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In Vitro Assessment of Stem Bark Extract of *Chrysophyllum Albidum* (G. Don) Against Bacterial Strains Implicated in Gastrointestinal Infections

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Abstract:- Gastrointestinal infections are a frequent cause of illness given the high global morbidity rate. This study examined the *in vitro* assessment of *Chrysophyllum albidum* stem bark extract against bacterial strains implicated in gastrointestinal infections. The antibacterial potentials of the fractions of *Chrysophyllum* stem bark extract were assessed using agar diffusion and dilution methods, time kill assay, potassium ions leakage and nucleotides leakage. The chemical constituents present in the fractions were determined using Fourier Transform Infrared Spectroscopy (FTIR) and Gas Chromatography-Mass Spectrometry (GC-MS). The inhibitory zones of the fractions at 10 mg/mL ranged from 10±0.82 mm to 27±0.00 mm. The minimum inhibitory concentrations of the fractions ranged from 0.31 mg/mL to 5 mg/mL while the minimum bactericidal concentrations ranged from 0.63 mg/mL to 10 mg/mL. The fractions at 1 x MIC leaked 0.16 µg/mL and 0.22 µg/mL of potassium ions and nucleotides respectively from bacterial isolates in 15 minutes while 66.7% of the bacterial isolates were eliminated. Similar trends of reactions were observed when the concentrations of the fractions were increased to 2 x MIC and 3 x MIC. The FTIR and GC-MS results showed that the compounds that were present in the fractions were biologically active. This study demonstrated significant biocidal potentials of *Chrysophyllum albidum* stem bark extract against bacterial strains implicated in gastrointestinal infections.

***Keywords*:-** *Gastrointestinal Infections, Chrysophyllum Albidum, Antibacterial Potentials, Time Kill Assay, Gas Chromatography -Mass Spectrometry*.

I. INTRODUCTION

Gastroenteritis, a gastrointestinal tract inflammation that affects the stomach and small intestine is caused by bacteria, virus and parasites (Burd and Hinrichs, 2015). The most common symptoms which can range in severity, include dehydration, anorexia, nausea, vomiting, fever, stomach discomfort and diarrhea (Mengelle *et al*., 2013). Dehydration is the main risk factor for gastroenteritis (Mengelle *et al*., 2013). The most well-known bacteria that can cause persistent gastrointestinal symptoms as well as other complications include *Shigella dysenteriae*, *Campylobacter jejuni*, *Salmonella typhi* and *Escherichia coli* (Rintala *et al*., 2016).

The leading cause of illness and mortality in humans worldwide is viral gastroenteritis, the most prevalent infectious cause of gastroenteritis (Rovida *et al*., 2016). They can spread quickly by person-to-person or fomite contact and are relatively persistent in the environment, especially in confined settings like hospitals, hostels, daycare centers and cruise ships (Burd and Hinrichs, 2015). Most commonly consuming contaminated food or water leads to gastrointestinal infections (Burd and Hinrichs, 2015). Travelers to nations where the disease is endemic run the danger of contracting these parasites because they are more prevalent there and in other tropical and subtropical regions of the world (Burd and Hinrichs, 2015).

The microorganisms that cause gastroenteritis differ depending on the geographical location, annual economic growth, sanitary conditions and hygienic standards (Burd and Hinrichs, 2015). Despite having the largest frequency in developing nations, gastrointestinal infections cause a significant percentage of morbidity worldwide (Sotelo-Coronado *et al*., 2016). In addition, gastrointestinal infections are a significant cause of death for children under the age of five, taking around two million lives annually (Moorin *et al*., 2010). In most parts of the world, even high-income countries with effective detection and control programs, gastrointestinal infections also contribute to economic loss (Moorin *et al*., 2010). Research is therefore required to develop more effective therapies that deal with both the infectious agents themselves as well as the long-term consequences of gastrointestinal infection on the gut and other organ systems throughout the body. In order to address the socio-economic and health effects caused by these infections, modern medicine must develop a novel antibiotic derived from natural products (Bakal *et al*., 2017). *Chrysophyllum albidum* (G. Don) is one of the therapeutic plants searched for by the scientists.

Chrysophyllum albidum (G. Don) referred to as the African Star apple, is a native plant. It is a member of the Sapotaceae family. It is commonly available in Nigeria, Uganda, Niger, Cameroon, and Cote d' ivoire (Duyilemi and Lawal, 2009; Adebayo *et al*., 2011). It is employed in Nigeria to treat a variety of illnesses (Adebayo *et al*., 2011).

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Previous research has also documented this plant's antibacterial, antioxidant and anti-inflammatory properties (MacDonald *et al*., 2014; Oputa *et al*., 2016). Due to the lack of information on plant extracts' ability to combat bacterial isolates implicated in gastrointestinal infections in Nigeria. Hence, this study was designed to evaluate the *in vitro* assessment of *Chrysophyllum albidum* (G. Don) stem bark extract against bacterial strains implicated in gastrointestinal infections.

II. MATERIALS AND METHODS

- *Ethical Approval*

Ethical approval with the approval number (NHREC/28/01/2020/AKTH/EC/2817) was obtained from the ministry of Health Research Ethical Committee, Aminu Kano Teaching Hospital, Kano, Kano State, Nigeria.

- *Collection of Bacterial Isolates*

Clinical strains and reference strains were both employed in this study. The clinical strains from stool samples were obtained from the culture collection of the Microbiology Laboratory at the Aminu Kano Teaching Hospital in Kano, Kano State, Nigeria. These strains comprised *Escherichia coli*, *Shigella dysenteriae* and *Salmonella typhi*. The reference strains were obtained from American Type Culture Collection (ATCC), Manassas, Virginia, United States of America. The reference strains are *Escherichia coli* (ATCC 25922), *Shigella dysenteriae* (ATCC 13313), and *Salmonella typhi* (ATCC 14208). The bacterial isolates' identities were determined through laboratory testing in the areas of culture, morphology, biochemistry and API 20E. The bacterial isolates were confirmed, sub-cultured in nutrient broth for 18 hours at 37 ^{o}C, and standardized to the 0.5 McFarland standard (10^{6} CFU/mL) before use.

- *Preparation of Culture Media*

Mueller Hinton agar medium was employed for the sensitivity tests while nutrient broth and nutrient agar medium were used for sub-culturing the test isolates. The bacterial isolates were re-confirmed using Salmonella Shigella agar, MacConkey agar, Eosin Methylene Blue agar and Xylose Deoxycholate agar.

- *Plant Sample Collection*

Fresh stem barks of *Chrysophyllum albidum* (G. Don) were collected in the month of December 2020 from a farm in Ile-Ife, Osun State, Nigeria. The stem barks were identified and authenticated at the Herbarium, Plant Biology Department, Bayero University, Kano, Kano State, Nigeria. A voucher specimen was prepared for the purpose of reference and deposited with the herbarium under the accession number BUKHAN 0522.

- *Drying and Extraction of the Plant Sample*

The plant sample was completely washed with tap water before being air dried in the shade, ground into a powder and sieved to produce fine ground particles. The powdered stem barks were employed in a precise amount of 1500 g and they were cold extracted over a period of four days using a 3:2 mixtures of methanol and sterile distilled water. Whatman's No. 1 filter paper was used to filter the collected supernatants and the filtrates were then concentrated *in vacuo* with a rotary evaporator to remove the methanol. The remaining aqueous residue was then lyophilized to provide 148.5 g of a crude dark brown extract.

- *Solvent Partitioning of the Stem Bark Extract of Chrysophyllum Albidum*

Exactly, 100 g of the stem bark extract were dissolved in exactly 250 mL of sterile distilled water. The mixture was partitioned into n-hexane, chloroform, ethyl acetate, n-butanol and aqueous in order of their polarity. The resultant n-hexane fraction was lyophilized after being concentrated *in vacuo* to dryness. The aqueous residue was kept in an airtight container. The aqueous residue was then further extracted with chloroform, ethyl acetate and n-butanol using the same technique. The aqueous fraction that was left over was concentrated, lyophilized and stored in the freezer for future use.

