28)	1	0.58
ECO3	1:9 n-hex: $CHCl_3$(500)	Pale yellow	1.29 (7.16)	1	0.82
ECO4	9:1 $CHCl_3$: EtOAc (500)	Light green	3.54 (19.67)	1	0.74
ECO5	1:1 $CHCl_3$: EtOAc(700)	Greenish	0.59 (3.28)	3	0.50-0.56
ECO6	1:1 $CHCl_3$: EtOAc(400)	Black	2.88 (16)	3	0.80-0.88
ECO7	100% EtOAc (400)	Light brown	1.24 (6.89)	1	0.66
ECO8	100% CH_3OH (400)	Dark brown	1.26 (7)	1	0.78
Residue	100% Water (300)	Off white	3.55 (19.72)	NA	NA

Key: n-hex: n-hexane, $CHCl_3$: Chloroform, EtOAc: Ethyl acetate, NA: Not applicable, NS: Number of spot, Rf: Retention factor.

Table 5. *Mean Inhibition Zones of Eucalyptus citriodora leaf Crude Extract and Fractions (mm).*

		***Eucalyptus citriodora* n-hexane fractions**								**Control**	
Org	**CE (20mg/mL)**	**ECO1 (40mg/mL)**	**ECO2 (40mg/mL)**	**ECO3 (40mg/mL)**	**ECO4 (40mg/mL)**	**ECO5 (40mg/mL)**	**ECO6 (40mg/mL)**	**ECO7 (40mg/mL)**	**ECO8 (40mg/mL)**	***Cpx (1mg/mL)**	**D (100µL)**
SpA	19.33 ±0.33^{b}	0.00^{a}	18.66 ±0.33^{e}	0.00^{a}	15.66 ±0.33^{c}	0.00^{a}	10.33 ±0.33^{b}	11.33 ±0.33^{bc}	8.66 ±0.33^{b}	23.50 ±1.50^{a}	0.00^{a}
SpB	20.66 ±0.33^{b}	0.00^{a}	17.66 ±0.66^{de}	0.00^{a}	17.66 ±0.33^{d}	0.00^{a}	7.33 ±0.33^{a}	12.00 ±0.00^{cd}	9.00 ±0.00^{b}	26.66 ±0.88^{a}	0.00^{a}
SpC	15.00 ±0.57^{a}	0.00^{a}	15.00 ±0.00^{a}	0.00^{a}	9.33 ±0.33^{a}	0.00^{a}	8.00 ±0.00^{a}	10.33 ±0.33^{ab}	9.33 ±0.66^{b}	24.66 ±1.45^{a}	0.00^{a}
ST	15.00 ±0.57^{a}	0.00^{a}	15.33 ±0.33^{ab}	0.00^{a}	13.66 ±0.33^{b}	0.00^{a}	11.00 ±0.00^{b}	9.66 ±0.33^{a}	11.33 ±0.33^{c}	25.33 ±0.33^{a}	0.00^{a}
Kp	19.00 ±0.57^{b}	0.00^{a}	17.33 ±0.33^{cd}	0.00^{a}	15.00 ±0.00^{c}	0.00^{a}	8.33 ±0.33^{a}	12.00 ±0.57^{cd}	9.33 ±0.33^{b}	25.3 3±0.33^{a}	0.00^{a}
Sp	19.33 ±0.88^{b}	0.00^{a}	18.00 ±0.00^{de}	0.00^{a}	16.33 ±0.33^{c}	0.00^{a}	12.66 ±0.66^{c}	11.66 ±0.66 ^{bcd}	7.00 ±0.00^{a}	25.33 ±0.88^{a}	0.00^{a}
Spy	16.00 ±0.57^{a}	0.00^{a}	16.33 ±0.33^{bc}	0.00^{a}	21.66 ±0.88^{e}	0.00^{a}	8.00 ±0.00^{a}	13.00 ±0.57^{d}	9.00 ±0.57^{b}	30.00 ±1.00^{b}	0.00^{a}

Key: Org: organism, ECO2 - 8: *Eucalyptus citriodora* Oil fraction 2 to 8, Cpx: ciprofloxacin, E: erythromycin, D: dimethyl sulfoxide, SpA: *Salmonella paratyphi* A, SpB: *Salmonella paratyphi* B, SpC: *Salmonella paratyphi* C, ST: *Salmonella typhi,* Kp: *Klebsiella pneumoniae*, Sp: *Stretococcus pneumoniae*, Spy: *Streptococcus pyogenes*, CE: Crude extract, *Specification for Cpx and E are: ≤15mm (resistance), 16-20mm (intermediate), and ≥21 (susceptible) (CLSI, 2012), Values on the same column with different superscript are significantly different ($p<0.05$), n = 3.

