POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 37(3): 311–344, Y. 2022

DOI: 10.31648/pjns.8143

THERAPEUTIC POTENCY OF LEMON-SCENTED GUM (*EUCALYPTUS CITRIODORA*) LEAF EXTRACT FOR THE DEVELOPMENT OF ANTIBACTERIAL DRUG

***Joel Uyi Ewansiha*[1], *Adebare Johnson Adeleke*[2], *Lilian Sopuruchi Orji*[3], *Ibiwumi Damaris Kolawole*[4]**

[1] ORCID: 0000-0003-0331-8885
[2] ORCID: 0000-0001-7586-5410
[3] ORCID: 0009-0000-5533-5578
[4] ORCID: 0000-0002-2025-7319
[1,2] Department of Microbiology
Modibbo Adama University, Yola, Adamawa State, Nigeria
[3] Bio-resources Development Centre
National Biotechnology Development Agency, Lugbe Abuja, Nigeria
[4] Department of Food and Experimental Nutrition
University of São Paulo, Brazil

Key words: *Eucalyptus citriodora*, ethyl acetate and aqueous fractions, acute toxicity, antibacterial, alkane, compounds.

Abstract

Objectives: The antibacterial activity, phytochemical components and safe dose of the leaf part of *Eucalyptus citriodora* was determined using standard methods.

Results: Ten phytochemicals (flavonoid, phenols, alkaloids, tannins, steroids, cardiac glycosides, saponins, terpenes, anthraquinones and resins) were present in other solvent extract but tannins and resinswere absent in the aqueous extract. All test organisms were susceptible to ethyl acetate crude extract with mean inhibition zone diameter (IZD) range from 14.00±0.00 to 21.66±0.88 mm but were not susceptible to the aqueous extract; minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values from 7.5 to 60 mg/ml respectively were obtained. Eight fractions were obtained from column chromatography of which 5 were active; however, 3 shows significant (p<0.05) activity with IZD of 26.00±0.00 mm, 23.6±0.33 mm and 20.33±0.33 mm respectively while their corresponding MIC/MBC were 3.75, 1.88 and 3.25 mg/ml/60, 30 and 30 mg/ml. In the same vein, 28, 18 and 17 compounds were detected by gas chromatography in the 3 significantly active fractions. Generally, the alkane group of compounds was the most detected, but for emphasis, Tetracosane (14.03% of ECE1), Octadecanal (59.54% of ECE4) and Decane (13.70% of ECE5) were the most abundant in the three active fraction. The LD_{50} for the extract was 707.10 mg/kg bw while mild tissue necrosis and degenerative glomeruli were observed in the liver and kidney pathology of experimental rats administered with 1000 mg/ml of extract (Fig. 1).

Address: Joel Uyi Ewansiha, Modibbo Adama University, Yola, Adamawa State, Nigeria, e- mail: ewansihajoel@mautech.edu.ng

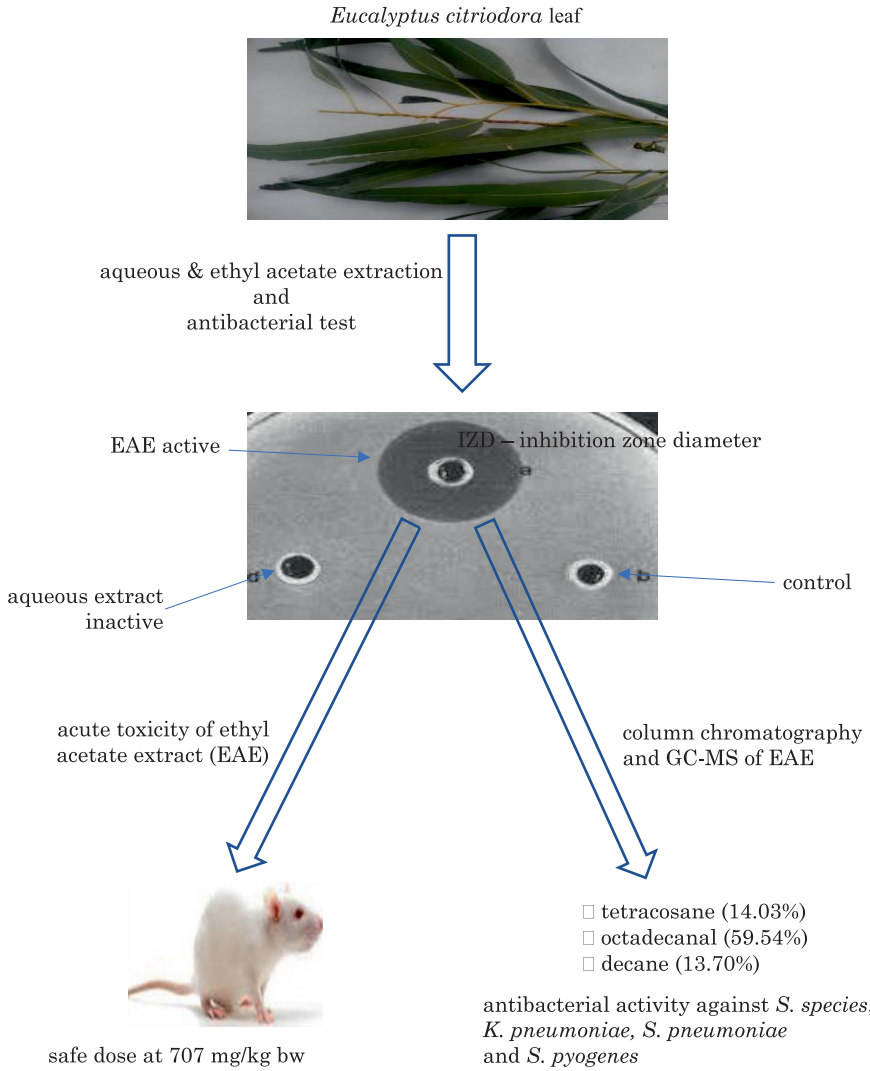


Fig. 1. Graphic abstract

Conclusion: Therefore, *Eucalyptus citriodora* leaf extract indicated antibacterial potency and therefore considered for drug development.

Introduction

Modern medicine in the 20th century was characterized by the introduction in the United Kingdom of the National Health Service (Hajir et al. 2016). Advances were also notable in the treatment of mental illness

through both psychotherapy and the administration of drugs (HAJIR et al. 2016) and plant-based medicines have been reported to have important therapeutic potency to cure human diseases (SARDAR et al. 2012). Plants have been seen as sources of medicine for thousands of years, since the creation of man. In Nigeria, almost all plants are medicinal and the application of medicinal plants especially in traditional medical practice has been well acknowledged and established as a viable profession (SARSWATI et al. 2013). Herbal medicines otherwise called herbal drugs are generally of natural plant parts such as stem, leaves, roots, flowers, stem bark, seeds, bulb (INNALEGWU et al. 2016). 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 values. Some of these metabolites include alkaloids, annins, phenols, steroids, saponins, flavonoids, anthraquinones, cardiac glycosides, terpenes, essential oils and resins (INNALEGWU et al. 2016). *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 (EWANSIHA et al. 2017). Plant oils from some *Eucalyptus* species (e.g *Eucalyptus pulverulenta*) comprise up to 90% cineol (EWANSIHA et al. 2020)

Materials and Methods

Harvesting, identification and processing of plant material

Fresh leaves of Lemon scented gum (*Eucalyptus citriodora*) – Figure 2 – were collected from house-hold gardens in Bosso Local Government, Minna, Niger State in the month of August, 2015. The plant materials were identified by local herbal practitioners in Minna, Niger State (with longitude 6.546316 and latitude 9.583555) while authentication of the plant samples was done by Dr. Ugbabe Grace and Mr. John Atogwe in the Herbarium Department of the National Institute of Pharmaceutical Research, and Development, Idu, Abuja, Nigeria where voucher specimens were deposited with voucher number: NIPRD/H/6787. The plant materials

(leaves) were dried at room temperature, until a constant weight was obtained. The plant samples were pulverized with a milling machine (Lab world Navbhart, with serial No. R66902 by Motor MFG. CO. Mumbai – India), and sieved with a 150 µm pore size filter to obtain a fine powder-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 (ACUNA 2015). The pulverized plant samples were then stored in amber bottles and kept in a cool and dry environment at room temperature (27ºC) until required for further use.

Fig. 2. Plate I – lemon scented gum *(Eucalyptus citriodora)*

Methods

Confirmation of test organisms identity

The test organisms, *Salmonella typhi, Salmonella paratyphi* A, B & C, *Klebsiella pneumoniae, Streptococcus pneumoniae* and *Streptococcus pyogenes* (the choice of these organisms was informed by the claims of local marketer/consumers of the use of extracts from the experiment plants to treat infections caused by the test organisms) obtained from stock cultures in the Microbiology laboratory Federal University of Technology, Minna. The bacteria were reconstituted by sub-culturing onto freshly prepared nutrient agar and then incubated at 37ºC for 24 hours, after which their identities were confirmed using gram staining and molecular identification.

Gram staining

An overnight culture of each test organisms was Gram stained according to the method described by (EWANSIHA et al. 2021, PRADHAN and TAMANG 2019). Gram-positive and Gram-negative organisms were recorded. A control smear of known Gram-positive organism (*Staphylococcus aureus*) and a known Gram-negative organism (*Escherichia coli*) were stained simultaneously for quality control.

Molecular identification of test organisms

The molecular confirmation of the identity of the test organisms was carried out according to the method stated in promega Technical Manual #TM050.

Treatment of Gram-positive cells with EDTA

One milliliter (1 ml) of an overnight culture of Gram-positive isolate was centrifuged for 2 minutes at 15000 x g and the supernatant was discarded. The pallet cells were suspended in 480 µl of 50 mM of ethylene diamine tetra acetic acid (EDTA), to aid the lysing process and 120 µl of lytic enzyme (lysozyme) was added. The mixture was incubated for 30 to 60 minutes at 37^{o}C. On completion of incubation, the mixture was centrifuged for 2 minutes at 15000 x g and the supernatant was decanted.

Lysing of gram-positive and gram-negative organisms

Six hundred microliter (600 µl) of nuclei lysis solution was added to the pallet cells separately and mixed gently by pipetting in and out of the tube. The mixture was incubated for 5 minutes at 80^{o}C and then allowed to cool to room temperature. Three microliters (3 µl) of RNase solution were added, mixed gently and incubated at 37^{o}C for 15 to 16 minutes after which they were allowed stand and attains room temperature at 27^{o}C.