- *Antibiotic Susceptibility Test of Fractions against Bacterial Isolates*

The fractions' antibiotic susceptibility test was evaluated using the agar-well diffusion method (Hugo and Russell, 2015) with little modifications. The Mueller Hinton Agar (Oxoid, UK) was melted, then the standardized bacterial isolates (0.1 mL) were added, pour into sterile Petri dishes and allowed to set. The wells were then bored into the agar medium using a sterile cork borer with a 6 mm diameter. The wells were filled with 0.1 mL prepared solutions that were 10 mg/mL in concentration and considerable care was taken to prevent solution spillage onto the medium's surface. The plates were allowed to stand on the laboratory bench for about an hour before being incubated upright at 37 ^{o}C for 24 hours. Zones of inhibition were measured after incubation using the millimeter rule. Streptomycin and ampicillin, which acted as positive control and standard antibiotics at 1 mg/mL each were used to compare the susceptibility of the bacterial isolates to the extracts. The experiment was run in triplicates and a negative control of 10% Dimethyl sulfoxide (DMSO) was used.

- *Determination of Minimum Inhibitory Concentrations*

The MICs of the fractions were determined by employing agar dilution method (EUCAST, 2000; Akinpelu *et al*., 2015). Two-fold dilution was made in sterile distilled water, and 2 mL of various aliquot concentrations were added to get a final concentration that varied from 0.16 mg/mL to 10 mg/mL. The medium was then poured into clean Petri dishes and allowed to set. The medium surfaces were allowed to dry before streaking with standardized bacterial isolates that were 18 hours old. The plates were examined to see if any growth had taken place after being incubated at 37 ^{o}C for up to 48 hours. The control was a sterile agar medium plate without the extract. The minimum inhibitory concentrations were determined to be the lowest concentration at which the bacteria could not grow.

➢ *Determination of Minimum Bactericidal Concentrations*

The Akinpelu *et al*. (2015) method was used to determine the MBCs of the fractions. The line of streak on the MIC plates that did not show any signs of growth was picked and it was subsequently sub-cultured onto freshly prepared Nutrient agar plates and incubated at 37 ºC for 48 hours. The minimum bactericidal concentrations of the fractions were taken to be the lowest concentration of extract that did not result in any growth on a fresh set of plates.

➢ *Determination of Rate of Kill of Bacterial Isolates by the Fractions*

The method of Odenholt *et al*. (2004) was slightly modified and used to carry out the rate of kill of bacterial isolates by the fractions. This was done on representative isolates of *Escherichia coli* and *Klebsiella pneumoniae*. The bacterial isolates' nutrient broth cultures (18–24 hours) were standardised and viable counts were determined. Precisely, 0.5 mL of a bacterial suspension with a known cell density (10^6 CFU/mL) and 4.5 mL of various concentrations of fractions in respect to the MIC were mixed. The killing rate was assessed over a 2 hours' period after the suspension had been fully mixed and kept at room temperature (28-30 SºC). To counteract the effects of the antimicrobial compounds that were transferred from the test suspensions, precisely 0.5 mL of each solution was taken out at the appropriate intervals and introduced to 4.5 mL of Nutrient broth recovery medium containing 3% Tween 80. The suspension was serially diluted in sterile physiological saline (0.9% NaCl) after being thoroughly shaken. Exactly, 0.1 mL of the final dilution was plated onto Nutrient agar plates and incubated at 37 ºC for 48 hours. The extract was not used in the control experiment's setup. For each sample, viable counts were performed in triplicate. The decrease in the viable counts indicated killing by the extract.

➢ *Determination of Potassium Ions Leakage*

Oladunmoye *et al*. (2007) method was used for the assay with little modifications. Test cells from representative isolates that were incubated for 18 hours were standardized to 0.5 McFarland's standard after being washed three times in 0.9% w/v NaCl (normal saline). Standardized washed suspensions of the representative isolates were exposed to various concentrations of the n-butanol fraction in comparison to the MICs over a period of 2 hours. Each sample was centrifuged at 10,000 rpm for 15 minutes and the supernatants were collected and assayed for potassium ions using an atomic absorption spectrophotometer at 260 nm wavelength. The standard curve was used to determine the percentage of potassium ions that was leaked. Sterile distilled water inoculated with the same quantity of inoculum without fractions was used as control.

➢ *Determination of Nucleotides Leakage*

The method described by da Silva Jr. *et al*. (2014) and Yang *et al*. (2015) was adopted to determine the nucleotides leakage from the bacterial isolates with little adjustments. Test cells from an 18-hour-old nutrient broth culture of the representative isolates were washed three times in 0.9% w/v NaCl (normal saline). Standardized washed suspensions of the representative isolates (0.5 McFarland) were exposed to various concentrations of the n-butanol fraction in comparison to the MICs over a period of 2 hours. Ultraviolet spectrophotometer was used to measure the absorbance of the supernatant collected after each suspension was centrifuged at 10,000 rpm for 15 minutes at 260 nm wavelength. Sterile distilled water was inoculated with the same amount of inoculum but without fractions as a control.

➢ *Fourier Transform Infrared Spectroscopy Analysis*

The fractions' functional groups were determine using FTIR (Fourier-transform infrared spectrometer) analysis. The spectra of each of the fractions were obtained using the FTIR Spectrometer (Agilent technologies). The annotated spectrum demonstrates that the chemical bond is significantly influenced by the wavelength of light that is absorbed. The analysis of infrared absorption spectra can be used to identify the chemical bonds that are present in a compound.

➢ *Gas Chromatography-Mass Spectrometry (GC-MS) Analysis*

Gas chromatography-mass spectrometry (GC-MS) was used to examine the n-butanol fraction using a GC-MS Model, QP 2010 PLUS, Shimadzu, Japan. The system includes a fused silica capillary column with a 30 m length, 0.25 mm internal diameter, and a 0.25 µm film thickness. The split ratio was 10:1, the injection volume was 2 µL and the flow rate of the carrier gas, helium (99.999% purity) was maintained at 1.0 mL/min. The injector temperature was maintained at 250 °C while the ion-source temperature was 200 °C. The oven's temperature was set to fluctuate between 80 °C and 280 °C for two minutes. Ionization was observed at a mass spectral energy of 70 eV. The solvent delay ranged from 0 to 6 minutes and the mass range from 40 to 1000 m/z was scanned at a rate of 0.5 seconds. A split injection technique was used to manually inject one microliter (1.0 µL) of the extract into the GC-MS for total ion chromatographic analysis (TIC) analysis. The GC-MS ran for a total of 27 minutes. The relative percentage of the fraction's constituents was determined by comparing the average peak area to total area. The National Institute of Standards and Technology (NIST) database and the Fatty Acids Methyl Esters Library version 1.0 (FAME library) were used to identify the chemical constituents present in the extract.

➢ *Statistical Analysis*

The results were statistically analyzed using the means ± SD (standard deviation) of three replicates. At $p \leq 0.05$, values were deemed significant.

III. RESULTS

Chrysophyllum albidum's stem bark extract was partitioned into three fractions: aqueous, ethyl acetate and n-butanol. This reveals that n-hexane and chloroform, which are not the best organic solvents, cannot be used to extract *Chrysophyllum albidum* stem bark extract of its bioactive constituents. The antibiotic susceptibility test revealed that 93%, 89%, and 96% of bacterial isolates were susceptible to

ISSN No:-2456-2165

aqueous, ethyl acetate, and n-butanol fractions at 10 mg/mL with varying degrees of zones of inhibition (Table 1). The fractions' zones of inhibition against the bacterial isolates ranged from 10±0.82 mm to 27±0.00 mm. *Salmonella typhi* (ST1) was not susceptible to any of the fractions at this concentration, however the n-butanol fraction showed the largest zone of inhibition (27±0.00 mm) against *Escherichia coli* (EC7) at this concentration. While some bacterial isolates were susceptible to the standard antibiotics employed in this study at 1 mg/mL, others were not. *Shigella dysenteriae* (83.3%) showed the highest ampicillin resistance followed by *Salmonella typhi* (45.5%) and *Escherichia coli* (36.4%). Streptomycin and ampicillin inhibited bacterial isolates growth by 68% and 50% at 1 mg/mL (Table 1). This study demonstrated that almost all of the bacterial isolates obtained from clinical samples were susceptible to the fractions.

