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Antimicrobial Efficacy, Secondary Metabolite Constituents, Ligand Docking of *Enantia chlorantha* on Selected Multidrug Resistance Bacteria and Fungi

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Authors' contributions

Author TOA prepares the final manuscript for constrictive criticism. Author OOT research into different arrays of medicinal plants and helps to designed the materials and methods of research work. Authors AOM and AFA helps managed and analyzed the collected data for statistical analysis. Author ARA prepares and proof-read the first manuscript. All authors read and approved the final manuscript.

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ABSTRACT

This study aimed at determining the phytochemical constituents and antimicrobial efficacy of *Enantia chlorantha* on multidrug resistance microorganisms.And also to study the interaction of plant secondary metabolite (phytochemicals) from *Enantia chlorantha* with three proteins. Antimicrobial activity of the extracts of *E. chlorantha* (leaf and stem bark) against selected microorganisms was done using agar well diffusion method. Minimum inhibitory concentrations (MICs), minimum bactericidal concentrations (MBCs) were also determined using standard methods. The qualitative

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and quantitative phytochemical screening of *E. chlorantha* were also determined. The molecular docking was determined using in-silico techniques and was elucidated. Protein generation, Ligand generation and Ligand Docking using GLIDE were determined. Standard precision (SP) flexible ligand docking was carried out in Glide of Schrödinger-Maestro 11.1 and the extra-precision (XP) mode. The crude extracts tested showed antimicrobial activities against all the test bacterial and fungal isolates for the stem bark extract while the leaf extract showed antimicrobial activities against some of the isolates with little differences. The zones of inhibition ranges between 9mm-24mm at 100mg/ml for the ethanol extract and 10mm-13mm at 12.5mg/ml. The Minimum Inhibitory Concentration (MIC) at which the isolates were sensitive to the various extracts differed and the MIC values ranged from 12.5mg/ml to 100mg/ml while the MBC for the organisms ranged from 25mg/ml to 100mg/ml.The qualitative phytochemical screening of *Enantia chlorantha* leaf and stem bark revealed the presence of medicinally active constituent such as cardiac glycoside, steroids, anthraquinone,tannin, saponin, phenol, and reducing sugar. The quantitative phytochemical screening of *E. chlorantha* stem bark and leaf using different solvents, showed the presence of different phytoconstituents in different quantities. Molecular docking results revealed some components of the plant to be more active compared to levofloxacin by inhibiting topoisomerase IV. Jartrorrhizine-1 and canadine-1 present in *Enantia chlorantha* have docking scores of -2.267 and -1.625 respectively which are greater than that of levofloxacin (-1.557) against *Salmonella typhi*. For *Staphylococcus aureus*, Argentine.sdf (-7.373) and Jartrorrhizine.sdf (-4.225) have high docking scores compared to Levofloxacin.sdf (-3.436) as well as *Candida albican*.The promising evidence for the antimicrobial effects of *E. chlorantha* against bacterial and fungal isolates in this study especially the stem bark extract showed that *Enantia chlorantha* is more effective at treating diseases caused by *Salmonella typhi* and other organisms and therefore can be used as an alternative source of therapeutic agents.

Keywords: Multidrug resistance; fungi; Enantia chlorantha; ligand docking; bacteria.

1. INTRODUCTION

Enantia chlorantha is a well-known medicinal plant that is used all over Nigeria. It is a fair sized ornamental forest tree that can grow to heights of 30 m. It grows in dense shade and may be recognized by its bright yellow slash and conspicuous black fruits. It is located in the West African region and extends from southern Nigeria to Gabon, Zaire and Angola. It is commonly called African white wood, Moambe Jaune and *Annikia chlorantha*. Locally, the name varies from place to place. The Ibibios of Akwa Ibom call it Uno eto; the Yorubas call it Osupupa or dokita, as referred to by the Igbos. The Edo people refer to it as Erenbavbogo while Ikale and Boki tribes refer to it as Osumolu and Kakerim respectively. The family is *Annonaceae* and the specie is *chlorantha*. There are over 40 different species that grow in Nigeria as is seen by the various names that it is called and the varied uses to which it is applied [1].*Enantia chlorantha* is used to treat a wide variety of conditions using its roots, stem bark, fruit and leaves. In Akwa Ibom, it is used to treat malaria fever, typhoid fever, jaundice, dysentery, wounds, infections, high blood pressure and convulsions. It has been used also as anti-viral, anti-candidal and for gastroenteritis. In traditional medicine, the plant is used in the treatment of coated tongue, typhoid fever, malaria, jaundice, ulcer, rickettsia and infective hepatitis [2, 3].

Enantia chlorantha is widely used in herbal medicine for the treatment of several ailments such as jaundice, malaria, fever, infective hepatitis, etc. They are also used as hemostatic agents and uterus stimulants [4].It is an ornamental tree which may grow up to 30 m high, with dense foliage and spreading crown. The outer bark which is thin and dark brown is fissured geometrically while the inner bark is brown above and pale cream beneath [1].

In the southern forest zone of Cameroon, *Enantia chlorantha* is also used for the management of stomach problems, as well as for the treatment of jaundice, tuberculosis, urinary tract infections, malaria, hepatitis and some forms of ulcer. In the western Cameroon, a mixture of *Enantia chlorantha* bark, cut into small pieces, *Citrus limonum* fruit also cut into small pieces with its peels and *Allium sativum* bulb crushed is macerated in water for two days and the resulting liquid taken twice daily to cure malaria.The stem bark of *Enantia chlorantha* plant has also been reported for treating leprosy spots, as hemostatic agent, and as uterine

stimulant [5]. The bark of *Enantia chlorantha* has several medicinal properties and has been used by traditional medical practitioners in Nigeria for the treatment of skin, gastric and duodenal ulcers, and as antimalarial [6]

Medicinal plants have been discovered and used in traditional medicine practices since prehistoric times. Plants synthesize hundreds of chemical compounds for functions including defence against insects, fungi, diseases, and herbivorous mammals. Numerous phytochemicals with potential or established biological activity have been identified. However, since a single plant contains widely diverse phytochemicals, the effects of using a whole plant as medicine are uncertain. Further, the phytochemical content and pharmacological actions of many plants having medicinal potential need to be assessed by rigorous scientific research to define efficacy and safety [7]. Therefore, the outbreak of enteric diseases, skin infections, and development of resistance of known pathogens to commercial antibiotics has resulted to this project study, Hence the use of *E. chlorantha.*

2. METHODOLOGY

2.1 Collection and Authentication of Plant Samples

The stem bark and leaves of *Enantia chlorantha* used in the study were collected from Owo forest reserve, Ondo State, Nigeria, with latitude and longitude of 6.96879 and 5.5626 respectively. The plant samples were authenticated at the Department of Plant Science and Biotechnology, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria.