Protein precipitation

Two hundred milliliters of protein precipitation solution was added to the mixture above and vortexed for 5 seconds after which it was incubated for 5 minutes. The mixture was centrifuged at 15000 g for 3 minutes. The supernatant was kept for DNA precipitation and rehydration.

DNA precipitation and rehydration

The supernatant from 3.3.2.3 was transferred into a clean tube containing 600 µl of isopropanol and gently mixed. The mixture was centrifuged for 2 minutes at 15000 g and the supernatant was decanted. Six hundred microliters (600 µl) of 70% ethanol was added and it was mixed and centrifuged for another 2 minutes at 15000 g. The ethanol was aspirated and the DNA pallet was rehydrated in 100 µl rehydration solution for 1 hour at 65ºC.

Polymerase chain reaction (PCR) reaction cocktail

Ten microliters (10 µl) of 5x Go Taq reagent, 3 µl of $MgCl_2$, 1 µl of 10 pmol each 27F 5'- AGA GTT TGA TCM TGG CTC AG-3' and - 1525R, 3′-AAGGAGGTGATCCAGCC-5′ primers and 0.3 units of Taq DNA polymerase (Promega, USA) was made up to 42 µl with sterile distilled water and 8 µl DNA template. PCR was carried out in a Gene Amp 9700 PCR System Thermal cycler (Applied Biosystem Inc., USA) PCR profile, an initial denaturation of 30 cycles at 94°C for 5 min; 50°C for 60 s and 72°C for 1-minute 30 s and a final extension at 72°C for 10 mins. It was then allowed to cool down to 4ºC.

Integrity check on agarose gel

The integrity of the amplified gene fragment was checked on a 1% Agarose gel to confirm amplification. This was carried out by mixing 8 µl of amplified product to 4µl of loading dye and ran on the solidified Agarose gel at 110 V for about 1 hour. Also, the amplified product was checked on a Nano drop of model 2000 from thermo scientific to quantify the concentration of the amplified product.

Purification of amplified product

After gel integrity, the amplified fragments were ethanol purified in order to remove the PCR reagents. Briefly, 7.6 µl of sodium acetate 3 M and 240 µl of 95% ethanol were added to each fragments of the PCR amplified products in a new sterile Eppendorf tube, it was vortexed for 5 seconds and kept at -20°C for 30 min. The mixture was centrifuged for 10 min at 13000 g and 4°C followed by removal of the supernatant (by inverting the tube on trash once) after which the pellets were washed by adding 150 µl of 70% ethanol, mixed and then centrifuged for 15 min at 7500 x g and 4°C. Again, the supernatant was decanted and the tube was inverted on blotting paper

and was allowed to dry in the fume hood at room temperature for 10–15 min. It was then suspended with 20 µl of sterile distilled water and kept in a refrigerator at – 20ºC prior to sequencing. The purified fragment was checked on a 1.5% Agarose gel, ran on a voltage of 110 V for about 1hr asdescribed above, to confirm the presence of the purified product before sequencing.

Sequencing and aligning

The amplified fragments were sequenced using a Genetic Analyser 3130 x l sequencer from Applied Biosystems using manufacturers' manual while the sequencing kit used was that of Big Dye terminator v 3.1 cycle sequencing kit. Bio-Edit software and MEGA 6 were used for all genetic analysis. Identities and accession numbers of the test organisms were determined by BLAST from the GENE bank through the NCBI web site.

Extraction

Crude extraction by reflux and decoction

The pulverized plant samples were subjected to successive reflux extraction method according to (ABOSHORA et al. 2014) to obtain the crude leaf extract using ethyl acetate as extraction solvent and water. This was achieved by transferring 100 g of the plant samples into a round-bottom flask of 1000 ml capacity using a Soxhlet apparatus. Ethyl acetate (polarity index = 4.1 P'), was gradually added until a ratio of 1:3 of the pulverized plant sample to ethyl acetate was attained. The power source was then switched on and the temperature was adjusted to 35ºC. The mixture was allowed to reflux for 2 hours in a round bottom flask with a reflux condenser attached at the top end, after which it was then filtered through a Whatman filter paper (pore size of 20 µm). The solvent (ethyl acetate) in the filtrate (containing the solvent and plant extract) was then evaporated using a rotary evaporator while the unevaporated plant extract was collected and transferred into universal containers and stored in the refrigerator at 4ºC (39.2ºF) for further use. The residue (merc obtained after filteration) was dried at room temperature (26ºC) and 100 g was transferred into the round-bottom flask containing water until a mixture of the merc and solvent attained a ratio of 1:4 (i.e., 100 g to 400 ml). The aqueous extract was obtained by decoction method considering the fact that water does not reflux. A total of 500 g each of the plant material/merc was com-

pletely extracted. The percentage yield of the crude extracts was determined using the equation below:

Percentage Yield [g] = Weight of Extract or Oil [g]/Weight of Dry Plant Material [g] ·100.

Phytochemical screening

Phytochemical analysis was performed using the method as described by HAJIR et al. (2016) to screen the extracts (hexane, ethyl acetate, methanol and aqueous leaf extracts of *Eucalyptus citriodora*) for the presence of the following active principles: alkaloids, tannins, saponins, flavonoids, anthraquinones, cardiac glycosides, volatile oils, terpenoids, resins, steroids and phenol.

Test for alkaloids

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

Test for tannins

One ml of each extracts was added (separately) to one ml of 3% $FeCl_3$. A greenish black precipitate is indicative of the presence of tannins.

Test for flavonoids

One ml of each extract was added (separately) to one ml of 10% KOH. It was gently shaken. Appearance of yellow colour is indicative of the presence of flavonoids.

Test for cardiac glycosides

Bontrager's test – To show the presence of free Anthraquinone, 0.5 g of the pulverized leaf extract was taken in dry test tubes. Ten milliliters of chloroform were added and the mixtures shaken for 5 minutes. The extracts were next filtered and an equal volume of ammonia was added to the filtrate and thoroughly shaken. A bright pink color in the upper aqueous layer indicates the presence of free anthraquinones.

Test for saponins

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

Test for terpenes and sterols

Five grams of the extracts was extracted by maceration with 95% ethanol and the extracts was filtered and the filtrates evaporated to dryness. The residues were dissolved in 10 ml of anhydrous chloroform and filtered. The filtrate was then divided into two equal portions and the following tests were carried out on it. i. Liebermann-Burchard test for the presence of terpenes. One portion of the chloroform solution from above was mixed with 1 ml of acetic anhydride, which was followed by the addition of concentrated sulphuric acid down the wall of the test-tube to form a lower layer. The formation of a reddish violet colour in the chloroform layer indicated the presence of terpenes. ii. Salkowski's Test – To show the presence of sterols. The other portion of the solution was mixed with 2 ml of concentrated sulphuric acid carefully so that the acid forms a layer. A reddish-brown colour at the interface indicated the presence of a steroidal ring.

Test for resins

Solutions of 5 ml petroleum ether was made using 0.1 g 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 volatility at room temperature. The plant material was heated 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 anthraquinone

One milliliter 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 colour in the ammoniacal layer (lower layer) indicated the presence of free anthraquinone.

Test for phenol

Two ml of extract was added to one ml of distilled water and warmed at 45–50ºC. Then 2 ml of 3% $FeCl_3$ was added. Appearance of green or blue color indicated the presence of phenols.

Antibacterial susceptibility assays of crude extracts

Standardization of inoculum

Two hundred microliter (200 µl) of overnight cultures of each test organism was transferred into 6 ml of sterile distilled water and it was thoroughly mixed. 100 µl (equivalent to 10^8 CFU/ml McFarland standardize) of the mixture was inoculated into freshly prepared Muller-Hinton agar, used for the antibacterial susceptibility assays.

Preparation of extracts concentration

One hundred milligram (150 mg) and 200 mg of the ethyl acetate and aqueous crude extract were weighed and dissolved in 5 ml each of 10% dimethyl sulfoxide (DMSO) (10 ml DMSO was made up to 100 ml with distilled water) to give 30mg/ml and 40 mg/ml concentrations respectively.

Determination of antibacterial activity of crude extracts

The antibacterial activity of the extracts was investigated using the agar-well diffusion method as described by (Clinical And Laboratory Standard Institute 2012) and as modified by (EWANSIHA et al. 2017). Muller-Hinton agar was prepared according to manufacturer instructions and inoculated with the standardized test organisms by the spread plate method using a sterile rod spreader to obtain homogenous microbial growth. Wells were made in the inoculated media using sterile cork-borer (6 mm diameter) after which molten medium was used to seal the base of the wells to prevent unwanted spread of the extracts. One hundred microliter (100 µl) each of the prepared extract was transferred into the wells with a sterile micropipette tip and it was well labelled, while 100 µl of 10% DMSO (free of extract) was transferred into wells to serve as the negative control. Ciprofloxacin (1 mg/ml) (a broad-spectrum antibiotic) was used as positive controls. This was done by transferring 100 µl of the prepared standard antibiotics into the well and the cultures were allowed to stand for 30 min after which it was incubated at 37°C for 24 hours. The experiment was carried out in triplicates and the mean values with the corresponding

standard deviation of the inhibition zone diameters (IZD) in millimeter were calculated.

Determination of the minimum inhibitory and minimum bactericidal concentration (MIC and MBC) of active crude extracts

Serial dilution of crude extracts

The tube dilution method as described by Clinical and Laboratory Standard Institute (CLSI 2012)with slight modification using spectrophotometer was used to determine the minimum inhibitory concentration. Two-fold serial dilutions of the crude plant extracts was prepared to give decreasing concentrations of 120, 60, 30, 15, 7.5, 3.75, 1.875 and 0.938 mg/ml. This was achieved by dissolving 480mg of the crude extract in 2 ml of 10% dimethyl sulfoxide and transferring the prepared 2 ml stock concentration (containing 240 mg/ml) of the plant leaf crude extract into a test tube containing 2 ml of nutrient broth labelled A (to give 120 mg/ml), from test tube A, 2 ml (containing 120 mg/ml) was next transferred into the next test tube containing 2 ml of sterile nutrient broth labelled B (to give 60 mg/ml). This process continued until a concentration of 0.938 mg/ml was obtained in the last test tube.