Table 1 Sensitivity Patterns Exhibited by the Fractions against Bacterial Isolates

	Zones of inhibition (mm)**					
Strains code	**Butanol (10 mg/mL)**	**Aqueous (10 mg/mL)**	**Ethyl acetate (10 mg/mL)**	**Streptomycin (1 mg/mL)**	**Ampicillin (1 mg/mL)**	**DMSO (10%)**
EC1	17±0.82	19±0.82	15±0.82	10±0.82	0	0
EC2	12±0.00	10±0.82	10±1.63	0	18±1.63	0
EC3	15±0.82	13±0.00	11±0.82	24±0.82	22±0.00	0
EC4	18±1.63	16±0.82	16±0.00	23±0.82	0	0
EC5	20±1.63	18±1.63	14±0.82	24±1.63	12±0.00	0
EC6	16±0.00	14±0.82	12±0.82	0	20±0.82	0
EC7	27±0.00	22±0.82	20±0.00	26±0.82	28±0.82	0
EC8	23±0.82	19±0.82	17±0.82	22±0.82	26±0.00	0
EC9	18±0.82	16±0.00	14±1.63	0	0	0
EC10	14±0.00	14±1.63	16±1.63	16±0.82	0	0
EC11	14±0.82	12±0.82	0	24±0.82	22±0.00	0
ST1	0	0	0	13±0.82	0	0
ST2	19±0.82	17±0.82	15±0.82	20±0.82	0	0
ST3	15±0.00	12±1.63	12±0.82	10±0.00	20±0.82	0
ST4	16±1.63	14±0.82	12±0.00	13±0.82	22±0.82	0
ST5	12±0.82	12±0.00	10±0.00	0	0	0
ST6	16±0.82	13±0.82	11±0.82	12±0.00	20±1.63	0
ST7	22±0.82	17±1.63	15±0.82	24±0.82	26±0.00	0
ST8	16±0.82	16±1.63	13±1.63	0	22±0.82	0
ST9	14±0.82	10±0.82	12±0.82	0	0	0
ST10	20±1.63	18±1.63	16±0.82	23±0.82	21±0.82	0
ST11	18±0.00	16±0.82	14±1.63	0	0	0
SDI	14±0.82	12±0.82	10±0.82	0	0	0
SD2	16±0.82	14±0.82	12±1.63	21±0.82	19±0.82	0
SD3	11±1.63	13±1.63	0	25±0.00	0	0
SD4	16±0.82	14±1.63	11±1.63	18±0.00	0	0
SD5	25±0.00	16±0.82	12±1.63	26±0.00	0	0
SD6	14±1.63	0	12±0.00	0	0	0

- ***Key*: EC1-EC10 = Strains of *Escherichia coli*, EC11 = *Escherichia coli* (ATCC 25922), ST1-ST10 = Strains of *Salmonella typhi*, ST11 = *Salmonella typhi* (ATCC 14028), SD1-SD5 = Strains of *Shigella dysenteriae*, SD6 = *Shigella dysenteriae* (ATCC 13313), ATCC = American type culture collection, 0 = Not sensitive, mm* = mean of three replicates, P≤ 0.05, DMSO = Dimethyl sulfoxide**

The minimum inhibitory concentrations and minimum bactericidal concentrations were also studied. The fractions used in this study expressed a range of MICs and MBCs against the bacterial isolates as shown in Table 2. The MICs for the n-butanol fractions ranged from 0.31 mg/mL to 5 mg/mL while the MICs for the aqueous and ethyl acetate fractions ranged from 1.25 mg/mL to 5 mg/mL. The n-butanol fraction exhibited the lowest MIC (0.31 mg/mL) with respect to *Escherichia coli* (EC7). The MBCs for n-butanol fraction ranged from 0.63 mg/mL to 10 mg/mL while the MBCs in the aqueous and ethyl acetate fractions ranged from 2.5 mg/mL to 10 mg/mL. The lowest MBC (0.63 mg/mL) was expressed against *Escherichia coli* (EC7) (Table 2).

ISSN No:-2456-2165

Table 2 Minimum Inhibitory and Bactericidal Concentrations of the Fractions of Chrysophyllum Albidum Stem bark Extract against Bacterial Isolates

	Butanol (mg/mL)		Aqueous (mg/mL)		Ethyl acetate (mg/mL)	
Bacterial Isolates	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
EC1	1.25	1.25	1.25	2.5	1.25	2.5
EC2	2.5	5	5	10	5	10
EC3	5	10	2.5	5	5	5
EC4	1.25	2.5	2.5	5	2.5	5
EC5	2.5	5	2.5	5	5	10
EC6	1.25	1.25	2.5	2.5	2.5	5
EC7	0.31	0.63	1.25	2.5	1.25	2.5
EC8	1.25	2.5	2.5	5	2.5	5
EC9	1.25	2.5	2.5	5	5	10
EC10	1.25	1.25	2.5	2.5	5	10
EC11	0.63	1.25	1.25	2.5	ND	ND
ST1	ND	ND	5	10	ND	ND
ST2	2.5	5	5	10	2.5	5
ST3	2.5	5	5	10	5	10
ST4	1.25	2.5	2.5	5	5	10
ST5	2.5	5	2.5	5	5	10
ST6	1.25	2.5	2.5	5	2.5	5
ST7	5	10	5	10	2.5	5
ST8	2.5	2.5	2.5	5	5	10
ST9	1.25	2.5	2.5	5	5	10
ST10	1.25	1.25	2.5	5	2.5	5
ST11	2.5	5	2.5	5	5	10
SD1	2.5	5	2.5	5	5	10
SD2	1.25	2.5	2.5	5	2.5	5
SD3	5	10	5	10	ND	ND
SD4	1.25	2.5	2.5	5	5	10
SD5	1.25	2.5	1.25	2.5	2.5	5
SD6	2.5	5	5	10	5	10

- *Key:* EC1-EC10 = Strains of *Escherichia coli*, EC11 = *Escherichia coli* (ATCC 25922), ST1-ST10 = Strains of *Salmonella typhi*, ST11 = *Salmonella typhi* (ATCC 14028), SD1-SD5 = Strains of *Shigella dysenteriae*, SD6 = *Shigella dysenteriae* (ATCC 13313), ATCC = American type culture collection, ND = Not determined

The modes of action were assessed using the time kill assay, potassium ions leakage, and nucleotides leakage from bacterial isolates by the fractions. *Escherichia coli* and *Klebsiella pneumoniae* were employed as representative isolates. At a concentration of 1 x MIC after 15 minutes of contact time, rate of kill of *Escherichia coli* by the fractions partitioned into aqueous, ethyl acetate and n-butanol were 59.1%, 60.8%, and 66.7%, respectively (Figures 1, Figure 2, and Figure 3). The percentage of bacterial isolates killed by the aqueous, ethyl acetate, and n-butanol fractions after 60 minutes of contact rose to 88.7%, 85.0% and 88.2% respectively. With increase in contact time and fractions concentrations, these bacterial isolates were killed more quickly. The percentage of bacterial isolates killed by the aqueous, ethyl acetate, and n-butanol fractions at 1 x MIC increased to 100%, 100%, and 100% respectively when the contact time was extended to 120 minutes. When the concentrations of the fractions were increased to 2 x MIC and 3 x MIC, similar trends of reactions were observed. Similarly, after 15 minutes of contact time, the killing rates of *Klebsiella pneumoniae* by the fractions partitioned into aqueous, ethyl acetate, and n-butanol were 52.5%, 57.1%, and 66.2% respectively (Figures 4, Figure 5 and Figure 6). The percentage of bacterial isolates killed by the aqueous, ethyl acetate, and n-butanol fractions after 60 minutes of contact time rose to 82.3%, 88.4%, and 87.4%, respectively. With increase in contact time and fractions concentrations, these bacterial isolates were killed more quickly. When the contact time was increased to 120 minutes, the percentage of bacterial isolates killed by the aqueous, ethyl acetate and n-butanol fractions at 1 x MIC increased to 98.5%, 96.5%, and 100% respectively. When the concentrations of the fractions were increased to 2 x MIC and 3 x MIC, similar trends of reactions were observed.