2.2 Preparation of Plant Samples

The leaves and stem bark of *Enantia chlorantha* were first washed thoroughly with sterile distilled water after collection, and appropriately air dried at room temperature for two weeks to ensure the samples lose its moisture content. The stem bark was powdered, after which the sample was then macerated using electronic blender. The leaf after being air dried was powdered and milled at the Department of Microbiology, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria [8].

2.3 Extraction of Plant

For the extraction of each plant part, 500g of each dried and powdered plant sample was weighed separately into corked containers containing 1500ml each of acetone and ethanol, the mixture was shaken rigorously and left for 9 days. The mixture was in ratio 1:1. All mixtures were filtered using sterile Whatman No. 1 filter papers, and the filtrates were collected directly into sterile crucibles. The filtrate was extracted using soxhlet extractor, and the residues obtained were kept at room temperature [8].

Plate 1. *Enantia chlorantha* leaf with stem

2.4 Standardization of Plant Extracts

At aseptic condition, the extracts were reconstituted by adding 1g of each extract to 2.5ml of DMSO and 7.5ml of sterile distilled water to make 100mg/ml. The serial concen tration was prepared to get concentration of 50mg/ml, 25mg/ml and 12.5mg/ml respectively [8].

2.5 Test Organisms

The human pathogenic test organisms used were: *Salmonella typhi, Shigella sonnei, Escherichia coli, Pseudomonas aeruginosa*, *Proteus mirabilis, Streptococcus pyogenes*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*. The fungi isolates include: *Aspergillus niger* and *Candida albican.*

2.6 Standardization of Test Organisms

The test organisms used were obtained from the stock culture of the laboratory of the Department of Microbiology, Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria. The test organisms were sub-cultured to obtain pure cultures of the organisms. The broth cultures of the test organism were prepared according to 0.5 McFarland's standard [9].

2.7 Antimicrobial Screening of the Extracts

The agar well diffusion method according to Perez *et al.* [10] was used. Overnight broth culture of the respective bacteria strains was adjusted to 0.5 McFarland standard. Mueller-Hinton agar plates were swabbed using sterile cotton swabs with the adjusted broth culture of the respective bacteria strains. Wells (6 mm in diameter) were made equidistance in each of the plates using a sterile cork borer. 100 µl (0.1 ml) of each concentration of the extract were respectively introduced into the wells using sterile automatic pipettes, with the stock solution in the center well with different concentration of the extracts (50, 25 and 12.5mg/ml). The plates were allowed to diffuse at room temperature for 2 hours, and were incubated at 37°C for 24 hours for the bacterial isolates and 24°C for 48 hours for the fungal isolates. The zones of inhibition were measured to the nearest millimeter (mm) using a standard transparent meter rule. The antibacterial activity was expressed as the mean zone of inhibition diameters (mm) produced by the plant extract [11]

2.8 Determination of Minimum Inhibitory Concentration (MIC)

The MIC of the extracts against the test organisms was determined using the broth dilution method as described by Coyle [9]. Aliquots of 1ml of stock extract at the concentration of 100mg/ml was added to 1ml of fresh nutrient broth and serially diluted to obtain extract concentrations of 100mg/ml,50mg/ml, 25mg/ml, 12.5mg/ml and 6.25mg/ml 3.125mg/ml.

2.9 Determination of Minimum Bacteri cidal/Fungicidal Concentration (MBC /MFC)

This was done according to the National Committee for Clinical Laboratory Standard (1990) method. One ml sample from tubes used in MIC determination without any visible growth after the period of incubation was streaked out on Nutrient Agar for 24 hours for bacteria and Sabouraud Dextrose Agar for72 hours for fungi to determine the minimum concentration of the extract required to kill the organisms. The lowest concentration of the extract that indicated a bactericidal effect after incubation was regarded as the minimum bactericidal concentration (MBC), while the lowest concentration that prevented fungal growth was taken as the Minimum Fungicidal Concentration (MFC).

2.10 Qualitative Secondary Metabolite (Phytochemical) Analysis of Medicinal Plant

Test for Reducing Sugars: One milliliter of the plant filtrate was mixed with Fehling A and Fehling B separately; a brown colour with Fehling B and a green colour with Fehling A indicated the presence of reducing sugars [12].

2.11 Test for Cardiac Glycosides (TLC Method)

The powdered test samples was extracted with 70% Ethanol on rotary shaker (180 thaws/min) for 10hr. 70% of the lead acetate was added to the filtrate and centrifuged at 5000rpm/10 min. The supernatant was further centrifuged by adding 6.3% Na_2CO_3 at 10000 rpm/10min. The retained supernatant was dried and redissolved in chloroform and used for chromatography. Then the glycosides were separated using EtOAc-MeOH-H2O (80:10:10) solvent mixture. The colour and hRf values of these spots were

recorded under ultraviolet (UV254 nm) light [13, 14].

2.12 Test for Alkaloid (TLC Method)

The powdered test samples were wet with a half diluted NH_4OH and lixiviated with EtOAc for 24hr at room temperature. Then the organic phase was separated from the acidified filtrate and basify with NH4OH (pH 11-12). Then it was extracted with chloroform (3X), condensed by evaporation and use for chromatography. The alkaloid spots were separated using the solvent mixture chloroform and methanol (15:1) and the spots were sprayed with Dragendorff's reagent. Orange spot indicated a positive result for alkanol[13].

2.13 Test for Phenol

The extract was spotted on a filter paper. A drop of phoshomolybdic acid reagent was added and exposed to ammonia vapors. Blue colouration of the spot showed a positive result for phenol [15].

2.14 Test for Anthraquinone (Borntrager's Test)

About 50mg of extract was heated with 1ml 10% ferric chloride solution and 1ml of concentrated hydrochloric acid. The extract was allowed to cool and filtered. The filtrate was shaken with equal amount of diethyl ether. The ether extract was further extracted with strong ammonia. A colour pink or deep red of aqueous layer indicated a positive result. [14, 15].