Assay for minimum inhibitory and minimum bactericidal concentration (MIC and MBC) of crude extracts

All the prepared extract dilutions were properly shaken to obtain homogenous mixtures and they were then inoculated with 100 µl of the test organisms appropriately. Positive and negative control tubes were also maintained for each test batch of extract concentration and test organisms respectively (ACEVEDO and STRONG 2012.). For the positive control, sterile nutrient broth was inoculated with 100 µl of the test organisms without the addition of the extract while for the negative control; serial dilutions of the extracts were prepared without the inoculation of the test organisms. The test tubes were all incubated at 37^{o}C for 24 h in a shaker incubator. At the end of the incubation period, the optical density of the cultures in the test tubes were read using spectrophotometer at a wavelength of 600 nm (this wavelength was used due to the fact that absorbance of light by other molecules in the microbial cells such as flavin's and carotenoids is minimal at this position (MOHANAPRIYA et al. 2013). Spectrophotometer was used due to the fact that the absorbance is directly proportional to the number of cells in the cultures and the colour intensity of the extract will

not allow an effective visual observation (GHADIR et al. 2014) 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 (see below formulae). The concentration/test tube where significant reduction in absorbance was first observed, was recorded as the MIC:

$$T\text{-}\ C_0 = C_1 \text{ (ACEVEDO and STRONG 2012)},$$

where:
T – absorbance of test
C_0 – absorbance of negative control
C_1 – absorbance of positive control.

Therefore, a deviation from the values of the positive control (C_1) is evidence of either inhibition (antibacterial activity) or resistance by the organism; by this, the MIC was determined. 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 37ºC, after incubation, the culture concentration without visible growth was regarded as the minimum bactericidal concentration.

Fractionation of crude extracts

Thin layer chromatography of crude extracts

The analytical thin layer chromatographic technique was done to spot, separate and determine the Rf (Retention factors) values and a suitable solvent system for fractionation of the phytochemical components by column chromatography on the crude extracts. This was achieved by using the pre-coated Thin Layer Chromatography silica gel (60 F254 Aluminum sheet, Merck KGaA, Millipore Corporation Germany) as the stationary phase for the active crude extracts of *Eucalyptus citriodora*. The mobile phase used for the Hexane extract was a mixture of Hexane and Chloroform in the ratio 4:1. The solvent for the separation was put in a glass tank and the tank was closed and allowed to stand for about 10 minutes so that the atmosphere in the tank becomes saturated with solvent vapor. A line was drawn on the pre-coated aluminum plates with a pencil at 1.5 cm from the origin and also 5 cm at the top of the plate from the 1.5 cm mark to mark the distance moved by the mobile phase. A capillary tube was used to place a drop of the extracts/fraction at the centre point of the drawn

line on the plates respectively and they were appropriately labelled and allowed to dry for 5 minutes. The plates were then inserted into the tank with the origin spot towards the bottom of the tank. The spots on the plate were higher than the solvent level in the tank and the glass tank was recapped while the ascension of the solvent was observed by capillary action. The plate was then removed from the tank as soon as the solvent got to the drawn line at the finishingspot (solvent front) and dried in an oven and were next sprayed with Vanillin in sulphuric acid (conc.), so as to locate the separated spots. The distance moved by the solvent and distance moved byeach spot were then measured in millimeter using a meter rule (TOJOLA et al. 2019). The solvent system that gave the best separation based on the Rf values were used to fractionate the crude extracts by column chromatography, while the number of spots seen was recorded. The Rf values were determined by dividing the distance moved by the substance by the distance moved by the solvent:

$$Rf = \text{Distance moved by substance [cm]/Distance moved by solvent [cm]},$$

where:
Rf – the retention factor.

Column chromatography (partial purification) of crude extract

The micro scale column chromatographic method described by (EWANSIHA 2020) was used to separate the fractions of the active crude extracts (ethyl acetate) of *Eucalyptus citriodora* that showed activity against the test organisms. The column (40 mm diameter width and 150 mm length) was prepared by packing; this was achieved by transferring prepared slurry of silica gel (150 g of 0.015–0.04 mm mesh size, dissolved in 500 ml n-hexane) into the column using the wet method. The column/silica gel was allowed to pack for about 1 hour and the excess n-hexane was drained off to almost dryness. A filter paper of 40 mm diameter was next inserted into the column at the top of the packed gel to further filter the extract into the column, this is to prevent blockage of the column. The extract was prepared by dissolving 3 g in 10 ml of chloroform (a moderately polar solvent) with the addition of 10 g of dried silica gel to aid adsorption and drying of the extract. The column was next loaded with the dried sample by the wet method after which a 40 mm diameter filter paper was placed on the extract. The extract was fractionated by the addition of eluting solvents, which include n-hexane, chloroform, ethyl acetate, methanol and water (as the mobile phases); beginning with the nonpolar solvent to the polar solvent systems until all the fractions were collected and they were bulked together according to their retention factor (Rf) values by TLC chromatography.

The fractions were collected in test tubes according to their color development and the eluting solvents were allowed to vaporize until a constant weight was obtained.

Antibacterial susceptibility test of fractions

The agar-well diffusion and the tube dilution methods as enumerated in the above section was used to determine the antibacterial activity, MIC and MBC of the active fractions obtained from the chromatographic procedure. The fractions obtained were designated as Eucalyptus citriodora and were numbered according to their number of elution (i.e., ECEn); where "n" is the number of fractions obtained.

Quantitative analysis and identification of compounds

The determination of the identity of active components in the fractions that shows considerable activity (ECO2 and ECO4) were done by gas chromatograph interface to a mass spectrometer (GC-MS) analysis using GC-MSQP 2010 Plus, Shimadzu system (SHIMADZU, JAPAN). The gas chromatograph interface to a mass spectrometer (GC-M5S) instrument was used while the Column elite-1 was fused with silica capillary column (30 m x 0.25 mm 1D x µldf, composed of 100% dimethyl polysiloxane). An electronic ionization system with ionization energy of 60 eV 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 2 µl (0.002 ml with split ratio of 1:40 and film thickness of 0.20 µm). The GC oven temperature was set at 70°C for 3.00 min and then programmed to rise from 70 to 250°C at a rate of 3°C min-1 and held isothermally for 3.00 min 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 70 eV; a scan interval of 0.5 s and fragments from 40 to 550 Da. Total GC running time was 28.00 minutes. Relative percentages and amount of each component 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).

Toxicity studies on the crude extract

Preparation and acclimatization of test animals

The rats used for the investigation were obtained from the animal house of the Department of Biological Sciences, at the Niger State Polytechnic, Zungeru, Nigeria. The rats were kept under observation for about 7 days before the onset of experiment to exclude any infection and for acclimatization. The temperature of the experimental animal room was maintained at 22ºC (+3ºC). The relative humidity was set at 30% and not allowed to exceed 70% with a fluctuating range of 50–60% during room cleaning. The lighting was made to be near artificial, with a sequence of 12 hours light and 12 hours darkness. The animals were fed with conventional laboratory diets with an unlimited supply of drinking water. All the experimental animals were group-caged by dose, and the number of animals per cage and not allowed to interfere with clear observations of each animal.

Acute oral toxicity studies

The acute toxicity studies were conducted to determine the safe dose of the extract. The studies were carried out using the Lorke's method and modified as per internationally accepted protocol drawn under OECD No 420 guidelines (ARAGE et al. 2022). This was achieved using 22 white female albino rats (120±20 g body weight) while the extracts were administered in two phases (phase I and phase II). In phase I, the overnight fasted rats (i.e. the rats were deprived of food for 12 hours) were divided into 4 groups, consisting of three test groups and one control group. The test animals were administered ethyl acetate extracts of the leaves of *Eucalyptus citriodora* in various doses (10, 100, 500 and 1000 mg/kg bw) while the control group was administered 1 ml each of normal saline by oral gavage route. After administration of the extract, the animals were observed for toxicity signs continuously for the first 30 minutes, then periodically for the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days in the case of delayed toxicities to detect any changes in the behavioral responses and also for tremors, convulsion, salivation, diarrhoea, lethargy, sleep and coma and monitored for mortality. The median lethal dose (LD_{50}) otherwise known as the safe dose were calculated as the geometric mean of the dose that causes 0% and 100% mortality according to Lorke's formula:

$$LD_{50} = \sqrt{a \cdot b},$$

where:

a – is the highest dose at which no death occurred

b – the least dosage at which death occurred.

Result and Discussion

Percentage yield of plant extracts

The extraction results as presented in Table 1 explains the details of the total and corresponding percentage yield respectively per five hundred grams of the dried plant leaves and or merc of *Eucalyptus citriodora*. The highest yield of 27.07 g (5.41%) was obtained with ethyl acetate while 20.09 g (4.02%) was obtained with water. The most widely-used solvent to obtain crude extract from plant sources are polar solvents which have fairly moderate to higher boiling point range ofapproximately 63–189°C and are excellent solvents in terms of solubility and ease of recovery as reported by GETANEH and GIRMA (2014) and this in part could explain the reason for a higher yield with ethyl acetate. Previous researches have shown that ethyl acetate is a good extraction solvent for medicinal plant-based drugs. WAZA (2015) reported that water also possess broad spectrum and relatively non-selective property for extraction and a high percentage of plant extracts from water have been observed to possess antibacterial activity; but in this case the result in terms of yield compared to ethyl acetate shows the opposite. The major factors that may be considered in the choice of suitable solvent for plant extraction, is the polarity of the solvent as reported by AMMAR et al. (2017) that like attracts like i.e. a polar solvent will extract polar constituents and vice vasa. Therefore, the fact that the extraction process in this research work was continuous i.e. the extraction was carried out in increasing order of polarity and more yields were obtained with the ethyl acetate solvents. This could mean that the moderately polar constituents might be more than the highly polar constituents in the plants extracts.

Table 1

Percentage yield of plant leaf extracts

Plant parts	Percentage yield		
	WPP/WtM [g]	ethyl acetate extract g [%]	AqEg [%]
Eucalyptus citriodora leaf	500	27.07 (5.41)	20.09 (4.02)

Abbreviations: WPP – weight of pulverized plant part; AqE – aqueous extracts; WtM – weight of merc

Qualitative phytochemical properties of plant crude extracts

The qualitative phytochemical analysis revealed the presence of 10 phytochemical constituents present in the ethyl acetate extract, while out of the 10,3 (tannins, volatile oil and resins) were absent in the aqueous extract (Table 2), while only volatile oil was absent in the ethyl acetate

extract. The phytochemical constituents present include alkaloids, flavonoids, tannins, phenols, anthraquinones, terpenes, resins, steroids, cardiac glycosides and saponins. Phytochemical constituents are the bases for the therapeutic potency of medicinal plants as reported by EWANSIHA et al. (2012), however, the types and the quantity of phytochemical constituents also determine the level, presence or absence of activity. The absence of some key constituents such as tannins, volatile oil and resins could give the ethyl acetate extract an edge over the aqueous extract. Secondly, this may also be attributed to the difference in the method of extraction (reflux and decoction) used; in the report of EWANSIHA et al. (2012), cold maceration extraction methodwas used which is void of heat and the solvent application was not successive unlike the extraction method used in this research studies. These differences confirm the fact that individual/separate use of solvents and the application of heat during extraction of medicinal plant might have some advantages over successive application of extraction solvent and cold maceration respectively (AMMAR et al. 2017) also reported on the effect of temperature on phytochemical components, that some of these components are thermo-stable in nature and they can be extracted with the application of heat without being destroyed.