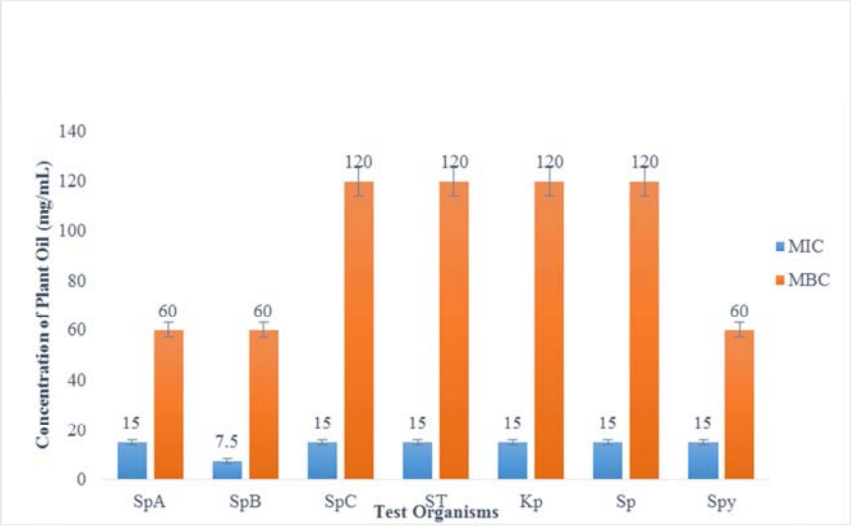


Figure 2. *Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of Eucalyptus citriodora crude oil extract.*

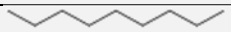
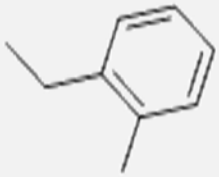
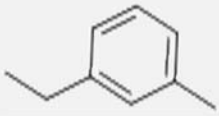
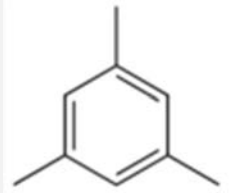
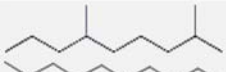
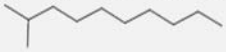
Key: SpA: Salmonella paratyphi A, SpB: Salmonella paratyphi B, SpC: Salmonella paratyphi C, ST: Salmonella typhi, Kp: Klebsiella pneumoniae, Sp: Stretococcus pneumoniae, Spy: Streptococcus pyogenes.

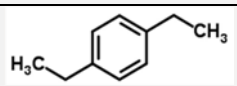
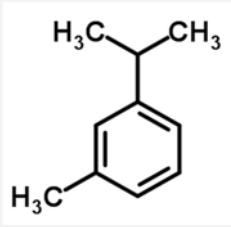
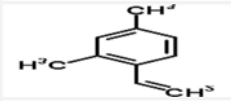
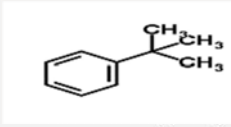
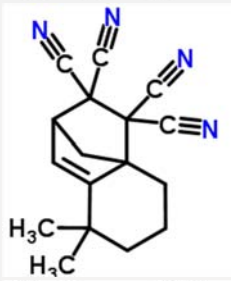
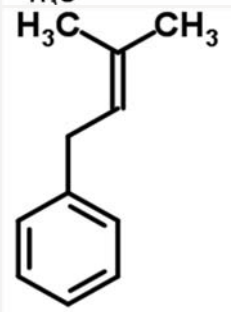
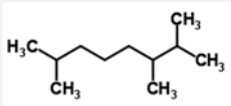
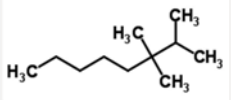
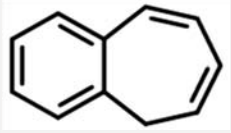
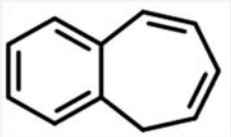
Table 6. *Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Eucalyptus citriodora Oil Fractions (mg/ml).*