ISSN No:-2456-2165

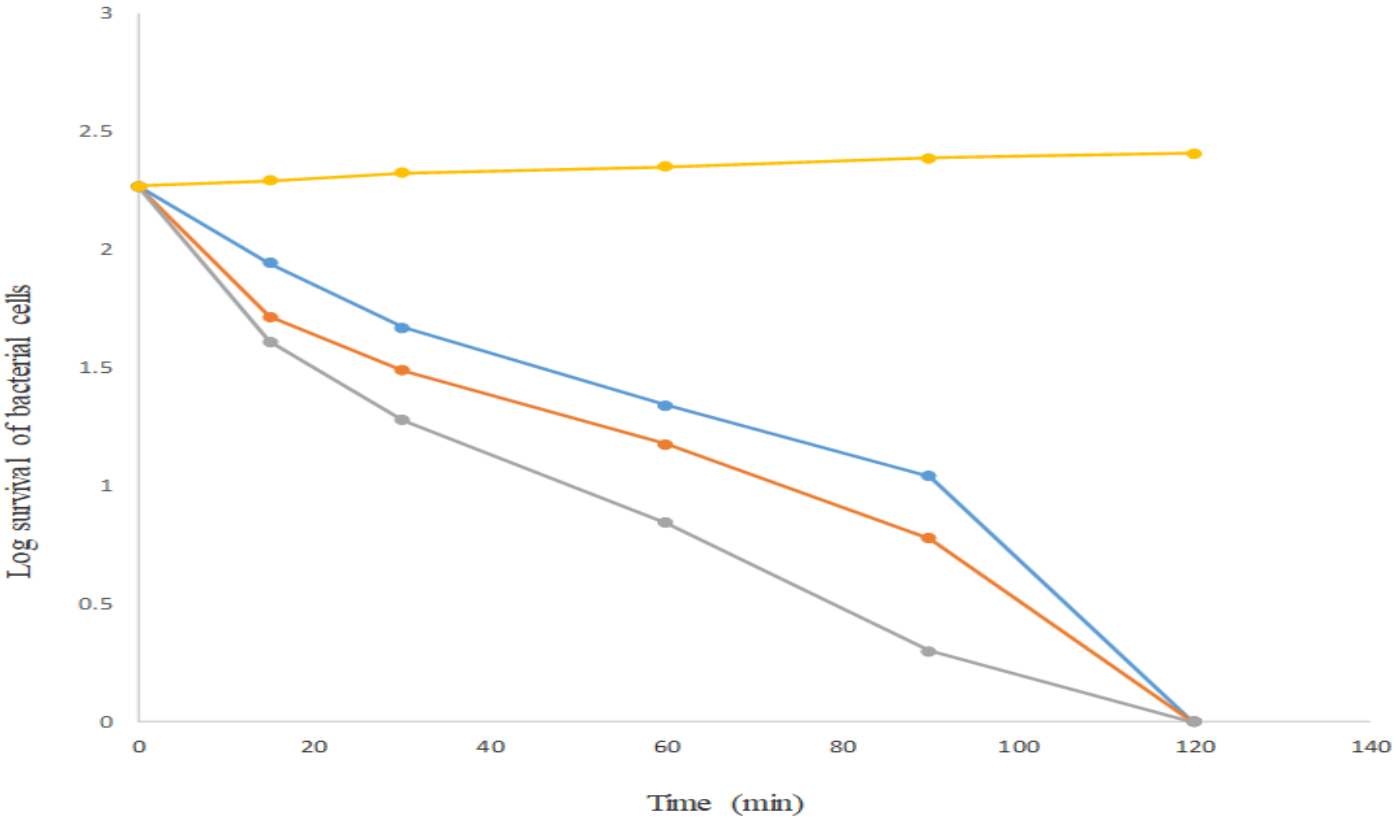

Fig 1 The rate of killing of *Escherichia coli* by n-butanol fraction of *Chrysophyllum albidum* stem bark extract at 1 x MIC ( ), 2 x MIC ( ), 3 x MIC ( ) and control ( ). Each point represents the $\log_{10}$ of mean survival of bacterial cells at a particular time interval in the presence of the fraction.

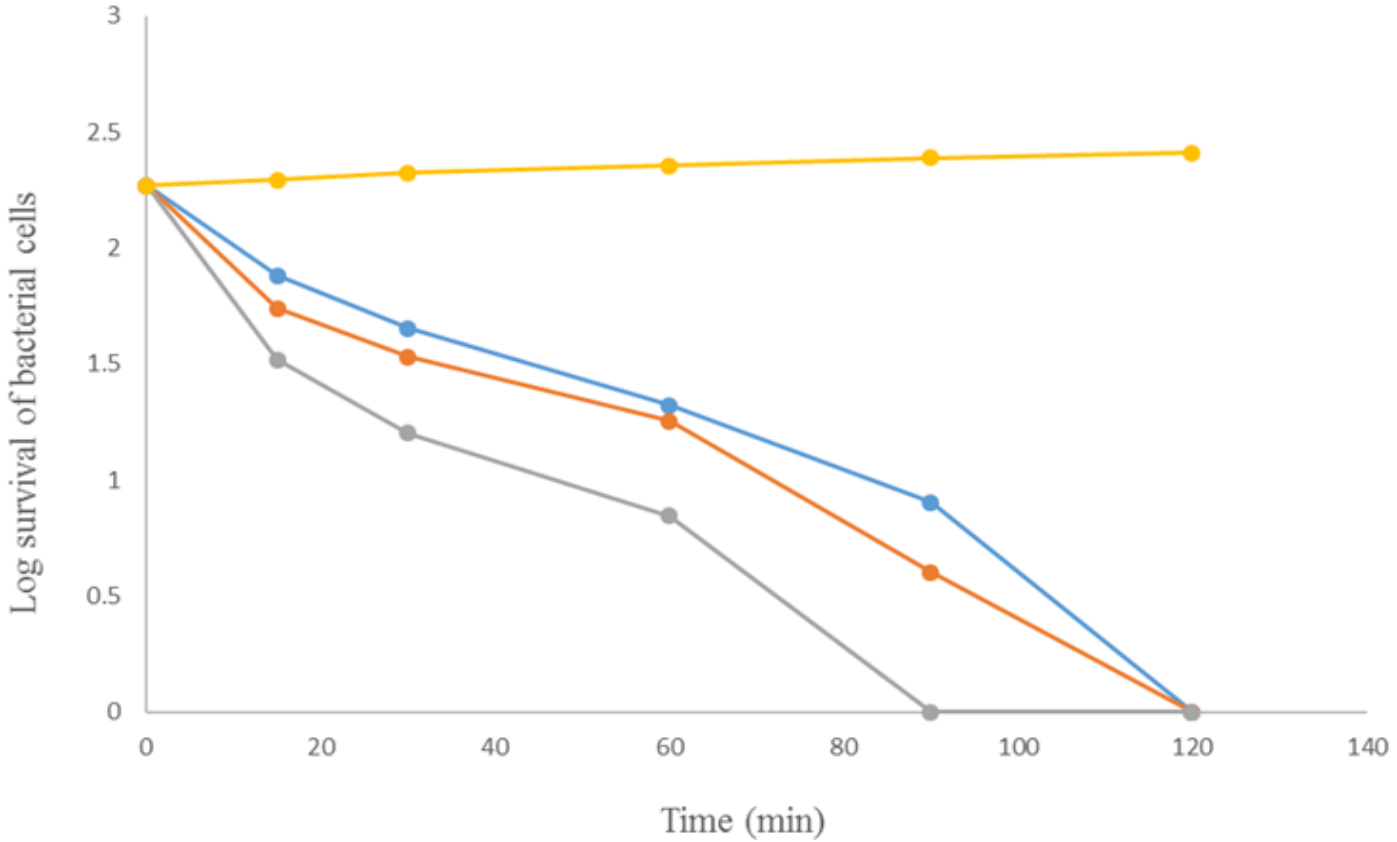

Fig 2 The rate of killing of *Escherichia coli* by aqueous fraction of *Chrysophyllum albidum* stem bark extract at 1 x MIC ( ), 2 x MIC ( ), 3 x MIC ( ) and control ( ). Each point represents the $\log_{10}$ of mean survival of bacterial cells at a particular time interval in the presence of the fraction.