2.15 Test for Flavonoid (TLC Method)

1g of the powdered test samples were extracted with 10ml methanol on water bath (60°C/ 5min). The filtrate was condensed by evaporation, and a mixture of water and EtOAc were added (10:1 mL), and mixed thoroughly. The EtOAc phase was retained and used for chromatography and the flavonoid spots was separated using chloroform and methanol (19:1) solvent mixture.The colour and hRf values of these spots was recorded under ultraviolet (UV254nm) light [13, 15, 16].

2.16 Test for Saponin (TLC Method)

About two grams of powdered test samples were extracted with 10 ml 70% EtOH by refluxing for 10min, the filtrate was condensed, enriched with saturated n-Butanol, and was mixed thoroughly. The butanol was retained, condensed and used for chromatography. The saponins were separated using chloroform, glacial acetic acid, methanol and water (64:34:12:8) solvent mixture. The chromatogram was exposed to the iodine vapors. The colour (yellow) and hRf values of these spots were recorded by exposing chromatogram to the iodine vapours[13, 17].

2.17 Test for Steroid

Two grams of powdered test samples was extracted with 10ml methanol in water bath (80°C/15min). The condensed filtrate was used for chromatography. The sterols were separated using chloroform, glacial acetic acid, methanol and water (64:34:12:8) solvent mixture. The colour and hRf values of these spots were recorded under visible light after spraying the plates with anisaldehyde- sulphuric acid reagent and heating (100°C/6 min). The color (Greenish black to Pinkish black) and hRf values of these spots were recorded under visible light [13].

2.18 Test for Tannin (Braemer's Test)

Two ml of methanolic extract was added to 10% alcoholic ferric chloride. A dark blue or greenish grey coloration of the solution indicated a positive test for tannin. [15, 17].

2.19 Quantitative Method of Secondary metabolite (Phytochemical) Analysis of *Enantia chlorantha* Medicinal Plant

2.19.1 Estimation of saponins

About 20 grams each of the dried plant samples were grounded and was put into a conical flask, after which 100 ml of 20 % aqueous and ethanol were added. The mixture was heated using a hot water bath at 55^{o}C for 4 hour**s** with continuous stirring, after which the mixture was filtered and the residue re-extracted further with a 200 ml of 20% ethanol. The combined extracts were reduced to 40 ml over a water bath at 90°C. The concentrate was transferred into a 250 ml separatory funnel and 20 ml of diethyl ether was added and then shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated three times. Then 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 m1 of 5% aqueous sodium

chloride. The remaining solution was heated in a water bath. After evaporation, the samples were dried in the oven to a constant weight; the saponin content was calculated as percentage of the starting material [18].

2.19.2 Estimation of total flavonoid concentration

The concentration of flavonoids in the extract was estimated spectrophotometrically. The extract (0.1 g) was dissolved in 20 ml of 70% (v/v) ethanol to give a final concentration of 0.5 mg/ml. To clean dry test tubes (in triplicate) were pipetted 0.5 ml of working solution of sample and diluted with 4.5 ml distilled water. To each test tube was added 0.3 ml of 5% (w/v) $NaNO_2$, 0.3 ml of 10% Aid_3 and 4 ml of 4% (w/v) NaOH. The reaction mixtures were incubated at room temperature for 15 minutes. The absorbance was read at 500nm against reagent blank. The standard calibration curve was prepared by pipetting 0.2, 0.4, 0.6, 0.8, 1.0 ml of 1 mg/ml rutin into clean dry test tubes. The volumes were made up to 5 ml with distilled water. To each of the tubes were added 0.3 ml of 5% (w/v) $NaNO_2$, 0.3 ml of 5% (w/v) Aid_3 and 4 ml of 4% (w/v) NaOH. The reaction mixture was incubated at room temperature for 15 min. Absorbance was taken at 500nm and was plotted against the concentration to give the standard calibration curve. The concentrations of the flavonoids in the extract was extrapolated from standard calibration curve and expressed as milligram rutin equivalent per g of extract (mg RE/g extract) [18,19].

2.19.3 Estimation of cardiac glucosides (Borntrager's Test)

To 2 ml of filtrate hydrolysate, 3ml of ethyl acetate was added and shaken, ethyl acetate layer was separated and 10% ammonia solution was added to it. Formation of pink color indicated the presence of anthraquinone glycosides [20].

2.19.4 Detection of alkaloid content

Five grams of the plant sample was weighed into a 250 ml beaker and 200ml of 10% acetic acid in ethanol was then added. The reaction mixture was covered and allowed to stand for 4 hour. This was filtered and the extract was concentrated on a water bath to one-quarter of the original volume. Concentrated ammonium hydroxide was added drop-wise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitate was collected, washed with dilute ammonium hydroxide and then filtered; the residue being the alkaloid was dried and weighed to a constant mass [18, 21].

2.19.5 Estimation of phlobatannins

About0.5 grams of each plant extracts was dissolved in distilled water and filtered. The filtrates were boiled in 2% HCl, Red precipitate showed the presence of phlobatannins[22].

2.19.6 Estimation of total phenolic concentration

Estimation of total phenolic content was carried out using Folin-Ciocalteu's phenol reagent reaction. The assay involved pipetting 0.2, 0.4, 0.6, 0.8, 1.0 ml of garlic acid solution (1.0 mg/l) in triplicate in clean dried test tubes. The volumes were made up to 1.0 ml with distilled water. To each of the test tube was added 1.5 ml of 10% (w/v) $NaHCO_3$ solution to give a total volume of 4.0 ml. The reaction mixtures were further incubated for additional one and half hours. The estimation of phenol in ethanolic extract of *S. mombin* involved pipetting 0.5 ml each of 5 mg/mlethanolic extract into clean dry test tubes in triplicate. The volumes were adjusted to 1.0 ml with distilled water. To each of the tubes was added 1.5 ml of Folin-Ciocalteu's phenol reagent (1:10). The reaction mixture was incubated at room temperature for 5 mimutes. To the reaction mixture was added 5 ml of 10% (w/v) $NaHCO_3$ solution. The reaction mixture was incubated for one and half hour. The absorbance was read at 725nm against the blank containing all reagents except the standard gallic acid. The absorbance at 725nm was plotted against the concentration to produce the standard curve. The concentrations of the phenolies in the extract was extrapolated from standard curve and expressed as milligram tannic acid equivalent per g of extract (mg TAE/g extract) [22, 23].