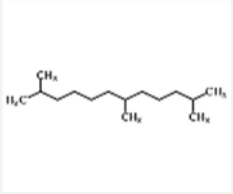
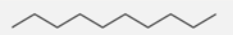
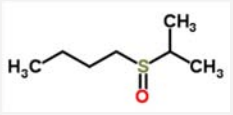
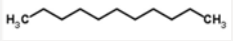
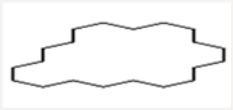
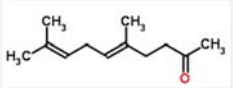
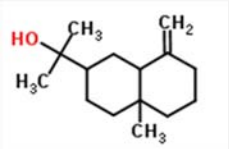
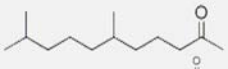
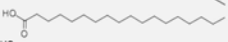
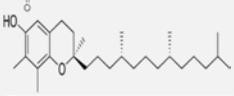
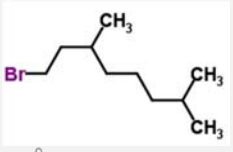
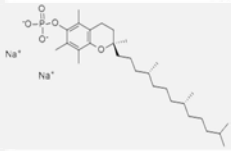
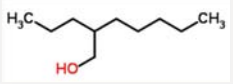
	ECO2		ECO4	
Organisms	**MIC**	**MBC**	**MIC**	**MBC**
SpA	3.25	120	7.5	120
SpB	3.25	60	3.25	60
SpC	15	60	15	120
ST	15	120	15	120
Kp	7.5	120	7.5	120
Sp	15	60	7.5	120
Spy	3.25	60	7.5	60

Key: SpA: Salmonella paratyphi A, SpB: Salmonella paratyphi B, SpC: Salmonella paratyphi C, ST: Salmonella typhi, Kp: Klebsiella pneumoniae, Sp: Stretococcus pneumoniae, Spy: Streptococcus pyogenes. ECO2 & ECO4: Eucalyptus citriodora Oil fractions 2 & 4.

Table 7. *Percentage composition and Structure of probable compounds Identified in Eucalyptus citriodora Oil Fraction (ECO2).*

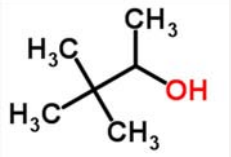
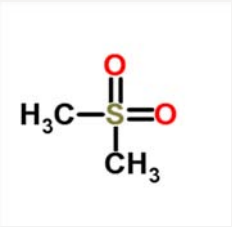
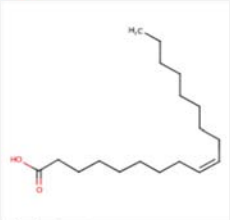
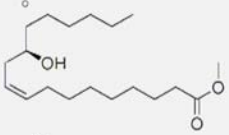
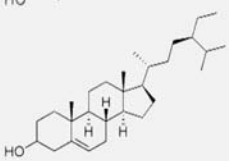
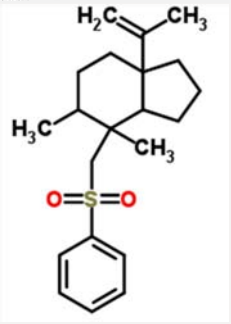
Peak No.	RT	PA (%)	MW (g/mol)	MF	Compound Name	Structure
1	3.606	0.31	128.2551	C_9H_{20}	Shellsol 140	
2	4.523	1.14	120.195	C_9H_{12}	Benzene, 1-ethyl-2-methyl-	
3	4.604	2.05	120.0	C_9H_{12}	m-Ethyltoluene	
4	4.944	8.64	120.0	$C9H_{12}$	Hemimellitene	
5	5.261	3.08	156.313	$C_{11}H_{24}$	Nonane, 2,6-dimethyl-	
6	5.875	5.13	156.188	$C_{11}H_{24}$	Decane, 2-methyl-	
7	6.406	6.82	142.0	$C_{10}H_{22}$	n-Decane	