ISSN No:-2456-2165

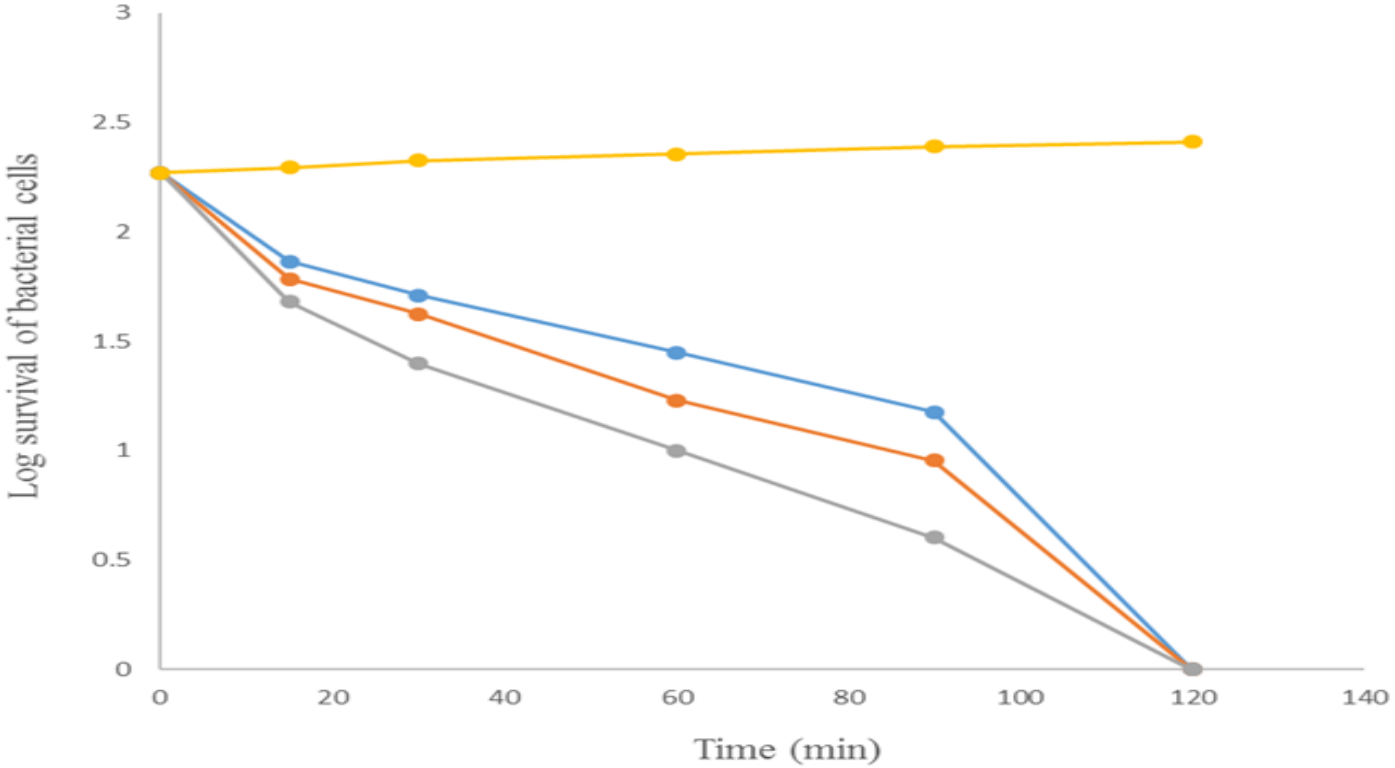

Fig 3 The rate of killing of *Escherichia coli* by ethyl acetate fraction of *Chrysophyllum albidum* stem bark extract at 1 x MIC ( ), 2 x MIC ( ), 3 x MIC ( ) and control ( ). Each point represents the $\log_{10}$ of mean survival of bacterial cells at a particular time interval in the presence of the fraction.

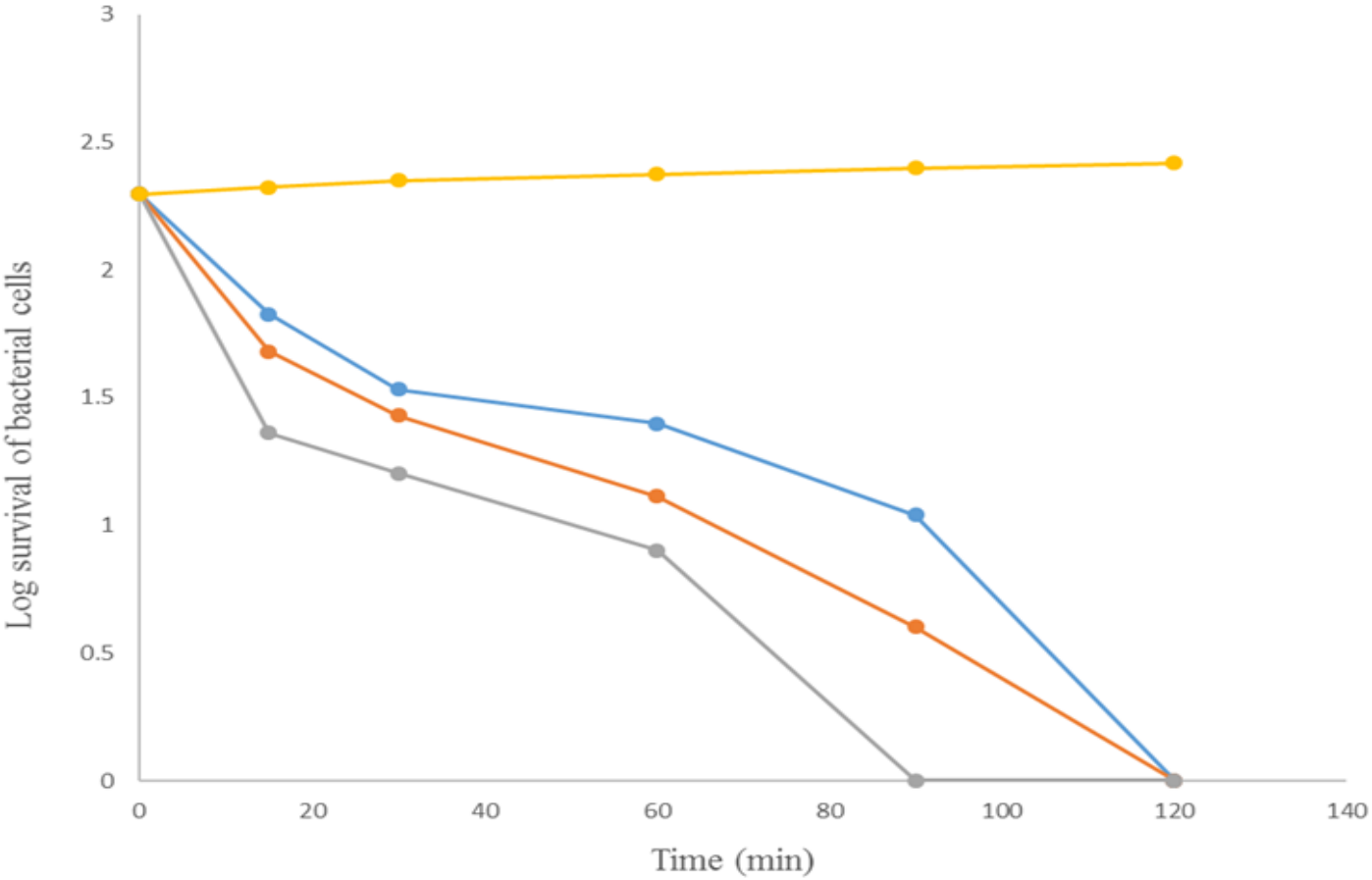

Fig 4 The rate of killing of *Klebsiella pneumoniae* by n-butanol fraction of *Chrysophyllum albidum* stem bark extract at 1 x MIC ( ), 2 x MIC ( ), 3 x MIC ( ) and control ( ). Each point represents the $\log_{10}$ of mean survival of bacterial cells at a particular time interval in the presence of the fraction.