Table 2

Phytochemical constituents of *Eucalyptus citriodora* extracts

Plant extracts	Phytochemical constituents										
	flavonoid	phenols	alkaloids	tannins	steroids	cardiac glycosides	saponins	terpenes	volatile oil	anthraquinones	resins
Ethyl acetate	+	+	+	+	+	+	+	+	-	+	+
Aqueous	+	+	+	-	+	+	+	+	-	+	-

Abbreviations: "+" = present, "-" = absent

Antibacterial activity of *Eucalyptus citriodora* crude plant extracts

The results of the antibacterial activity of *Eucalyptus citriodora* crude extracts are presented in Table 3. The most active extract was the ethyl acetate extracts which shows considerable activity at a concentration of 30 mg/ml compared to aqueous extracts (40 mg/ml). Result in Table 3 showed that ethyl acetate extract was most active with mean diameter zone of inhibition (DZI) of 21.66±0.88 mm (p<0.05) against *Salmonella paratyphi B*, followed by 20.66±1.20 mm DZI against *K. pneumoniae* while the least DZI of 14.00±0.00 mm (p<0.05) was obtained against *S. typhi*.

The aqueous extract showed no activity against all the test organisms while the positive control is significantly (p<0.05) higher than the extract. The results of the antibacterial activity revealed that all the crude extracts have antibacterial activity against the test organisms and this could be attributed to the presence of the phytochemical constituents' present. ALTEMIMI et al. (2015) reported that the presence of phytochemical constituents in medicinal plants is the reason for their therapeutic potency. Also, the absence of some vital constituents (tannins and resins) could also be responsible for the ethyl acetate extract to be more active than the aqueous extracts. The antibacterial activity of the plant extracts which is attributed to the abundant presence of phytochemicals constituents, authenticate the use of the leaf and the fruit parts of the plants under study by local herbal practitioners and consumers either singly or in combination for the treatment of typhoid fever and respiratory tract infections as reported by OKOKON et al. (2018), MAMMEN et al. (2012) and GRIFFITH and GINTER (2017) reported on a study implicating some of the phytochemical constituents as possessing inhibitory activity against organisms that causes plant diseases e.g. *Fusarium oxysporum*. The presence of particular secondary metabolite in medicinal plants may not necessarily guarantee its antimicrobial potency owing to the fact that there are different types of secondary metabolites and the presence of the active type may not be certain. A good example is the existence of different types of tannins such as the hydrolysable and the non-hydrolysable tannins in plant cells as reported by ODELEY and OBAMESO (2022).

Table 3

Mean diameter zones of inhibition of *Eucalyptus citriodora* crude leaf extract[mm]

Organisms	Plant extracts		Control	
	EtOAc (30 mg/ml)	AqE (40 mg/ml)	Cpx* (1 mg/ml)	DMSO (100 µl)
S. paratyphi A	18.00 ± 0.57^{b}	0.00^{a}	23.50 ± 1.50^{c}	0.00^{a}
S. paratyphi B	21.66 ± 0.88^{b}	0.00^{a}	26.66 ± 0.88^{c}	0.00^{a}
S. paratyphi C	14.00 ± 0.57^{b}	0.00^{a}	24.66 ± 1.45^{c}	0.00^{a}
S. typhi	14.00 ± 0.00^{b}	0.00^{a}	25.33 ± 0.33^{c}	0.00^{a}
K. pneumoniae	20.66 ± 1.20^{b}	0.00^{a}	25.33 ± 0.33^{c}	0.00^{a}
S. pneumoniae	19.33 ± 0.66^{b}	0.00^{a}	25.33 ± 0.88^{c}	0.00^{a}
S. pyogenes	20.33 ± 0.66^{b}	0.00^{a}	30.00 ± 1.00^{cd}	0.00^{a}

Abbreviations: EtOAc – ethyl acetate extract; AqE – aqueous extract; Cpx – ciprofloxacin; values on the same row with different superscript are significantly (p<0.05) different, n = 3

Minimum inhibitory concentrations and minimum bactericidal concentrations of *Eucalyptus citriodora* leaf crude ethyl acetate extract

The results of the MIC and MBC of the crude ethyl acetate extract against the test organisms at different concentrations of the ethyl acetate crude plant extract is presented in Table 4. Tubes with low optical density corresponding to 7.5 mg/ml was recorded as the lowest MIC value of the crude extract against *Salmonella paratyphi* A*, Salmonella paratyphi* B and *Streptococcus pyogenes* while 15 mg/ml was recorded as the highest MIC value of the crude extract against *Salmonella paratyphi* C, *Salmonella typhi, Klebsiella pneumoniae* and *Streptococcus pneumoniae*. The MBC values was 60 mg/ml against *Salmonella paratyphi* B and *Streptococcus pyogenes* and 120 mg/ml against *Salmonella paratyphi* A, *Salmonella paratyphi* C, *Salmonella Typhi, Klebsiella pneumoniae* and *Streptococcus pneumoniae*. MIC of drugs guides the choice of antimicrobial to be used against a given infection or in therapy by predicting efficacy (Rehman et al. 2022).

Table 4

Minimum inhibitory concentrations and minimum bactericidal concentrations (MIC and MBC) of *Eucalyptus citriodora* ethyl acetate crude extract

Organism	Concentrations (mg/ml)/(OD600 nm)									EA extract	
										MIC [mg/ml]	MBC [mg/ml]
	+ve C	120	60	30	15	7.5	3.25	1.625	0.938	NA	NA
	-ve C	0.368	0.188	0.095	0.048	0.026	0.014	0.005	0.003		
S. paratyphi A	0.590	0.516	0.538	0.550	0.571	**0.584**	0.592	0.591	0.593	7.5	120
S. paratyphi B	0.587	0.497	0.521	0.555	0.571	**0.582**	0.589	0.589	0.588	7.5	60
S. paratyphi C	0.563	0.479	0.508	0.539	**0.556**	0.562	0.563	0.563	0.562	15	120
S. typhi	0.535	0.451	0.480	0.505	**0.528**	0.535	0.536	0.538	0.537	15	120
K. pneumoniae	0.527	0.443	0.443	0.509	**0.520**	0.526	0.528	0.528	0.529	15	120
S. pneumoniae	0.690	0.623	0.652	0.670	**0.681**	0.689	0.690	0.690	0.690	15	120
S. pyogenes	0.669	0.582	0.614	0.642	0.663	**0.661**	0.669	0.669	0.669	7.5	60

Abbreviations: OD – optical density; NA – not applicable; +ve C – positive control; -ve C – negative control; EA – ethyl acetate

Retention factor of *Eucalyptus citriodora* leaf ethyl acetate crude extract revealed by thin layer chromatography (TLC)

The result of thin layer chromatography on the ethyl acetate crude extract reveals the possible number of fractions contained in the extract which may be deduced from the number of spot and retention factor.

In Table 5, the highest number of spot (that is the possible number of fraction) seen with absolute chloroform was 8 while the lowest number (1) was recorded with ethyl acetate: methanol and the highest retention factor recorded was 0.98 cm while the lowest retention factor recoded was 0.8 cm. The result also reveals an increased number of spots towards the middle of the table i.e., where the moderately polar solvents such as chloroform and ethyl acetate were used while fewer spots were obtained with the highly polar solvent. FAIR and KORMAS (2008) reported that substances that produces retention factors between 0.3 and 0.7 cm need not adjusted in terms of solvent system.

Table 5
Solvent system, number of spots and retention factors of *Eucalyptus citriodora* leaf ethyl acetate crude extract

Solvent system (2 ml)	NS	DMS [cm]	DMF [cm]	Rf [cm]
100% n-hexane	2	5.0	0.6, 0.9	0.12–0.18
9:1 hexane: chloroform	3	5.0	0.6, 2.1, 3.1	0.12–0.62
4:1 hexane: chloroform	4	5.0	0.8, 2.1, 2.8, 3.5	0.16–0.70
1:1 hexane: chloroform	5	5.0	0.6, 1.8, 3.4, 4.0, 4.6	0.12–0.92
1:4 hexane: chloroform	7	5.0	0.7, 1.0, 1.2, 1.4, 2.3, 3.0, 4.6	0.14–0.92
1:9 hexane: chloroform	7	5.0	0.8, 1.1, 1.7, 2.4, 2.7, 3.1, 4.7	0.16–0.94
100% chloroform	8	5.0	0.7,1.0, 1.5, 1.8, 3.1, 4.3, 4.6, 4.9	0.14–0.98
9:1 chloroform: ethyl acetate	4	5.0	0.8, 3.1, 4.2, 4.9	0.16–0.98
4:1 chloroform: ethyl acetate	4	5.0	1.0, 3.6, 4.1, 4.9	0.20–0.98
1:1 chloroform: ethyl acetate	6	5.0	0.8, 1.2, 1.7, 1.9, 2.3, 4.6	0.16–0.92
1:4 chloroform: ethyl acetate	5	5.0	0.7, 1.9, 2.8, 3.3, 4.6	0.14–0.92
1:9 chloroform: ethyl acetate	4	5.0	2.5, 3.0, 3.7, 4.2	0.25–0.84
100% ethyl acetate	3	5.0	2.8, 3.5, 4.2	0.56–0.84
9:1 ethyl acetate: methanol	3	5.0	3.0, 3.8, 4.6	0.6–0.92
4:1 ethyl acetate: methanol	1	5.0	4.6	0.92
1:1 ethyl acetate: methanol	1	5.0	4.9	0.98
1:4 ethyl acetate: methanol	NSp	5.0	NSp	NSp
1:9 ethyl acetate: methanol	NSp	5.0	NSp	NSp
100% methanol	NSp	5.0	NSp	NSp

Abbreviations: DMS – distance moved by the solvent (mobile phase); DMF – distance moved by fraction; Rf – retention factor; NS – number of spots; NSp – no spot seen

Column chromatogram and retention factor of *Eucalyptus citriodora* ethyl acetate fractions

A total of 9 fractions were eluted (ECE1 TO ECE9) from *Eucalyptus citriodora* ethyl acetate extract (Table 6). Fraction 9 (ECE9) had the highest yield of 11.4 (57%) followed by fraction 8 (ECE8) with 2.05 g (8.20%) while fraction 2 (ECE2) had the lowest yield of 0.39 g (1.56%). The highest retention factor was recorded for fraction ECE7 and fraction ECE8 followed by fraction ECE9 and ECE7 (spot number two) while fraction ECE3 had the lowest retention factor. Also, fraction ECE9 used up a higher volume of solvent (800 ml) for its elution followed by fractions ECE8, ECE6 and ECE5 (700 ml) while fraction ECE2 consumed less solvent (300 ml). All fractions had one spot except for fraction ECE2, ECE4, ECE5 and ECE7 which had two spots each.