2.19.7 Determination of Tannins

About 500 mg of the plant sample were weighed into a 50-ml plastic bottle. 50 ml of distilled water was added and shaken for 1 hour on a mechanical shaker. This was filtered into a 50-ml volumetric flask and made up to the marked level. Then, 5 ml of the filtrate was transferred into a test tube and mixed with 2 ml of 0.1 M FeCl in 0.1 M Hcl and 0.008 M potassium ferrocyanide. The absorbance was measured at

120 nm within 10 minutes. The tannins content was calculated using a standard curve of extract [24].

2.20 Molecular Docking

In this study, in-silico approach was employed to study the interaction of plant phytochemicals from *Enantia chlorantha* with three proteins; the *Staphylococcus aureus* topoisomerase iv, *Salmonella typhi* topoisomerase iv, and the yeast *Candida albican*14α demethylase. It is a computational screening technique known as Virtual high throughput screening (HTS) used to screen a pool of compound libraries to probe the binding affinity of the target receptor with library of compounds [25]. Computer-aided docking is an important tool for understanding the binding between a ligand and a target protein/receptor [26, 27]. This tool has emerged a reliable, cost-effective and time-saving technique in drug design by discovering lead therapeutic compounds [28, 29, 30].

2.20.1 Protein generation and preparation

The 3-dimensional crystallized structures of *Staphylococcus aureus* topoisomerase iv and yeast *Candida albican* 14α demethylase were downloaded from the Protein Data Bank (PDB) repository (www.rcsb.org) with the PDB ID of 4URN and 5FSA with crystallographic resolutions of 2.30**Aº** and 2.86**Aº** respectively. The 3D structure of the *Salmonella typhi* topoisomerase iv (not found on the PDB repository) was retrieved by modelling the FASTA sequence of the protein gotten from the NCBI database (www.ncbi.nlm.nih.gov/protein/) using the swiss model server (www.swissmodel.expasy.org). The downloaded proteins were viewed with Schrödinger Maestro11.1. Proteins were prepared using Protein Preparation Wizard tool of the Schrödinger suite. The missing side-chains within the protein residues and the missing loops were filled using Prime (Schrödinger). The Co-crystallized water molecules, ions and cofactors were deleted, hydrogen atoms were added, and formal charges along with bond orders were assigned to the structures. The grid coordinate was generated around the co-crystallized ligand of the proteins with a grid box of 20A×20A×20A [30].

2.20.2 Ligand generation and preparation

A list of phytochemical constituents of *Enantia chlorantha* was gotten from various literatures. The 2D structure of the ligands was retrieved from the NCBI Pubchem database. Under Schrödinger-Maestro tools, the respective 3D conformers of ligands were generated using the LigPrep. It also applies sophisticated rules to correct Lewis structures and to eliminate mistakes in ligands in order to reduce downstream computational errors [31]. Moreover, it optionally expands tautomeric and ionization states, ring conformations, and stereoisomers to produce broad chemical and structural diversity from a single input structure.

2.20.3 Ligand docking

This was carried out using GLIDE (Grid-based Ligand Docking with Energetics). Glide searches for favorable interactions between one or more ligand molecules and a receptor molecule, usually a protein. Glide was run in rigid or flexible docking modes, and automatically generated conformations for each input ligand. The selection of the best pose was done on the interaction energy between the ligand and the protein as well as on the interactions the ligand shows with experimentally proved important residues(Schrodinger).Standard precision (SP) flexible ligand docking was carried out in Glide of Schrodinger-Maestro11.1 followed by the extra-precision (XP) mode which was used to combine a powerful sampling protocol with a custom scoring function designed to identify ligand poses that would be expected to have unfavorable energies, based on well-known principles of physical chemistry [31].

3. RESULTS

The zones of inhibition (mm) of bacterial and fungal growth at different concentrations of ethanol extract of *Enantia chlorantha* was depicted in Fig. 1. The stem bark extract inhibited all the isolates with zone diameter ranging from 10-30mm, while the leaf extract showed diameters ranging from 9-24mm at 100mg/ml respectively. *Proteus mirabilis* showed the widest zone (30mm at 100mg/ml) for the stem bark, while for the leaf, *Candida albican* showed the widest zone (28mm at 100mg/ml).However, *Pseudomonas aeruginosa* and *Shigella sonnei* showed the least zones of 10mm and 9mm respectively at 100mg/ml for the stem bark and leaf extracts respectively. Fig. 1 also showed the MIC and MBC values. Both extracts had MIC values ranging from 12.5-100 mg/ml, and the MBC values ranged from 25-100 mg/ml.

Fig. 2 showed the zones of inhibition of bacterial and fungal growth at different concentrations (100mg/ml, 50mg/ml, 25mg/ml and 12.5mg/ml) of acetone extract of *Enantia chlorantha*. The stem bark extract inhibited all isolates except one of the isolates with diameter ranging from 12.0-28.0mm, while the leaf extract showed diameters ranging from 12-20mm at 100mg/ml respectively. *Proteus mirabilis* had the widest zone for the stem bark (28mm at 100mg/ml), while *Candida albican* showed the widest zone of 20mm at100mg/ml for the leaf. *Escherichia coli* showed the least zone at 100mg/ml for the leaf extract, while *Klebsiella pneumonia* showed the least zone for the stem bark extract of 12mm at 100mg/ml. The Figure also depicted the MIC and MBC values. Both extracts had MIC values ranging from 12.5-100mg/ml, and the MBC values were either 50 or 100mg/ml.

Fig. 3 Showed the zones of inhibition of growth of multiple resistant bacteria at different concentrations of ethanol extract of *E. chlorantha.* The most sensitive resistant bacteria to the leaf was *Salmonella subsp 3b* with a diameter of 42mm at 100mg/ml, *Enterobacter agglomerans* was resistance with diameter of 10mm at 100mg/ml. *Proteus mirabilis* showed no inhibition. The stem bark extract also inhibited all except one of the resistant bacteria tested.