ISSN No:-2456-2165

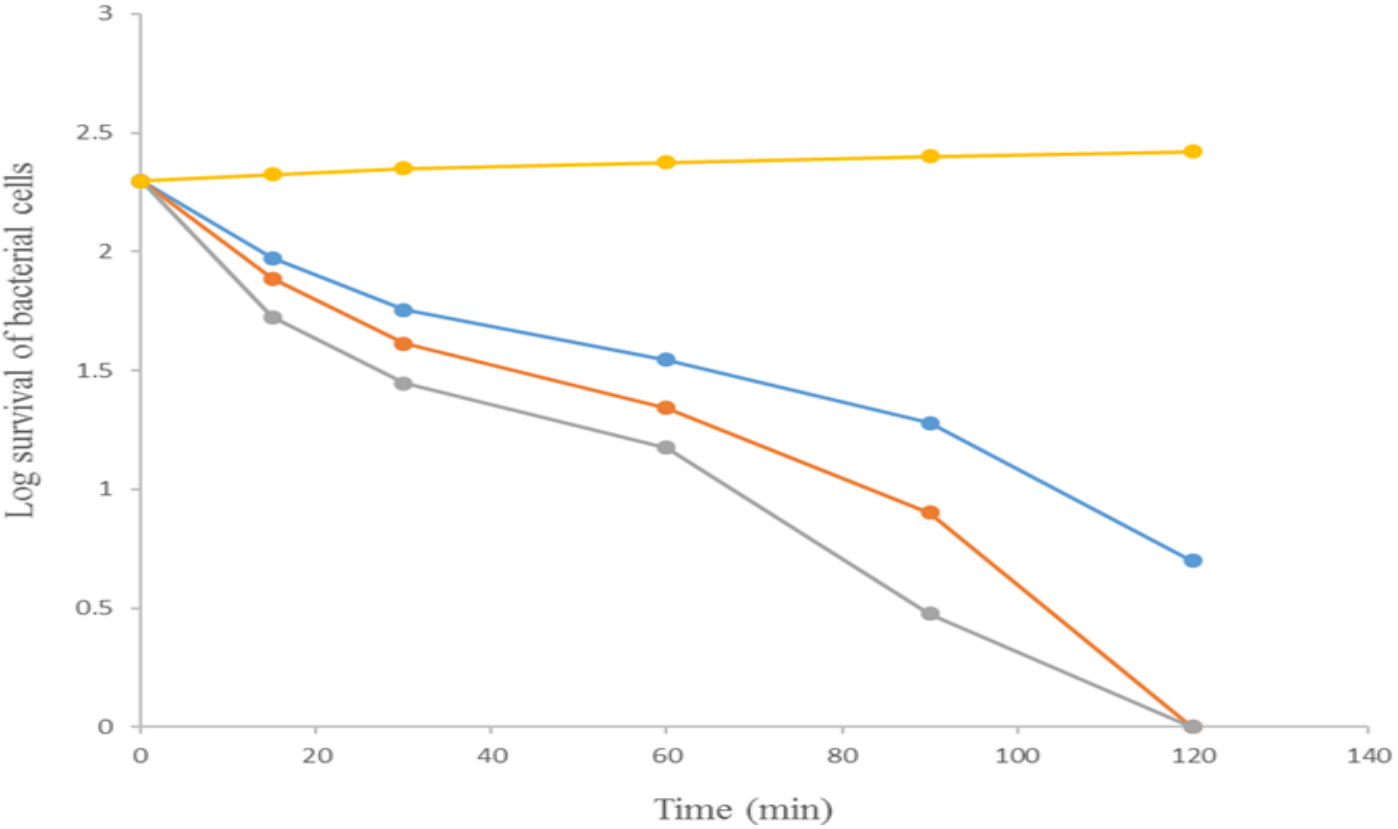

Fig 5 The rate of killing of *Klebsiella pneumoniae* by aqueous fraction of *Chrysophyllum albidum* stem bark extract at 1 x MIC ( ), 2 x MIC ( ), 3 x MIC ( ) and control ( ). Each point represents the $\log_{10}$ of mean survival of bacterial cells at a particular time interval in the presence of the fraction.

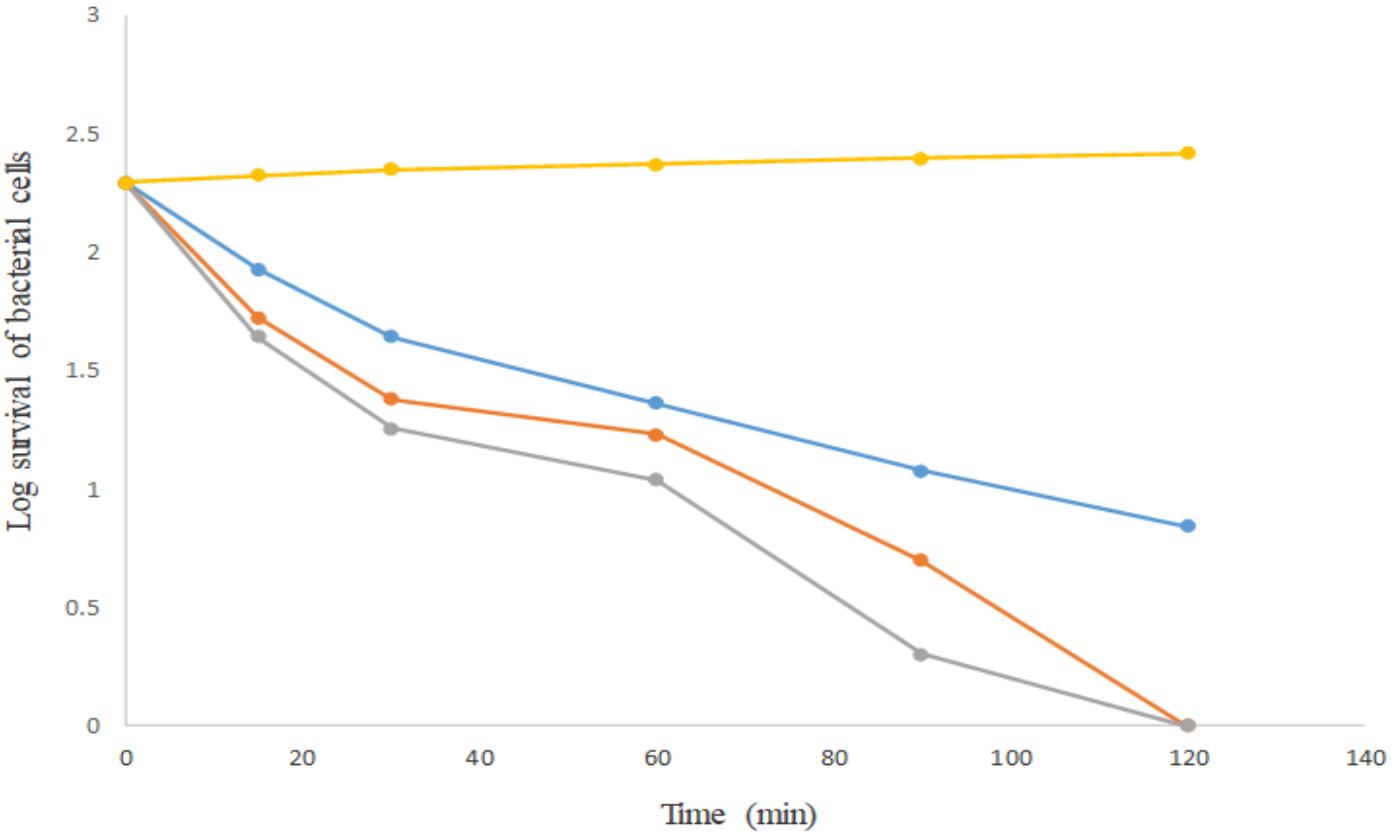

Fig 6 The rate of killing of *Klebsiella pneumoniae* by ethyl acetate fraction of *Chrysophyllum albidum* stem bark extract at 1 x MIC ( ), 2 x MIC ( ), 3 x MIC ( ) and control ( ). Each point represents the $\log_{10}$ of mean survival of bacterial cells at a particular time interval in the presence of the fraction.