Table 6

Percentage yield, solvent systems and retention factor of *Eucalyptus citriodora* ethyl acetate fractions (20 g)

Solvent systemand volume [ml]	Description	Percentage yield [%]	NS	Rf [cm]	Fraction eluted
100% n-hexane (500)	yellow and oily	0.7 (3.5)	1	0.48	ECE 1
100% n-hexane (300)	red and oily	0.39 (1.95)	1	0.44	ECE 2
100% n-hexane (400)	grey and oily	0.60 (3.0)	1	0.38	ECE 3
9:1 n-HEX: $CHCl_3$ (600)	light green	0.56 (2.80)	1	0.70	ECE 4
1:1 n-HEX: $CHCl_3$ (700)	dark green oily	0.86 (4.30)	1	0.46	ECE 5
1:9 $CHCl_3$: EtOAc (700)	dark green	0.91 (4.55)	1	0.58	ECE 6
9:1 $CHCl_3$: EtOAc (600)	light green	1.02 (5.10)	2	0.80; 0.76	ECE 7
100% EtOAc (700)	black	2.05 (10.25)	1	0.80	ECE 8
100% water (500)	off white	12.33 (61.65)	NSp	NSp	ECE 9

Abbreviations: n-HEX – N-hexane; $CHCl_3$ – chloroform; EtOAc – ethyl acetate; NSp – no spot seen; NS – number of spots; Rf – retention factor

Antimicrobial activity of *Eucalyptus citriodora* ethyl acetate leaf fractions

A total of 9 fractions were eluted by column chromatography from the ethyl acetate crude extract of *Eucalyptus citriodora*. Of the 9 fractions eluted, 5 were active against the test organisms while 4 were not active as shown in Table 7 and Table 8. The mean diameter zone of inhibition (DZI) of the active fraction's ranges from 13.33±0.33 mm (ECE5 against *Streptococcus pneumoniae*) to 26.00±0.00 mm (ECE1 against *Streptococcus pyogenes*). ECE1 shows a greater activity against the test organisms as com-

pared to the other fractions and also its activity is more comparable to the positive control drug. This was followed closely by fraction ECE4 (15.33±0.66 mm to 23.66±0.33 mm) and ECE5 (13.33±0.33 mm to 20.33±0.33 mm) while fraction ECE2 (11.33±0.33 mm to 24.33±0.33 mm) was the least in activity when compared to the positive control drugs. Streptococcus pyogenes was more susceptible to the fractions and standard drugs among all other test organisms while *S. paratyphi* C and *S. typhi* were the least susceptible. The antimicrobial activity of several *Eucalyptus* plants has been attributed to their possession of certain specific Terpenes and Phenolic compounds (BEN-ARFA et al. 2006) The majority of compounds in *Eucalyptus citriodora* ethyl acetate fractions was alkane hydrocarbons of which decane is their basic structure ($C_{10}H_2$). It is reported that decane is the basic structure of the novel antibiotic drug Aranorosin with molecular formula $C_{23}H_{33}NO_6$ (1-oxaspiro 4, 5 decane) isolated from the fungi *Pseudoarachniotus roseus* (MALIK et al. 2014). The result of this investigation revealed large qualitative and quantitative differences in the phytochemical composition of same plants grown in Algerian origins as reported by BAYNESAGNE (2017). In his report, vital phytochemical constituents with evidence of antibacterial activity were reported absent in the plant extracts. This may be as a result of environmental factors such as the, time of harvest, drying method, type of soil, plant part used, solvent used for extraction, methods of extraction and the amount of plant material used for extraction.

Table 7

Mean diameter zones of inhibition of *Eucalyptus citriodora* ethyl acetate leaf fractions [mm]

Organisms	*Eucalyptus citriodora* ethyl acetate leaf fractions				Control	
	ECE1 (40 mg/ml)	ECE2 (40 mg/ml)	ECE3 (40 mg/ml)	ECE4 (20 mg/ml)	Cpx (1 mg/ml)	DMSO (100 µL)
S. paratyphi A	23.66±0.33^{d}	8.33±0.33^{b}	9.66±0.33^{b}	15.33±0.66^{c}	23.50±1.50^{d}	0.00^{a}
S. paratyphi B	25.00±0.00^{d}	9.33±0.33^{b}	8.00±0.00^{b}	19.00±0.00^{cd}	26.66±0.88^{d}	0.00^{a}
S. paratyphi C	23.66±0.33^{d}	8.33±0.88^{b}	8.00±0.57^{b}	23.66±0.33^{d}	24.66±1.45^{d}	0.00^{a}
S. Typhi	25.66±0.33^{d}	5.66±0.33^{b}	5.33±0.33^{b}	22.00±0.00^{cd}	25.33±0.33^{d}	0.00^{a}
K. pneumonia	21.33±0.66^{cd}	8.00±0.57^{b}	7.33±0.33^{b}	19.00±0.57^{c}	25.33±0.33^{d}	0.00^{a}
S. pneumoniae	25.66±0.33^{d}	10.00±0.00^{b}	9.66±0.33^{b}	15.66±0.66^{c}	25.33±0.88^{d}	0.00^{a}
S. pyogenes	26.00±0.00^{d}	10.00±0.57^{b}	11.33±0.33^{b}	20.66±0.33^{cd}	30.00±1.00^{e}	0.00^{a}

Abbreviations: Org – organism; ECE 1–8 – *Eucalyptus citriodora* ethyl acetate fraction 1 to 8; Cpx – ciprofloxacin; DMSO – dimethyl sulfoxide. Values on the same row with different superscript are significantly different ($p<0.05$), n – 3

Table 8

Mean diameter zones of inhibition of *Eucalyptus citriodora* ethyl acetate leaf fractions [mm].
Eucalyptus citriodora ethyl acetate leaf fractions control

Organisms	*Eucalyptus citriodora* ethyl acetate leaf fractions				Control	
	ECE5 (40 mg/ml)	ECE6 (40 mg/ml)	ECE7 (40 mg/ml)	ECE8 (40 mg/ml	Cpx (1 mg/ml)	DMSO (100 µl)
S. paratyphi A	15.00 ± 0.57^{c}	0.00^{a}	0.00^{a}	0.00^{a}	23.50 ± 1.50^{d}	0.00^{a}
S. paratyphi B	18.66 ± 0.66^{cd}	0.00^{a}	0.00^{a}	0.00^{a}	26.66 ± 0.88^{d}	0.00^{a}
S. paratyphi C	18.33 ± 0.33^{c}	0.00^{a}	0.00^{a}	0.00^{a}	24.66 ± 1.45^{d}	0.00^{a}
S. Typhi	14.66 ± 0.33^{bc}	0.00^{a}	0.00^{a}	0.00^{a}	25.33 ± 0.33^{d}	0.00^{a}
K. pneumoniae	20.33 ± 0.33^{cd}	0.00^{a}	0.00^{a}	0.00^{a}	25.33 ± 0.33^{d}	0.00^{a}
S. pneumoniae	13.33 ± 0.33^{bc}	0.00^{a}	0.00^{a}	0.00^{a}	25.33 ± 0.88^{d}	0.00^{a}
S. pyogenes	16.66 ± 0.66^{c}	0.00^{a}	0.00^{a}	0.00^{a}	30.00 ± 1.00^{e}	0.00^{a}

Abbreviations: ECE1–8 – *Eucalyptus citriodora* ethyl acetate fraction 1 to 8; Cpx – ciprofloxacin; DMSO – dimethyl sulfoxide. Values on the same row with different superscript are significantly different ($p<0.05$), $n = 3$.

Minimum inhibitory and minimum bactericidal concentration (MIC and MBC) of *Eucalyptus citriodora* ethyl acetate fractions [mg/ml]

The results of the MIC and MBC for ECE1 (*Eucalyptus citriodora* leaf ethyl acetate fraction 1), ECE4 (*Eucalyptus citriodora* leaf ethyl acetate fraction 4) andECE5 (*Eucalyptus citriodora* leaf ethyl acetate fraction 5) are presented in Table 9 – Table 11. The lowest MIC recorded was 1.88 mg/ml for ECE1 and ECE4 against *Salmonella paratyphi* B and *Streptococcus pyogenes* (ECE1 and ECE4) and *Streptococcus pyogenes* (ECE4). This was followed by MIC of 3.75 mg/ml for ECE1 (against *Salmonella paratyphi* A) and ECE4 (against *Salmonella paratyphi* A and *Streptococcus pneumoniae*) while 15 mg/ml MIC was recorded for the 3 fractions (ECE1, ECE4 & ECE5) against *Salmonella paratyphi* C, *Salmonella typhi and Streptococcus pneumoniae* (ECE1), *Salmonella paratyphi* C, *Salmonella typhi, Klebsiella pneumoniae and Streptococcus pneumoniae (*ECE5), *Salmonella typhi* (ECE4). The MBC of the fractions against the test organisms ranges from 30 mg/ml to 120 mg/ml.