The zones of inhibition of growth of multiple resistant bacteria at different concentrations of acetone extract of *E. chlorantha* was shown in Fig. 4. The leaf extract inhibited only *Salmonella subsp 3b* at 100mg/ml with a diameter of 40mm. All other isolates showed no inhibition, i.e resistant to the extract. The stem bark extract on the other hand inhibited only three of the tested resistant isolates with measurable zones of inhibition. The most sensitive test organism were *Enterobacter agglomerans* and *Providencia stuartii* with diameter of 30mm at 100mg/ml, followed by *Proteus mirabilis* with a diameter of 28mm at 100mg/ml. *Acinetobacter baumannii* and *Salmonella subsp 3b* showed no inhibition.

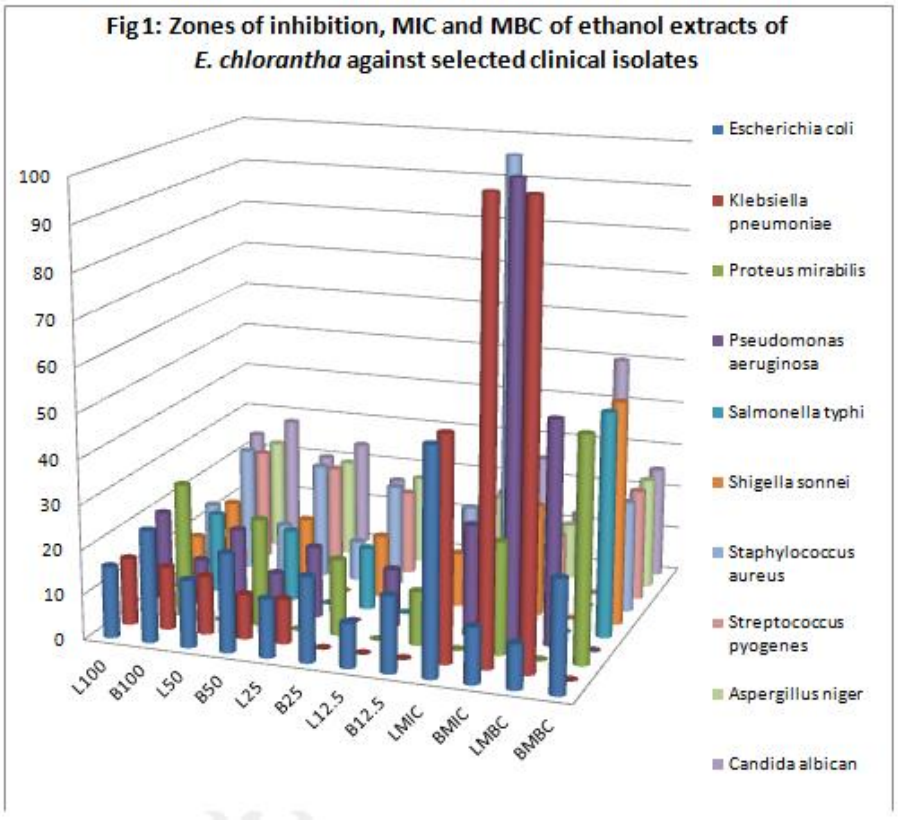

Fig. 1. Zones of inhibition, MIC and MBC of ethanol extracts of *E. chlorantha* against selected clinical isolates(Concentration (mg/ml))

Key ;L = E. chlorantha leaf B = E. chlorantha stem bar

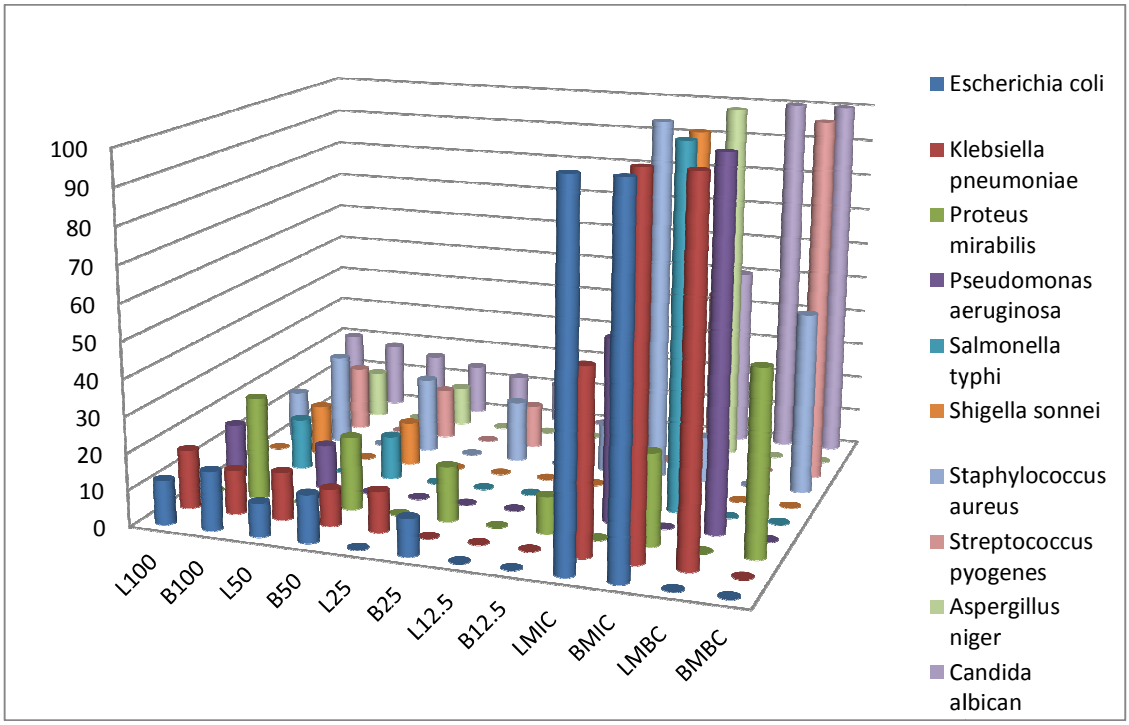

Fig. 2. Zones of inhibition, MIC and MBC of acetone extracts of *E. chlorantha* against selected clinical isolates