Peak No.	RT	PA (%)	MW (g/mol)	MF	Compound Name	Structure
8	6.619	3.30	134.0	$C_{10}H_{14}$	para-Diethylbenzene	
9	6.860	2.70	134.218	$C_{10}H_{14}$	Benzene, 1-methyl-3-(1-methylethyl)-	
10	7.144	2.79	132.202	$C_{10}H_{12}$	2,4-Dimethylstyrene	
11	7.338	5.33	134.11	$C_{10}H_{14}$	tert-Butylbenzene	
12	7.864	3.67	142.28	$C_{10}H_{22}$	2-Methylnonane	
13	8.516	1.62	276.33	$C_{17}H_{16}N_4$	5,5-Dimethyltricyclo[6.2.1.0(1,6)] undec-6-ene-9,9,10,10-tetracarbonitrile	
14	8.759	1.29	146.23	$C_{11}H_{14}$	Benzene, (3-methyl-2-butenyl)-	
15	8.885	1.34	156.31	$C_{11}H_{24}$	Octane, 2,3,7-trimethyl-	
16	9.269	1.94	156.31	$C_{11}H_{24}$	2,3,3-Trimethyloctane	
17	9.492	2.38	142.19	$C_{11}H_{10}$	5H-Benzo[a]cycloheptene	
18	9.732	1.86	142.19	$C_{11}H_{10}$	5H-Benzo[a]cycloheptene	

Peak No.	RT	PA (%)	MW (g/mol)	MF	Compound Name	Structure
19	10.309	0.84	212.42	$C_{15}H_{32}$	2,6,11-Trimethyldodecane	
20	10.617	1.61	142.0	$C_{10}H_{22}$	n-Decane	
21	11.421	0.70	148.266	$C_7H_{16}OS$	1-(Isopropylsulfinyl)butane	
22	11.899	0.39	156.31	$C_{11}H_{24}$	Undecane	
23	13.036	0.52	280.53	$C_{20}H_{40}$	Cycloicosane	
24	13.282	1.33	180.29	$C_{12}H_{20}O$	(5E)-5,9-Dimethyl-5,8-decadien-2-one	
25	14.083	0.31	222.36		Eudesm-4(14)-en-11-ol	
26	15.528	0.59	210.4	$C_{15}H_{30}$	1-Pentadecene	
27	16.385	0.30	198.34	$C_{13}H_{26}O$	Pseudoionone, hexahydro-	
28	17.910	4.22	270.45	$C_{17}H_{34}O_2$	Methyl 14-methylpentadecanoate	
29	19.043	3.63	256.42	$C_{16}H_{32}O_2$	n-Hexadecanoic acid	
30	19.246	0.55	210.39	$C_{15}H_{30}$	1-Pentadecene	
31	20.937	4.34	322.52	$C_{21}H_{38}O_2$	Methyl (11E,14E)-11,14-icosadienoate	
32	21.047	6.56	296.48	$C_{19}H_{36}O_2$	11-Octadecenoic acid, methyl ester	
33	21.369	2.08	298.50	$C_{19}H_{38}O_2$	Octadecanoic acid, methyl ester	
34	21.806	3.54	282.46	$C_{18}H_{34}O_2$	Oleic Acid	
35	22.081	1.05	284.48	$C_{18}H_{36}O_2$	Octadecanoic acid	
36	25.112	10.25	416.68	$C_{28}H_{48}O_2$	gamma.-Tocopherol	
37	25.959	0.71	221.18	$C_{10}H_{21}Br$	1-Bromo-3,7-dimethyloctane	
38	26.937	0.68	430.38	$C_{29}H_{50}O_2$	Vitamin E	
39	27.335	0.40	158.28	$C_{10}H_{22}O$	2-Propyl-1-heptanol	
Total	-	100.0	-	-	-	-