Table 9

Minimum inhibitory and minimum bactericidal concentration (MIC and MBC) of *Eucalyptus citriodora* ethyl acetate fractions [mg/ml]

Organism	Concentrations [(mg/ml) /(OD600 nm)]									ECE1	
										MIC [mg/ml]	MBC [mg/ml]
	+ve C	120	60	30	15	7.5	3.75	1.88	0.938	NA	NA
	-ve C	0.300	0.160	0.082	0.045	0.024	0.011	0.006	0.003	NA	NA
S. paratyphi A	0.502	0.383	0.405	0.429	0.450	0.468	**0.480**	0.498	0.497	3.75	120
S. paratyphi B	0.524	0.401	0.422	0.442	0.471	0.494	0.510	**0.522**	0.530	1.88	60
S. paratyphi C	0.516	0.442	0.458	0.488	**0.504**	0.520	0.521	0.522	0.520	15	120
S. typhi	0.551	0.448	0.485	0.504	**0.521**	0.539	0.539	0.540	0.539	15	120
K. pneumonia	0.560	0.447	0.493	0.516	0.527	**0.549**	0.561	0.561	0.560	7.5	120
S. pneumonia	0.644	0.544	0.581	0.610	**0.630**	0.644	0.645	0.645	0.644	15	120
S. pyogenes	0.628	0.489	0.518	0.552	0.588	**0.611**	0.628	0.627	0.627	7.5	60

Abbreviations: OD – optical density; NA – not applicable; +ve C – positive control; -ve C – negative control; ECE1 – *Eucalyptus citriodora* ethyl acetate fractions 1

Table 10

Minimum inhibitory and minimum bactericidal concentration (MIC and MBC) of *Eucalyptus citriodora* ethyl acetate fractions [mg/ml]

Organism	Concentrations [(mg/ml) /(OD600 nm)]									ECE4	
										MIC [mg/ml]	MBC [mg/ml]
	+ve C	120	60	30	15	7.5	3.75	1.88	0.938	NA	NA
	-ve C	0.300	0.160	0.082	0.045	0.024	0.011	0.006	0.003	NA	NA
S. paratyphi A	0.502	0.383	0.405	0.429	0.450	0.468	**0.480**	0.498	0.497	3.75	120
S. paratyphi B	0.524	0.401	0.422	0.442	0.471	0.494	0.510	**0.522**	0.530	1.88	60
S. paratyphi C	0.516	0.442	0.458	0.488	**0.504**	0.520	0.521	0.522	0.520	15	120
S. typhi	0.551	0.448	0.485	0.504	**0.521**	0.539	0.539	0.540	0.539	15	120
K. pneumonia	0.560	0.447	0.493	0.516	0.527	**0.549**	0.561	0.561	0.560	7.5	120
S. pneumonia	0.644	0.544	0.581	0.610	**0.630**	0.644	0.645	0.645	0.644	15	120
S. pyogenes	0.628	0.489	0.518	0.552	0.588	**0.611**	0.628	0.627	0.627	7.5	60

Abbreviations: OD – optical density; NA – not applicable; +ve C – positive control; -ve C – negative control; ECE4 – *Eucalyptus citriodora* ethyl acetate fractions 4

Table 11

Minimum inhibitory and minimum bactericidal concentration (MIC and MBC) of *Eucalyptus citriodora* ethyl acetate fractions [mg/ml]

Organism	Concentrations [(mg/ml) /(OD600 nm)]									MIC [mg/m])	MBC [mg/ml]
	+ve C	120	60	30	15	7.5	3.75	1.88	0.938	NA	NA
	-ve C	0.297	0.146	0.080	0.039	0.019	0.009	0.005	0.003	NA	NA
S. paratyphi A	0.580	0.470	0.508	0.530	0.559	**0.571**	0.580	0.580	0.581	7.5	60
S. paratyphi B	0.530	0.301	0.436	0.488	0.512	**0.526**	0.531	0.530	0.530	7.5	60
S. paratyphi C	0.575	0.379	0.411	0.542	**0.568**	0.577	0.578	0.577	0.577	15	120
S. typhi	0.522	0.432	0.480	0.502	**0.515**	0.523	0.523	0.523	0.523	15	120
K. pneumonia	0.504	0.390	0.428	0.460	**0.498**	0.507	0.507	0.508	0.508	15	120
S. pneumonia	0.618	0.534	0.568	0.591	**0.610**	0.620	0.620	0.620	0.620	15	120
S. pyogenes	0.630	0.411	0.538	0.567	0.593	0.611	**0.625**	0.631	0.631	3.25	30

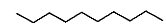
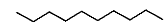
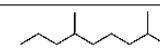
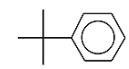
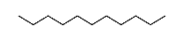
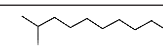
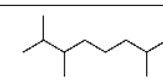
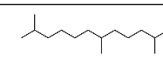
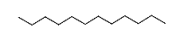
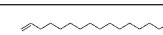
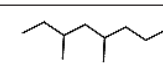
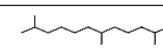
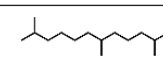
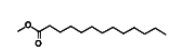
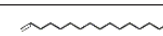
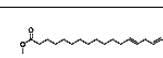
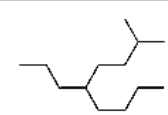
Abbreviations: OD – optical density; NA – not applicable; +ve C – positive control; -ve C – negative control; ECE5 – *Eucalyptus citriodora* ethyl acetate fractions 5

Compounds identified in *Eucalyptus citriodora* ethyl acetate fraction (ECE1)

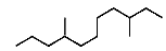
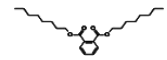
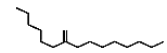
The result of the gas chromatography mass spectrometry (GC-MS) of ethyl acetate fraction (ECE1) of *Eucalyptus citriodora* is presented in Table 12. A total of 28 compounds were identified in the ethyl acetate fraction. The result shows the peak number, retention time, peak area in percentage, molecular weight, molecular formula and structure of each identified compounds. Peak number 28 corresponding to Tetracosane was the most abundant compound with peak area of 14.03% followed by peak number 2 that is Decane, with peak area of 11.49%; others were between the ranges of 1.17% to 5.06% while peak number 14 that is 1-Octanol, 2-butyl- was the least abundant with peak area of 0.6%. The most abundant compounds present in the ethyl acetate fractions ECE1 are Tetracosane (14.03%) and Decane (14.49%), some are in limited amount ranging from 1.17% to 6.59% while others are in trace amount. These group of compounds identified are reported to possess antibacterial activity against Gram negative and positive pathogens. The test organisms were more susceptible to fraction ECE1 with mean diameter zones of inhibition ranged from 15 mm to 26 mm compared to fraction ECE5 which exhibited intermediate activity with mean zones of inhibition ranging from 15 mm to 20 mm.

Table 12

Percentage composition and suspected structures of compounds identified in *Eucalyptus citriodora* ethyl acetate fraction (ECE1)

Peak No.	RT	P.A [%]	MW [g/mol]	MF	Compound name	Suspected structures
1	3.607	3.77	142.28	$C_{10}H_{22}$	decane (A)	
2	4.923	11.49	142.28	$C_{10}H_{22}$	decane (B)	
3	5.245	3.39	156.31	$C_{11}H_{24}$	nonane, 2,6-dimethyl-	
4	5.847	4.50	134.22	$C_{10}H_{14}$	benzene, tertbutyl-	
5	6.368	6.59	156.31	$C_{11}H_{24}$	undecane	
6	7.783	3.63	156.31	$C_{11}H_{24}$	decane, 2-methyl	
7	8.808	2.17	156.31	$C_{11}H_{24}$	octane, 2,3,7-trimethyl-	
8	9.251	3.01	184.36	$C_{13}H_{28}$	tridecane	
9	10.298	2.50	212.41	$C_{15}H_{32}$	2,6,11-trimethyldodecane	
10	10,607	5.38	142.28	$C_{10}H_{22}$	decane (C)	
11	11.412	3.78	170.33	$C_{12}H_{26}$	dodecane	
12	11.895	3.22	212.41	$C_{15}H_{32}$	n-Pentadecane	
13	11.117	1.27	224.43	$C_{16}H_{32}$	hexadecane	
14	13.682	0.71	142.26	$C_{10}H_{22}$	octane, 3,5-dimethyl-	
15	14.346	1.94	212.41	$C_{15}H_{32}$	2,6,11-trimethyldodecane	
16	15.619	1.60	170.33	$C_{12}H_{26}$	dodecane	
17	17.348	1.22	212.41	$C_{15}H_{32}$	2,6-dimethylheptadecane	
18	17.895	1.54	228.37	$C_{14}H_{28}O_2$	tridecanoic acid, methyl ester	
19	19.237	1.17	256.47	$C_{17}H_{36}O$	1-Heptadecanol	
20	19.344	1.69	224.43	$C_{16}H_{32}$	hexadecane	
21	20.987	4.36	294	$C_{19}H_{34}O_2$	13,16-octadecadienoic acid, methyl ester	
22	22.310	1.64	184	$C_{13}H_{28}$	nonane, 2-methyl-5-propyl-	

cont. Table 12

23	23.480	1.38	184	$C_{13}H_{28}$	undecane, 3,8-dimethyl-	
24	24.522	2.16	282.55	$C_{20}H_{42}$	eicosane	
25	25.470	4.90	282.55	$C_{20}H_{42}$	n-eicosane	
26	25.949	1.91	390.56	$C_{24}H_{38}O_4$	di-n-octyl phthalate	
27	26.354	5.06	310	$C_{22}H_{46}$	pentadecane, 8-heptyl-	
28	27.322	14.03	338.65	$C_{24}H_{50}$	tetracosane	

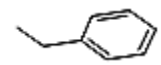
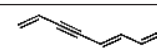
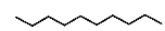
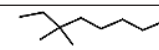
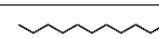
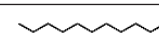
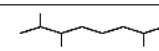
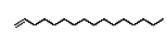
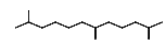
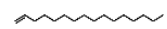
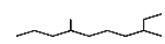
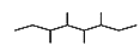
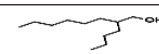
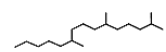
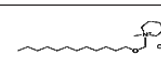
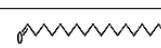
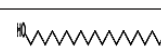
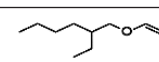
Abbreviations: RT – retention time; PA – peak area; MW – molecular weight; MF – molecular formula

Compounds identified in *Eucalyptus citriodora* ethyl acetate fraction (ECE4)

The result of the gas chromatography mass spectrometry (GC-MS) of ethyl acetate fraction (ECE4) of *Eucalyptus citriodora* is presented in Table 13. A total of 18 compounds were identified in fraction (ECE4). The result in Table 13 shows the peak number, retention time, peak area in percentage, molecular weight, molecular formula and structure of each identified compounds. Peak number 16 corresponding to Octadecanal was the most abundant compound with peak area of 59.54% followed by peak number 17 that is 1-Eicosanol, with peak area of 7.90%; others were between the ranges of 0.80% to 4.35% while peak number 13 that is 1-Octanol, 2-butyl- was the least abundant with peak area of 0.16%. The high activity of ECE1 and ECE4 could be attributed to the presence of Tetracosane, eicosane, n-eicosane, Decane and Octadecanal which are reported by BRINDA and MOHAN (2016) to be bactericidal in activity. Pentadecane, 2, 6, 10-trimethyl identified in ECE4, though in trace amount was also reported by BELAYNEH et al. (2019) to possess antibacterial activity against Gram-negative bacteria.