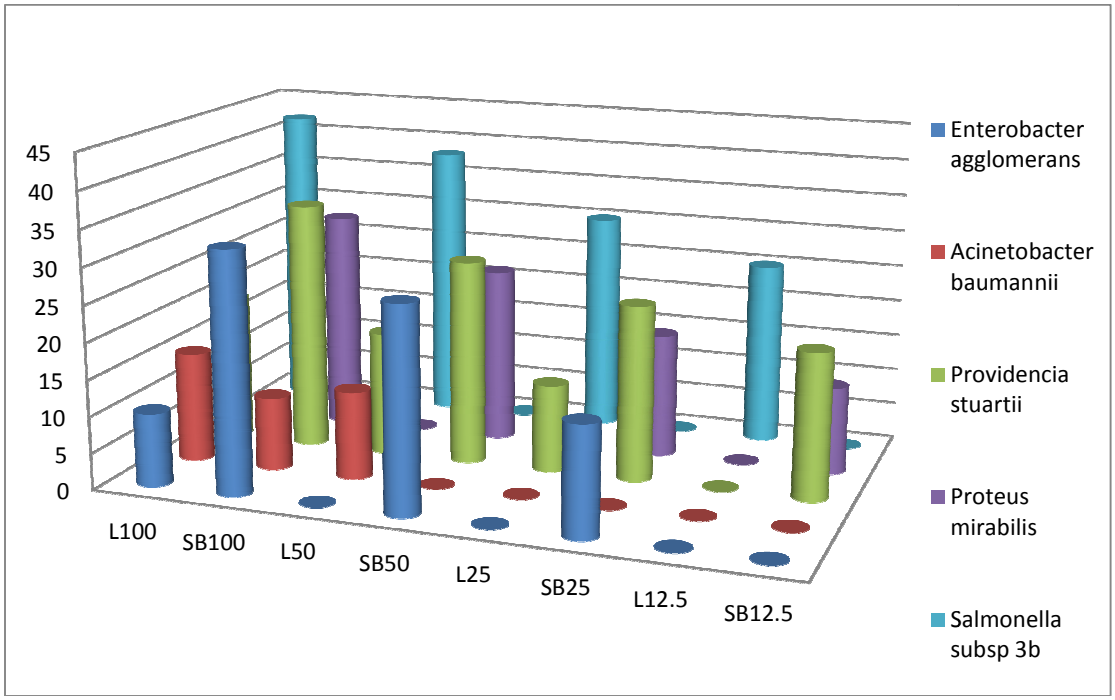

Fig. 3. Zones of inhibition of ethanol extracts of *E. chlorantha*

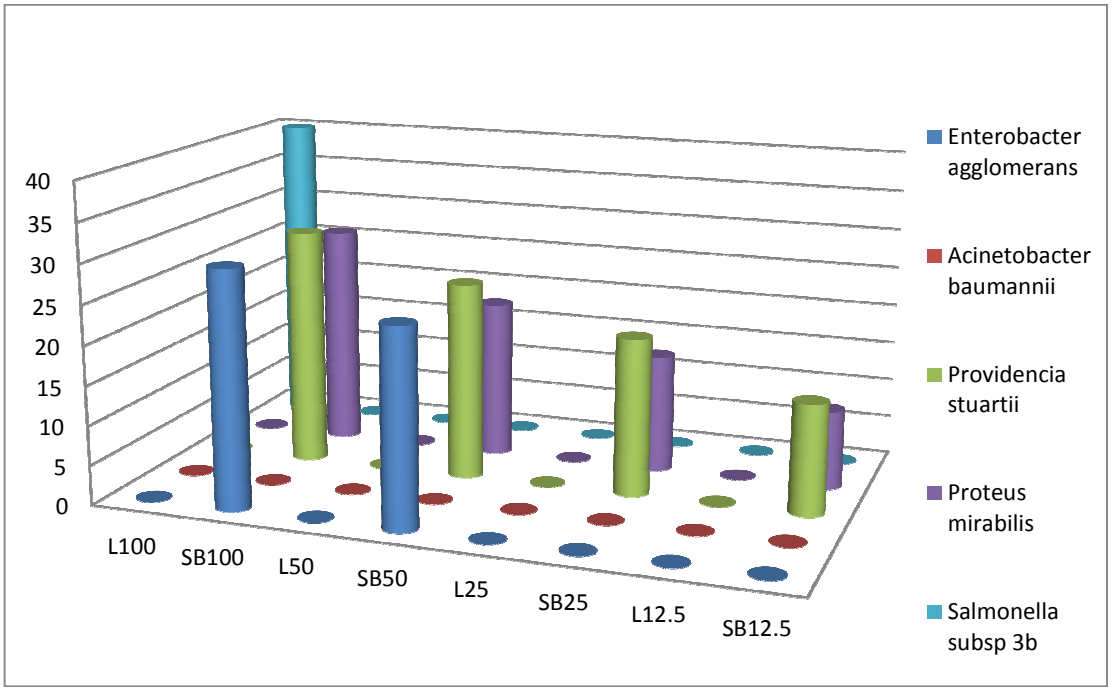

Fig. 4. Zones of inhibition of acetone extracts of *E. chlorantha* against multiple resistant isolates

Table 1 showed the qualitative phytochemical analysis of *Enantia chlorantha* using methanol, acetone, dichloromethane and ethyl acetate. It was observed that using methanol, anthraquinone was not detected while reducing sugar was negative in the leaf. In the stem bark, cardiac glycoside, steroids, phenol and flavonoids were negative, and other screened phytoconstituents were all present. Using acetone, alkaloids, anthraquinone and reducing sugar were negative, all other screened phytoconstituents were present in the leaf and for other solvent are recorded as depicted in Table 1.

The quantitative phytochemical analysis of *Enantia chlorantha* using methanol, acetone, dichloromethane and ethyl acetate showed that using methanol, flavonoid was the most abundant phytochemical in the leaf with 4.55, and others such as alkaloid, phlobatannins, cardiac glycoside, phenol, tannins and saponin ranged between 2.10 and 2.37, with phlobatannins being the least with 2.10. From the stem bark, flavonoid was the most abundant compound with 5.20, while alkaloid, cardiac glycoside, phenol, tannins and saponin varied between 3.23-4.37. Phlobatannin was the least with 1.25 as depicted in Table 2.