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The amounts of potassium ions leaked from *Escherichia coli* and *Klebsiella pneumoniae* by n-butanol fraction (most active fraction) were 0.22 µg/mL and 0.51 µg/mL respectively after 15 minutes of interaction (Figure 7 and Figure 8). When the contact time was raised to 30, 60, 90, and 120 minutes, there was an increase in the amounts of potassium ions leaked from the bacterial isolates by the fraction. At 1 x MIC for 120 minutes of contact time, the potassium ions leak rates from *Escherichia coli* and *Klebsiella pneumoniae* were 1.59 µg/mL and 2.22 µg/mL respectively. When the fraction' concentrations were increased to 2 x MIC and 3 x MIC, the same reactive patterns were observed. Similarly, the fraction leaked 0.16 µg/mL and 0.32 µg/mL of nucleotides respectively from *Escherichia coli* and *Klebsiella pneumoniae* after 15 minutes of contact time (Figures 9 and Figure 10). The amounts of nucleotides that were leaked from the bacterial isolates by the fraction increased when the contact time was increased to 30, 60, 90, and 120 minutes. When the contact time was increased to 120 minutes, the fraction at 1 x MIC leaked 0.71 µg/mL and 1.26 µg/mL of nucleotides from the bacterial isolates respectively. When the fraction' concentrations were increased to 2 x MIC and 3 x MIC, the same reactive patterns were observed.

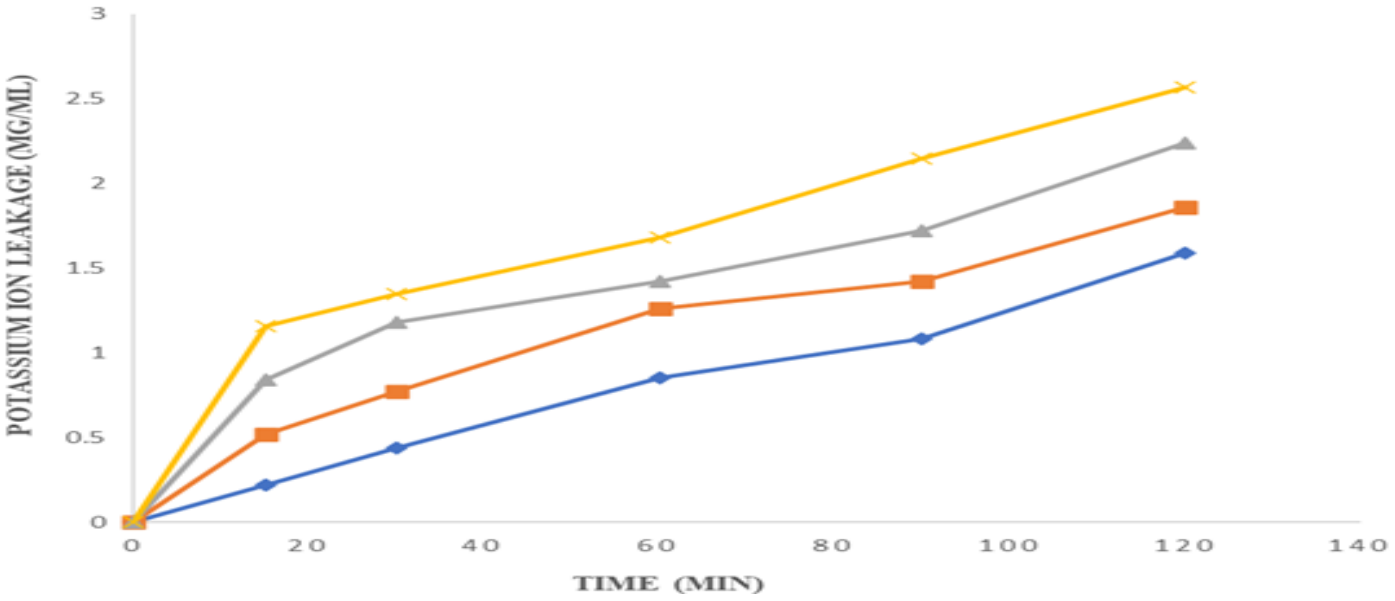

Fig 7 Leakage of potassium ions from *Escherichia coli* by n-butanol fraction at 1 x MIC ( ), 2 x MIC ( ), 3 x MIC ( ) and control ( ). Each point represents the quantity of potassium ions leaked (µg/mL) from the bacterial strains at a particular time interval in the presence of the fraction.

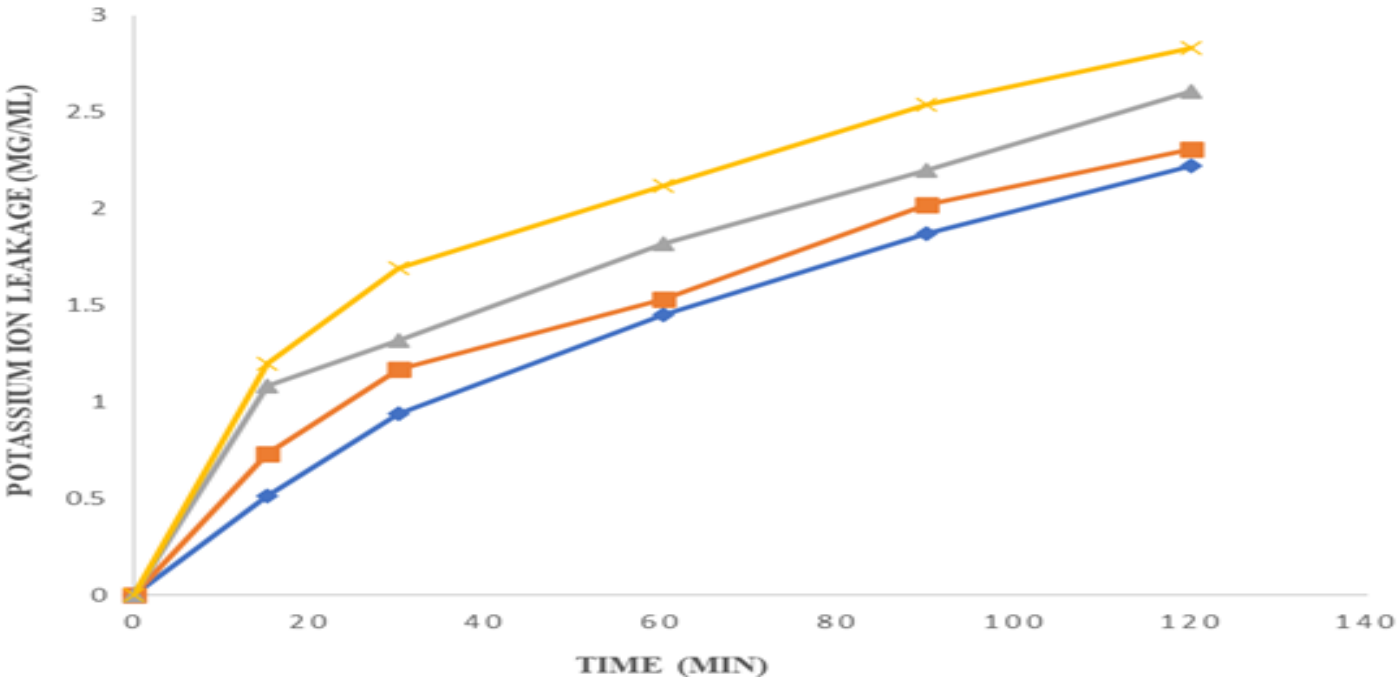

Fig 8 Leakage of potassium ions from *Klebsiella pneumoniae* by n-butanol fraction at 1 x MIC ( ), 2 x MIC ( ), 3 x MIC ( ) and control ( ). Each point represents the quantity of potassium ions leaked (µg/mL) from the bacterial strains at a particular time interval in the presence of the fraction.