Table 13

Percentage composition and suspected structures of compounds identified in *Eucalyptus citriodora* ethyl acetate fraction (ECE4)

Peak No.	RT	PA [%]	MW [g/mol]	MF	Compound name	Suspected structures
1	3.351	1.44	106.17	C_8H_{10}	ethylbenzene	
2	3.616	1.86	104	C_8H_8	1,3,7-octatrien-5-yne	
3	4.934	4.35	142.28	$C_{10}H_{22}$	decane	
4	5.258	0.97	142.28	$C_{10}H_{22}$	3,3-dimethyloctane	
5	6.378	3.15	156.31	$C_{11}H_{24}$	undecane	
6	7.839	1.53	156.31	$C_{11}H_{24}$	undecane	
7	8.875	1.25	156	$C_{11}H_{24}$	octane, 2,3,7-trimethyl-	
8	9.257	1.77	226	$C_{16}H_{32}$	hexadecane (a)	
9	10.303	1.25	212.41	$C_{15}H_{32}$	2,6,11-trimethyldodecane	
10	10.613	3.01	226	$C_{16}H_{32}$	hexadecane (b)	
11	11.418	2.07	170	$C_{12}H_{26}$	3,7-dimethyldecane	
12	11.907	1.99	170.34	$C_{12}H_{26}$	octane, 3,4,5,6-tetramethyl	
13	13.128	0.61	186.33	$C_{12}H_{26}O$	1-octanol, 2-butyl-	
14	14.356	0.80	254.49	$C_{18}H_{38}$	pentadecane, 2,6,10-trimethyl-	
15	21.024	3.42	242	$C_{15}H_{30}O_2$	oxirane, [(dodecyloxy)methyl]-	
16	23.211	59.54	268.48	$C_{18}H_{36}O$	octadecanal	
17	25.576	7.90	298.55	$C_{20}H_{42}O$	1-eicosanol	
18	25.961	3.10	156.27	$C_{10}H_{20}O$	heptane, 3-[(ethenyloxy)methyl]-	
Total	–	100.0	–	–	–	–

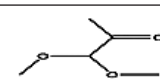
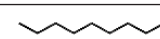
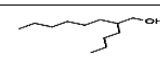
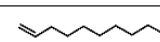
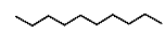
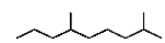
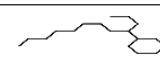
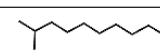
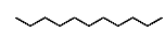
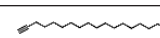
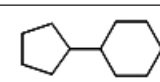
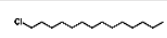
Abbreviations: RT – retention time; PA – peak area; MW – molecular weight; MF – molecular formula

Compounds identified in *Eucalyptus citriodora* ethyl acetate fraction (ECE5)

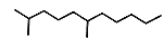
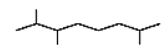
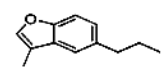
The result of the gas chromatography mass spectrometry (GC-MS) of ethyl acetate fraction (ECE5) of *Eucalyptus citriodora* is presented in Table 14. A total of 23 compounds were identified in fraction ECE5. The result in Table 14 shows the peak number, retention time, peak area in percentage, molecular weight, molecular formula and structure of each identified compounds. Peak number 5 corresponding to Decane was the most abundant compound with peak area of 13.70% followed by peak number 9 that is Undecane, with peak area of 11.68%; others were between the ranges of 0.42% to 8.98% while peak number 22 that is Nonanoic acid, methyl ester was the least abundant with peak area of 0.36%. Most of the compounds identified in the *Eucalyptus citriodora* fractions (ECE1, ECE4 and ECE5) belongs to the alkanes group, which are reported to exhibit antimicrobial activity in combination with other elements (LA et al. 2012).

Table 14

Percentage composition and suspected structures of compounds identified in *Eucalyptus citriodora* ethyl acetate fraction (ECE5)

Peak No.	RT	PA [%]	MW [g/mol]	MF	Compound name	Suspected structure
1	3.212	0.42	118.13	$C_5H_{10}O_3$	2-propanone, 1,1-dimethoxy-	
2	3.602	1.24	128.26	C_9H_{20}	nonane	
3	4.051	1.76	186.33	$C_{12}H_{26}O$	1-octanol, 2-butyl-	
4	4.811	5.01	140.27	$C_{10}H_{20}$	1-decene	
5	4.929	13.70	142.28	$C_{10}H_{22}$	decane	
6	5.252	4.90	156	$C_{11}H_{24}$	nonane, 2,6-dimethyl-	
7	5.484	5.18	364	$C_{18}H_{36}$	dodecane, 4-cyclohexyl-	
8	5.857	7.05	156.31	$C_{11}H_{24}$	decane, 2-methyl-	
9	6.380	11.68	156.31	$C_{11}H_{24}$	undecane	
10	6.723	2.51	250.46	$C_{18}H_{34}$	1-octadecyne	
11	6.971	3.57	152.28	$C_{11}H_{20}$	cyclopentylcyclohexane	
12	7.316	3.83	232.83	$C_{14}H_{29}Cl$	tetradecane, 1-chloro-	

cont. Table 14

13	7.840	8.98	170.33	$C_{12}H_{26}$	dodecane (a)	
14	8.093	3.34	184.36	$C_{13}H_{28}$	2,6-dimethylundecane	
15	8.875	4.86	156	$C_{11}H_{24}$	octane, 2,3,7-trimethyl-	
16	9.258	6.18	184.36	$C_{13}H_{28}$	tridecane	
17	10.302	3.86	174.23	$C_{12}H_{14}O$	nonane, 3-methyl-5-propyl	

Abbreviations: RT – retention time; PA – peak area; MW – molecular weight; MF – molecular formula

Toxicity of plant extracts

The result of the acute toxicity and LD_{50} of *Eucalyptus citriodora* (ethyl acetate) extracts and the corresponding histopathology of the kidney & liver of the dead rats is presented in Table 15 and Plate II & III respectively (Fig. 3 and Fig. 4). There were no observable signs of toxicity in the experimental rats in response to the first three doses administered (10 mg, 100 mg and 1000 mg/kg bw) but at 1000 mg/kg bw, the animals reacted with shivering within the first 1 to 3 h and 1 death after 48 h. The result shows that the minimum tolerated and maximum lethal doses for both *Eucalyptus citriodora* ethyl acetate extract was 500 mg/kg bw and 1000 mg/kg bw respectively therefore, the LD_{50} for the crude extract was 707.10 mg/kg bw which is far lower than the recommended lethal dose of 5000 mg/kg bw (ZHAO et al. 2019). Furthermore, the fact that the kidney and liver plays vial roles in the processing of drugs in the human system, makes it very important to determine the state of these vital organs in the dead animals. These changes on the organs could be as a result of toxic agents present in the plant extracts considering the fact that such changes were not observed in the control rats and this may affect the kidney and impair its physiological functions. But the result of this study reveals that the effect is dose dependent. This result is similar to the result of BIN ZHAO et al. (2019) who reported that the same pathological changes were observed in experimental rats' organs administered with oil extracts from *Eucalyptus viminalis*. ODELEY and OBAMESO (2022)· reported that physiological changes in kidney histology may not be sensitive enough in detecting renal toxicity or damage. OKOKON et al. (2018) who reported that degenerative glomeruli were observed in kidney section of male albino rats administered with *Eucalyptus citriodora* acetone extract but were less effective with lower doses.

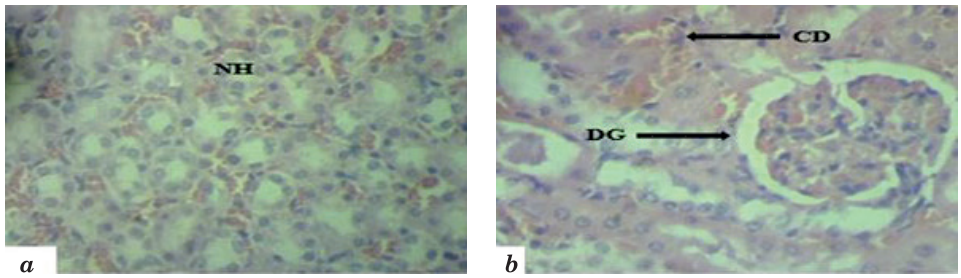


Fig. 3. Plate II. Photomicrographs of normal and experimental rat liver sections (*a*) section of kidney administered with normal saline showing normal histology (NH); (*b*) section of kidney administered with 1000 mg/kg bw *Eucalyptus citriodora* oil extract showing mild degenerative glomerulus & renal tubules and cytoplasmic distortion

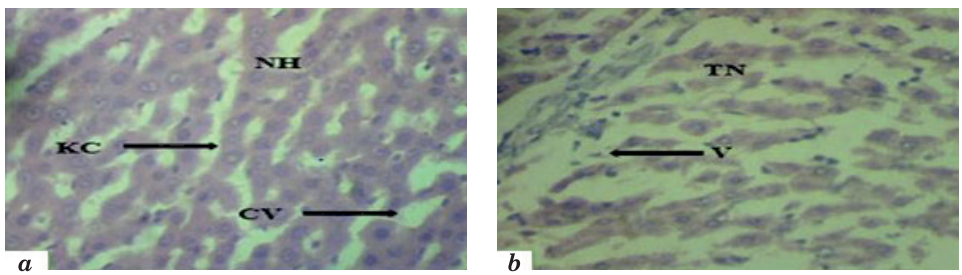


Fig. 4. Plate III. Photomicrographs of normal and experimental rat kidney sections (*a*) section of liver administered with normal saline showing normal hepatocyte (NH), Central vein (CV) and kupfer cell (KC); (*b*) section of liver administered with 1000 mg/kg bw *Eucalyptus citriodora* oil extract showing mild tissue necroses (TN) and vacuolation (V)

Table 15

Acute toxicity and LD_{50} of plant extracts

Extract	Number of animals	Dose [mg/kg bw]	Motality/ survival	Toxicity reactions
ECE	3	10	0/3	no sign of toxicity
	3	100	0/3	no sign of toxicity
	3	500	0/3	no sign of toxicity
	3	1000	1/2	shivering within the first 1–3 hrs and 1 death after 48 h
NS3	3	10	0/3	no sign of toxicity
	3	100	0/3	no sign of toxicity
	3	500	0/3	no sign of toxicity
	3	1000	0/3	no sign of toxicity

Abbreviations: ECE – *Eucalyptus citriodora* ethyl acetate fraction; NS – normal saline; mg/kg bw – milligram per kilogram body weigh N.B:LD_{50} – √(*minimum tolerated dose*) (*maximum lethal dose*), route of administration: oral, LD_{50} = √(500)(1000) = 707.10 mg/kg bw

Conflict of interest. The authors declare that there is no conflict of interest.