Key: TR: Retention time; PA: Peak area; MW: Molecular weight; MF: Molecular formular

Table 8. *Percentage composition and Structure of probable compounds Identified in Eucalyptus citriodora Oil Fraction (ECO4).*

Peak No.	RT	PA (%)	MW(g/mol)	MF	Compound Name	Structure
1	3.764	0.67	102.18	$C_6H_{14}O$	3,3-Dimethylbutane-2-ol	
2	3.940	6.71	94.13	$C_2H_6O_2S$	Dimethyl sulfone	
3	4.785	2.94	154.29	$C_{11}H_{22}$	(3E)-3-Undecene	
4	4.904	0.95	142.28	$C_{10}H_{22}$	Decane	
5	17.905	1.78	270.45	$C_{17}H_{34}O_2$	Methyl 14-methylpentadecanoate	
6	19.000	8.62	256.24	$C_{16}H_{32}O_2$	n-Hexadecanoic acid	
7	20.918	2.85	322.53	$C_{21}H_{38}O_2$	Methyl (11E,14E)-11,14-icosadienoate	
8	21.003	6.98	296.49	$C_{19}H_{36}O_2$	11-Octadecenoic acid, methyl ester	
9	21.365	2.15	294.47	$C_{19}H_{34}O_2$	Octadecenoic acid, methyl ester	
10	21.790	21.02	282.26	$C_{18}H_{34}O_2$	delta.(Sup9)-cis-Oleic acid	
11	22.081	7.64	284.48	$C_{18}H_{36}O_2$	Hydrofol Acid 150	
12	23.492	7.61	312.49	$C_{19}H_{36}O_3$	Ricinoleic acid methyl ester	
13	24.183	1.60	184.28	$C_{11}H_{20}O_2$	Undecylenic Acid	
14	25.670	14.35	414.70	$C_{29}H_{50}O$	Stigmasterol, 22,23-dihydro-	
15	27.459	14.13	346.53	$C_{21}H_{30}O_2$	6.beta.Bicyclo[4.3.0] nonane, 5.beta.-iodomethyl-1.beta.-isopropenyl-4.alpha.,5.alpha.-dimethyl-	
Total	-	100.0	-	-	-	-

Key: TR: Retention time; PA: Peak area; MW: Molecular weight; MF: Molecular formular

Table 9. *Acute toxicity and LD_{50} of Plant Extracts.*

PHASE I				
Extract	**No. of mice**	**Dose (mg/kgbw)**	**Motility/Survival**	**Toxicity reactions**
	3	10	0/3	No observable sign of toxicity
EC (n-hex)	3	100	0/3	Shivering and inactive at first 20mins
	3	1000	1/3	Shivering, Loss of sensitivity and inactive at first 45mins and one dead.
	1	10	0/3	No observable sign of toxicity
NS	1	100	0/3	No observable sign of toxicity
	1	1000	0/3	Inactive Within 1st 30mins
PHASE II				
	1	2000	1/1	Shivering, inactive after the 1-4hrs, but later died before 24hrs.
EC (n-hex)	1	1500	1/1	Inactive after the 1-4hrs, but later died before 24hrs
	1	1250	0/1	Inactive after the 1-4hrs but no observable sign of toxicity.
	1	2000	0/1	No observable sign of toxicity
NS	1	3500	0/1	No observable sign of toxicity
	1	5000	0/1	No observable sign of toxicity

N.B: $LD_{50}=\sqrt{(minimum\ tolerated\ dose)(max.lethal\ dose)}$
Route of Administration: Oral
EC (n-hex): $LD_{50}=\sqrt{(1250)(1500)}$ = 1369mg/kgbw

4. Conclusion

Based on the result of this research study, it can therefore be concluded that *Eucalyptus citriodora* possess phytochemical constituents which can be used to formulate or develop drugs for the treatment of infections caused by the test organisms. Also, the activity exhibited by the crude extract reveals that the individual compounds might be acting synergistically in the crude to give the desirable activity than the fractions. Furthermore, the safe dose which is 1369mg/kgbw, is within the acceptable limits

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