Table 1. Qualitative Secondary metabolite (phytochemical) analysis of *Enantia chlorantha* using different solvents

Leaf extract of *E. chlorantha*(%)					**Stem bark extract of *E. chlorantha* (%)**			
Phytochemicals	**Lm**	**La**	**Ld**	**Le**	**Bm**	**Ba**	**Bd**	**Be**
Alkaloid	2.20	4.89	1.03	4.03	3.23	3.79	2.00	10.00
Phlobotannins	2.10	2.89	2.14	0.14	1.25	3.69	2.19	16.19
Cardiac glycosides	2.32	3.89	2.31	2.31	4.25	3.69	2.49	1.49
Phenol	2.37	4.01	2.09	6.09	4.31	3.70	2.08	2.08
Tannins	2.30	5.01	2.09	8.09	4.36	2.10	5.07	5.07
Saponin	2.25	4.89	6.70	6.70	4.37	3.79	5.75	5.75
Flavonoids	4.55	2.89	6.42	6.42	5.20	3.69	7.23	7.23

Using acetone, tannins was the most abundant phytochemical (Secondary metabolite) in the leaf with value of 5.01 while the least abundant compounds was recorded in phlobatannins and flavonoid with a value of 2.89. Saponin and alkaloid were the most abundant in the stem bark with a value of 3.79, while the least was phenol (2.10).

Table 3 showed that jartrorrhizine-1 and canadine-1 which are phytochemicals present in *Enantia chlorantha* are the more active phytochemicals against *Salmonella typhi* by inhibiting the topoisomerase IV than levofloxacin with docking scores of -2.267 and -1.625 respectively while levofloxacin has a docking score of -1.557. The table also showed that argentine, jartrorrhizine and berberine present in *Enantia chlorantha*have docking scores of -7.373, -4.225 and -3.69 respectively. These phytochemical (Secondary metabolite) are more active than levofloxacin by inhibiting the topoisomerase IV of *Staphylococcus aureus*. Levofloxacin has a docking score of -3.436. The argentinine-1, columbamine-1, jartrorrhizine-1 and pseudo columbamine-1 present in *Enantia chlorantha* have docking scores of -9.993, -8.976, -8.509 and -8.006 respectively which is higher than the docking score of levofloxacin, which is -7.372. This indicated that argentinine-1, columbamine-1, jartrorrhizine-1 and pseudocolumbamine-1 are more active against *Candida albican* than levofloxacin by inhibiting the DNA gyrase (Table 3).

Table 2. Quantitative phytochemical (Secondary metabolite) **analysis of *Enantia chlorantha* using different solvents**

Leaf extract of *E. chlorantha*					**Stem bark extract of *E.chlorantha***			
Phytochemicals	**Lm**	**La**	**Ld**	**Le**	**SBm**	**SBa**	**SBd**	**SBe**
Alkaloid	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
Cardiac glycoside	+ve	+ve	-ve	+ve	-ve	+ve	+ve	+ve
Steroids	+ve	+ve	+ve	+ve	-ve	-ve	+ve	-ve
Anthraquinone	ND	-ve	+ve	-ve	+ve	+ve	-ve	+ve
Phenol	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve
Tannins	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve
Saponin	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
Flavonoids	+ve	ND	+ve	+ve	-ve	-ve	+ve	-ve
Reducing sugars	-ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve

Key: m = Methanol, a = Acetone, d = Dichloromethane, e = Ethyl acetate, L = E. chloranthaleaf, B = E. chlorantha stem bark

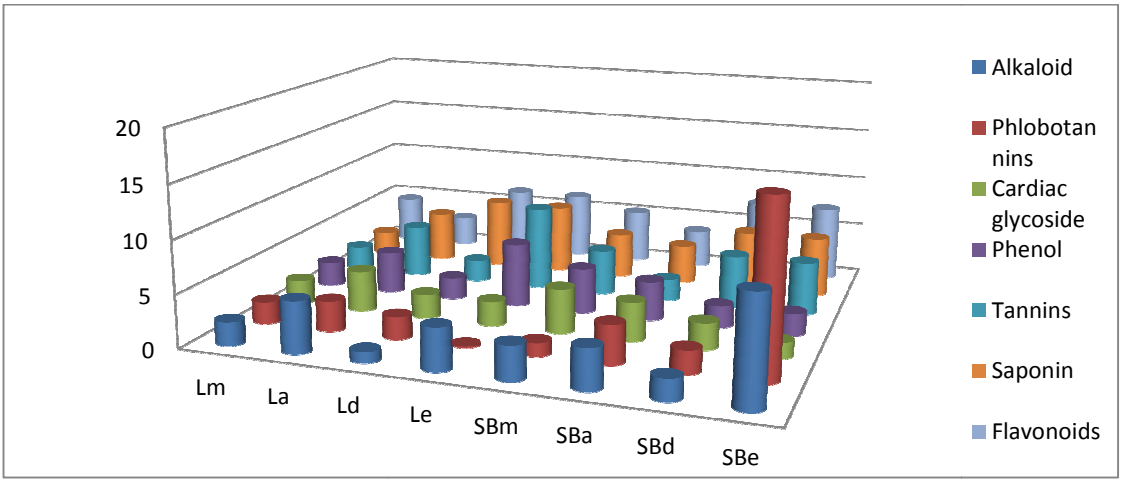

Fig. 5. Quantitative phytochemical (Secondary metabolite) **analysis of *Enantia chlorantha* using different solvents**

Key: m = Methanol, a = Acetone, d = Dichloromethane, e = Ethyl acetate, L = E. chlorantha leaf, B = E. chlorantha stem bark

Table 3. Docking scores of the phytochemical **(**Secondary metabolite) **present in *Enantia chlorantha* against clinical isolates**

Salmonella typhi		***Staphylococcus aureus***		***Candida albican***	
Phytochemicals	**Docking scores**	**Phytochemicals**	**Docking scores**	**Phytochemicals**	**Docking scores**
Jartrorrhizine-1	-2.267	Argentine.sdf	-7.373	Argentinine-1	-9.993
Canadine-1	-1.625	Jartrorrhizine.sdf	-4.225	Columbamine-1	-8.976
Levofloxacin-3	-1.557	Berberine.sdf	-3.69	Jartrorrhizine-1	8.509
Atherosperminine-1	-1.144	Levofloxacin.sdf	-3.436	Pseudocolumbamine-1	-8.006
Palmatine-1	-1.131	Antherosperminine.sdf	-3.315	Levofloxacin-1	-7.372
Berberine-1	-0.467	Pseudocolumbamine.sdf	-3.308	Berberine-1	-6.359
Argentinine-1	0.343	Palmatine.sdf	-3.287	Canadine-1	-6.18
Columbamine-1	1.004	Canadine.sdf	-2.819	Atherosperminine-1	- 5.853
Canadine-2	1.201	Tetradihydroprotoberberine.sdf	-2.743	Tetradihydroprotoberberine-2	-5.328
		Columbanine.sdf	-0.315	Palmatine-1	-5.216
				Tetradihydroprotoberberine-1	-4.576