Acknowledgment. The authors are grateful to Prof S.A. Garba of the Department of Microbiology FUT, Minna for his encouragement and support needed to complete this work.

Accepted for print 30.12.2022

References

Aboshora W., Lianfu Z., Dahir M., Qingran M., Qingrui S., Jing L., Al-Haj Nqm, Ammar A. 2014. *Effect of extraction method and solvent power on polyphenol and flavonoid levels in Hyphaene thebaica* L. *Mart Arecaceaedoum fruit, and its antioxidant and antibacterial activities*. Tropical Journal of Pharmaceutical Research,13: 2057–2063.

Acevedo R.P., Strongm. T. 2012. *Catalogue of the seed plants of the West Indies*. Smithsonian Contributions to Botany, 98: 1192.

Acuna G.J. 2015. *Undesirable plants in Cuban crops*. Academia de Ciencias de Cuba, Havana, pp. 241.

Altemimi A.W., Watson D.G., Kinsel M., Lightfoot D.A. 2015. *Simultaneous extraction, optimization, and analysis of flavonoids and polyphenols from peach and pumpkin extracts using a tlc-densitometric method*. Chemistry Central Journal, 9: 1–15.

Altemimi A., Lakhssassi N., Baharlouei A., Watson D. 2017. *Phytochemicals: Extraction, isolation, and identification of bioactive compounds from plant extracts*. Plants, 6: 42, doi: 10.3390/plants6040042.

Arage M., Eguale T., Giday M. 2022. *Evaluation of antibacterial activity and acute toxicity of methanol extracts ofArtemisia absinthium*, *Datura stramonium*, and *Solanum anguivi*. Infection and Drug Resistance, 15: 1267–1276.

Baynesagne S., Berhane N., Sendeku W., Ai L. 2017. *Antibacterial activity of Datura stramonium against standard and clinical isolate pathogenic microorganisms*. Journal Medicinal Plants Research, 11(3): 501–506.

Belayneh Y.M., Birhanu Z., Birru E.M, Getenet G. 2019. *Evaluation of in vivo antidiabetic, antidyslipidemic, and in vitro antioxidant activities of hydromethanolic root extract of Datura stramonium* L. (Solanaceae). Journal of Experimental Pharmacology, 11: 29–38.

Zhao B., Jiang X., Cao J., Huang K. 2019. *Biostatistical analysis on medicinal plant toxicity*. Biomedical Journal of Scientific and Technical Research, 18(5): 13890–13896, doi: 10.26717/BJSTR.2019.18.003212.

Brinda C., Mohan K.R. 2016. *Evaluation of antioxidant, antibacterial activity of ethanolic extract in the leaves of Combretum Albidum and gas chromatography-mass spectrometry analysis*. Asian Journal of Pharmaceutical and Clinical Research, 9(4): 325–329.

Clinical and Laboratory Standard Institute (CLSI). 2012. *Performance standards for antimicrobial susceptibility testing; seventeenth information supplement*, 27(1): 100–117.

Ewansiha J.U., Garba S.A., Galadima M., Daniyan S.Y., Busari M.B., Doughari J.H. 2017. *Bioactivity of chromatographic fractions from eucalyptus citriodora leaf against some bacterial pathogens*. International Journal of Clinical Medicine Research, 4(1): 1–14.

Ewansiha J.U., Tilon G., Kolawole I.D., Orji S.L. 2020. *Inhibitory potential of coconut and carrot oil extracts on some bacterial skin microflora*.African Journal of Biology and Medical Research, 3(3): 15–21.

Ewansiha J.U. 2020. *Evaluation of antibacterial potency of Citrus Limon (Lemon) juice against some pathogenic organisms as alternative source of chemotherapy*.European Journal of Biology and Biotechnology, doi: 10.24018/2020.1.1.12.

Ewansiha J.U., Garba S.A., Galadima M., Daniyan S.Y, Busari M.B., Doughari J.H. 2017. *Bioactivity of chromatographic fractions from Eucalyptus citriodora leaf against some bacterial pathogens*. International Journal of Clinical Medicine Research, 4(1): 1–14.

Ewansiha J.U., Garba S.A., Mawak J.D., Oyewole O.A. 2012. *Antimicrobial activity of Cymbopogon citratus (Lemon Grass) and its phytochemical properties*. Frontiers in Science, 2(6): 214–220.

Geleta B., Eyasu M., Kebamo A., Mekonnen E., Abebe A. 2015. *In vitro vasodilatory effect of aqueous leaf extract of Thymus serrulatuson thoracic aorta of Guinea pigs*. Asian Pacific Journal of Tropical Biomedicine, 5(1): 15–18.

Getaneh S., Girma Z. 2014. *Ethnobotanical study of medicinal plants in Debre Libanosereda, central Ethiopia*. African Journal of Plant Science, 8(7): 366–379,doi:10.5897/AJPS2013.1041.

Ghadir A.E, Abeer F.A, Eman S.R. 2014. *Evaluation of the antioxidant and antibacterial properties of various solvents extracts of A. squamosal* L. leaves. Arabian Journal of Chemistry, 2: 227–233.

Griffith O.W, Ginter P.W. 2017. *The placenta as a model for understanding the origin and evolution of vertebrate organs*. Nature Ecology & Evolution, 1(4): 72.

Hajir B.A., Alaa I.M., Khansa A.A, Naga I., Wdeea A.A., Monier N.H. (2016). *Evolution of antimicrobial, antioxidant potentials and phytochemical studies of three solvent extracts of five species from Acacia used in sudanese ethnomedicine*. Advances in Microbiology, 6: 691–698.

Hajir B.A., Alaa I.M, Khansa A.A. 2016. *Evolution of antimicrobial, antioxidant potentials and phytochemical studies of three solvent extracts of five species from acacia used in sudanese ethnomedicine*. Advances in Microbiology, 6: 691–698.

Innalegwu D.A., Ewansiha J.U., Amuda O.A., Vivian N. 2016. *Phytochemical and anti-microbial study of seventy percent methanol leaf extract of Terminalia microptera against selected pathogenic bacteria*. Journal of Advances in Medical and Pharmaceutical Sciences, 9(1): 1–8.

Ewansiha J.U., Ugo C.E., Kolawole D.I, Orji L.S. 2021. *Antibacterial activities of Hyphaene thebaica (*Doum Palm) *fruit extracts against intestinal microflora and potential constipation associated pathogens in Yola Metropolis*, Nigeria,Tanzania Journal of Science, 47(1): 104–111.

La M.P, Li C., Li L. 2012. *New bioactive sulfated alkenes from the sea cucumber Apostichopus japonicus*. Chemistry & Biodiversity, 9(6): 1166–1171, doi: 10.1002/cbdv.201100324.

Malik M., Li L., Zhao X. 2014. *Lethal synergy involving bicyclomycin: an approach for reviving old antibiotics*. Journal of Antimicrobial Chemotherapy, 9(12), 3227–3235.

Mammen D., Bapat S., Sane R. 2012. *An investigation to variation in constituents in the fruits of Terminalia chebula Retz at different maturity stages*. International Journal of Pharmacy and Biological Sciences, 3(1): 416–419.

Mohanapriya M., Lalitha R., Rajendran R. 2013. *Health and medicinal properties of lemon* (*Citrus limonum*). International Journal of Ayurvedic and Herbal Medicine, 3(1), 1095–1100.

Odeleye A.A, Obameso J.O. 2022. *Activities ofDatura stramonium extracts against Clinical Pathogens*. South Asian Journal of Research in Microbiology, 13(2): 12–18, doi: 10.9734/SAJRM/2022/v13i230292.

Okokon J.E, Simeon J.O, Umoh E.E. 2018. *Hepatoprotective activity of extract of Homalium Letestui stem against carbon tetrachloride-induced liver injury*. Avicenna Journal of Phytomedicine, 3(4): 1–11.

Okokon J.E, Simeon J.O., Umoh E.E. 2018. *Hepatoprotective activity of extract of Homalium Letestui stem against carbon tetrachloride-induced liver injury*. Avicenna Journal Phytomed, 3(4): 1–11.

Pradhan P., Tamang J.P. 2019. *Phenotypic and genotypic identification of bacteria isolated from traditionally prepared dry starters of the Eastern Himalayas*. Frontiers in Microbiology, 10: 1–15, doi: 10.3389/fmicb.2019.02526.

Rehman S., Nadeem U., Mehwish J., Riffat Z., Hina A. 2022. *Light and scanning electron microscopy of Datura stramonium* L. *extract and its biological applications*. Microscopy Research and Technique, 85(8): 2997–3004, doi: 10./jemt.24148.

Sardar A.F., Mujaddadur-Rehman K., Naumank., Khan S.A. 2012. *Antimicrobial activity of Eucalyptus Tereticornisand comparison with daily life antibiotics*.International Journal of Pharmaceutical Sciences Review and Research, 12(1): 21–29.

Sarswati N.O., Kuldeep S.N., Lalit M.T., Puran S.M., Jai C.R. Veena P., Sandeep S., Amit P. 2013. *Variation in essential oil composition and anti-microbial activity of Indian Oregano* (*Origanum vulgare* L.) *population from Indian Himalayan Region (IHR)*. Journal of Medicinal Plants Research, 7(46): 3375–3384.

Sarswati, N.O., Kuldeep, S.N. and Lalit S.N. 2013. *Variation in essential oil composition and antimicrobial activity of Indian Oregano* (*Origanum vulgare* L.) *population from Indian Himalayan Region (IHR)*. Journal of Medicinal Plants Research, 7(46), 3375–3384.

Tojola O.B, Lajide L. Owolabi B.J, Olaleye M.T., Okoh S.O. 2019. *Phytochemical screening & antibacterial activity of ethyl acetate & methanol extracts of Annona muricata* aerial part. Journal of Medicinal Plants Studies, 7(6): 1–5.

Waza S.A., Anthony P., Dar S. 2015. *Phytochemical analysis, antioxidant and antimicrobial activities of methanolic extract of Datura stramonium seeds*. International Journal of Pharmaceutical Sciences and Research, 6: 3021–3026.