4. DISCUSSION

The crude extracts tested in this study showed antimicrobial activities against all the test bacterial and fungal isolates for the stem bark extract. The leaf extract showed antimicrobial activities against some of the isolates. However, differences were observed between the antimicrobial activities. These differences could be attributed to the differences in their chemical composition and amount of the bioactive compounds extracted by the solvents. These compounds usually accumulate in different parts of the plant [32], and such secondary metabolites have been found to produce many effects including antibacterial and antifungal properties, this is in line with the observation of [33].

The stem bark extracts (ethanol and acetone) of *E. chlorantha* inhibited the growth of all the bacteria and fungi tested. This suggests that the plant extract has broad spectrum of activity and its mode of action may not be due to inhibition of cell wall synthesis. This corroborated the study of Adebayo et al. [34].

In this study,generally higher antimicrobial activity of the extracts was observed on *Proteus mirabilis, Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli, Aspergillus niger, Candida albican, Salmonella typhi, Shigella sonnei,* and *Klebsiella pnuemoniae*, this is similar to the results obtained by Oloke *et al.,*[35] when extracts from *Aramomum melegueta* fruit was used as the medicinal plant of interest. The strong activity of the extract on *Staphylococcus aureus,Shigella sonnei* and *Escherichia coli* suggested that it can be used for the treatment of wound infection and diarrhea caused by these organisms. Similar results have been obtained with extracts from leaves of *Kalanchoe pinnate* when tested against *Staphylococcus aureus, Escherichia coli*, *Klebsiella pnuemoniae*, *Pseudomonas aeruginosa* and *Candida albican*[36].

The MIC values obtained for the entire test organisms were very high when compared to the values of 0.01-10µg/ml usually recorded for typical antibiotics; these differences may be due to the fact that the extract used was in the impure form and would definitely contain substances which do not have antibacterial activities [37]. The MBC varied from the MIC, indicating that a different concentration is needed to inhibit the growth of the bacteria and an entirely different concentration to kill them [3,4].

The ethanol extracts of both leaf and stem bark was more effective against multiple resistant organisms, compared to the acetone extracts. This antimicrobial activity could be attributed to the presence of alkaloids and flavonoids in the plant [43].The mechanisms of resistance generally fall into three categories: antimicrobial-inactivating enzymes, reduced access to bacterial targets, and mutations that change targets or cellular functions [38].

The qualitative phytochemical screening of *Enantia chlorantha* leaf revealed the presence of medicinally active constituent such as cardiac glycoside, alkaloids, anthraquinone, steroids, tannin, saponin, phenol, and reducing sugar. This is similar to the results of Dawodu *et al.,* [39]who reported the presence of phenol, flavonoid, cardiac glycoside, alkaloids, reducing sugar and saponin, while tannin was not detected. On a phytochemical analysis of aqueous and ethanol extracts of *E. chlorantha* stem bark, Adesokan*et al,*[40]also reported the presence of phenolics, flavonoids, alkaloids, glycosides and saponins constituents [40].These biologically active constituents are known to act by different mechanism and exert antimicrobial action [41, 42]. Alkaloids are medicinally useful, possessing analgesic, antispasmodic and bactericidal effects. Flavonoids are hydroxylated phenolic substance known to be synthesized by plants in response to microbial infection and that is the reason they have been found *in-vitro* to be effective antimicrobial substances against a wide array of microorganisms. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell walls [43]. The antimicrobial property of saponin is due to its ability to cause leakage of proteins and certain enzymes from the cell [44]. Steroids have been reported to have antibacterial properties the correlation between membrane lipids and sensitivity for steroidal compound indicates the mechanism in which steroids specifically associate with membrane lipid and exerts its action by causing leakages from liposomes [45].

The quantitative phytochemical analysis of *E. chlorantha* stem bark and leaf using different solvents, showed the presence of different phytoconstituents in different quantities. For leaf and stem bark using methanol, flavonoid was shown to be present in the largest quantity while phlobatannins was present in the low quantity.Tannin was present in the highest quantity using ethyl acetate while the least was in

cardiac glycosides. This is in contrast to the results of Dawodu *et al.,* [39]which showed that alkaloid is 146.21%, cardiac glycoside to be 14.31%, phenol 26.41%, saponin 12.98% and flavonoid 7. 63% in ethanolic extract of the stem bark of *E. chlorantha*[46].

Jartrorrhizine-1 and canadine-1 present in *Enantia chlorantha* have docking scores of -2.267 and -1.625 respectivelyare greater than that of levofloxacin with docking score of -1.557. This implies that *Enantia chlorantha* is more effective at treating diseases caused by *Salmonella typhi.* The same plant contains other phytochemicals that are more effective than levofloxacin against *Staphylococcus aureus* and *Candida albican* from the results. The results supports the study of Lewis [47]. Previously, Lewis [47] extracted palmatine, coloumbamine and jatrorrhizine, which are alkaloids, as major phytochemicals with amphipaticcations from *E.chlorantha.* These amphipaticcation are said to be preferred substrates by most multidrug resistance pumps [47]. Earlier workers [48] also discovered that these phytochemicals intercalate DNA of microorganisms, as the mechanism of their antibacterial activity.

5. CONCLUSION

Due to the challenges associated with drug resistance which have made scientists to search for effective and sustainable means of managing the problem, plants have emerged as an alternative to synthetic antibiotics which is prone to reoccurring drug resistance. In conclusion, the promising evidence for the antimicrobial effects of *E. chlorantha* against bacterial and fungal isolates in this study especially the stem bark extract proved that *Enantia chlorantha* is more effective at treating diseases caused by *Salmonella typhi* and other organisms and therefore can be used as an alternative source of therapeutic agents and natural antimicrobial agents with potential applications in pharmaceutical industries for controlling pathogenic organisms.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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