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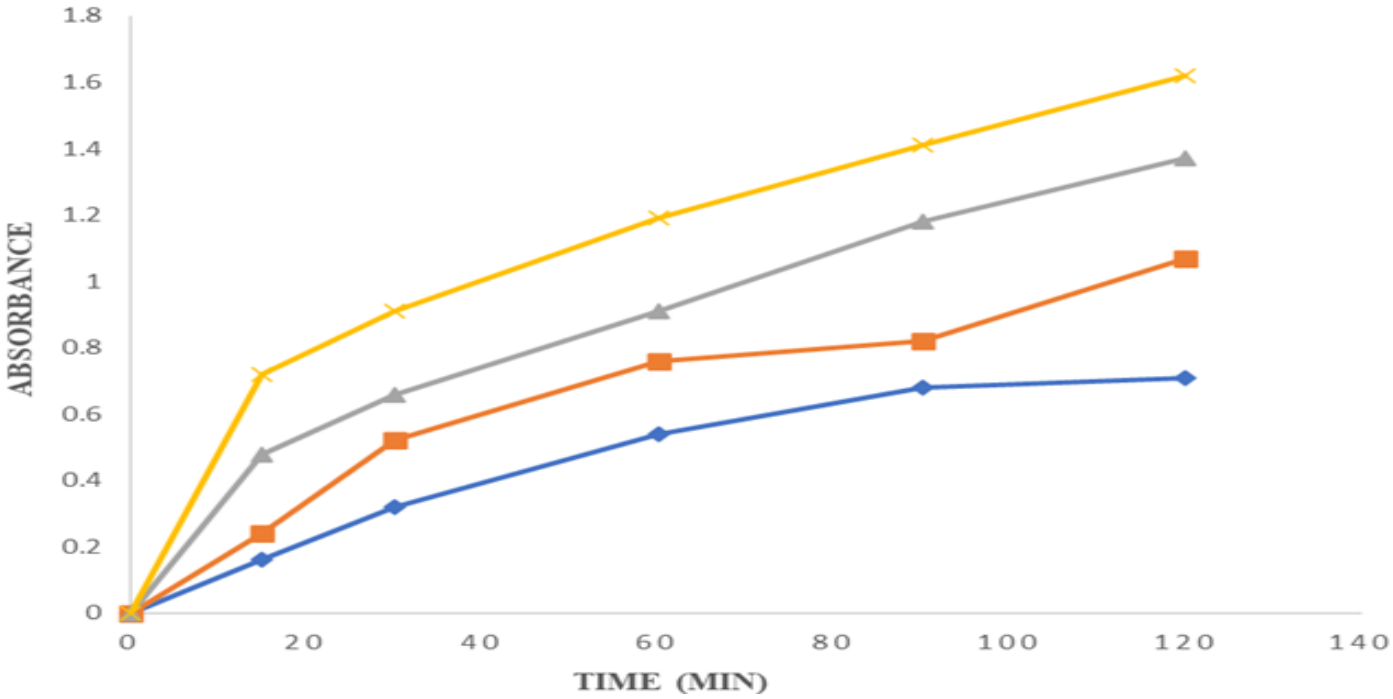

Fig 9 Leakage of nucleotides from *Escherichia coli* by n-butanol fraction at 1 x MIC ( ), 2 x MIC ( ), 3 x MIC ( ) and control ( ). Each point represents the quantity of potassium ions leaked (µg/mL) from the bacterial strains at a particular time interval in the presence of the fraction.

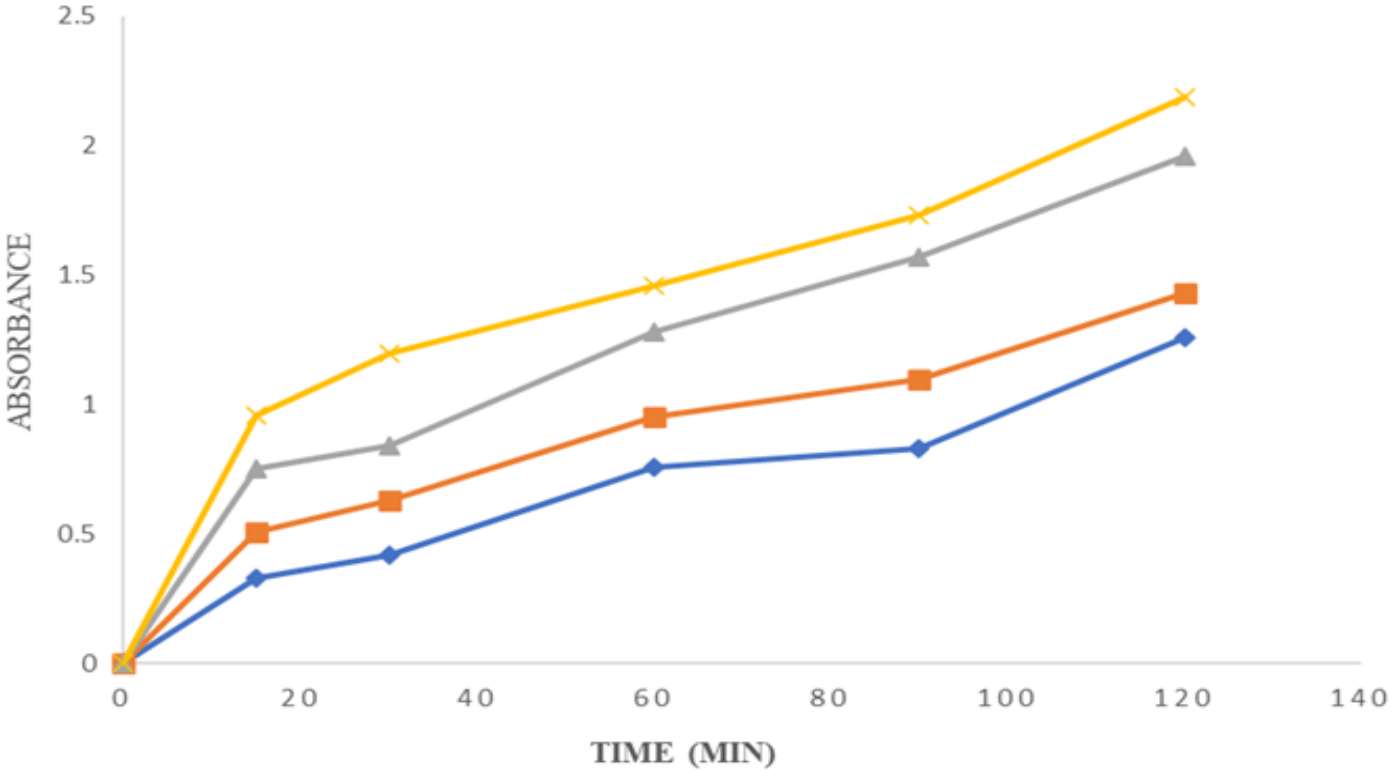

Fig 10 Leakage of nucleotides from *Klebsiella pneumoniae* by n-butanol fraction at 1 x MIC ( ), 2 x MIC ( ), 3 x MIC ( ) and control ( ). Each point represents the quantity of potassium ions leaked (µg/mL) from the bacterial strains at a particular time interval in the presence of the fraction.

The functional groups present in the fractions of the stem bark extract of *Chrysophyllum albidum* were identified by FTIR analysis based on the peak values in the infra-red radiation region. The spectra from the FTIR analyses of the fractions of the stem bark extract are shown in Figure 11 to Figure 13. Alcohol, phenol, alkanes, alkenes, aliphatic ether, amines, aromatics, carboxylic acids, esters, and sulfoxides were among the functional groups identified by FTIR analysis of the plant extract's active fractions (Table 3).

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Table 3 FTIR Analysis of the Potent Fractions of the Chrysophyllum Albidum Stem Bark Extract

Functional groups	Functional groups description	BUT	ETH	AQU
		Absorption Frequency (cm-1)		
Alcohol, Phenol	O-H bending	1343	1346	-
	O-H bending	1402	-	1406
	O-H Stretching	3242	3235	3223
Alkanes	C-H stretching	2933	-	2936
	CH_3 bending	1454	1451	1406
Alkenes	C-H out of plane bend	747	747	870
	C=C Stretching	1614	1607	1611
Aliphatic ether	C-O stretching	-	1097	-
Amines	C-N stretching	1164	1145	1156
	C-N stretching	-	1197	-
Aromatics	C-H out of plane bend	747	747	870
	C=C stretching	1525	1518	1529
Carboxylic acids	C=O stretching	1722	1722	-
Esters	C-O stretching	1205	1283	1208
Sulfoxides	S=O stretching	1033	1037	1033

➢ *Key*: BUT= n-Butanol fraction, ETH= Ethyl acetate fraction, AQU=Aqueous fraction

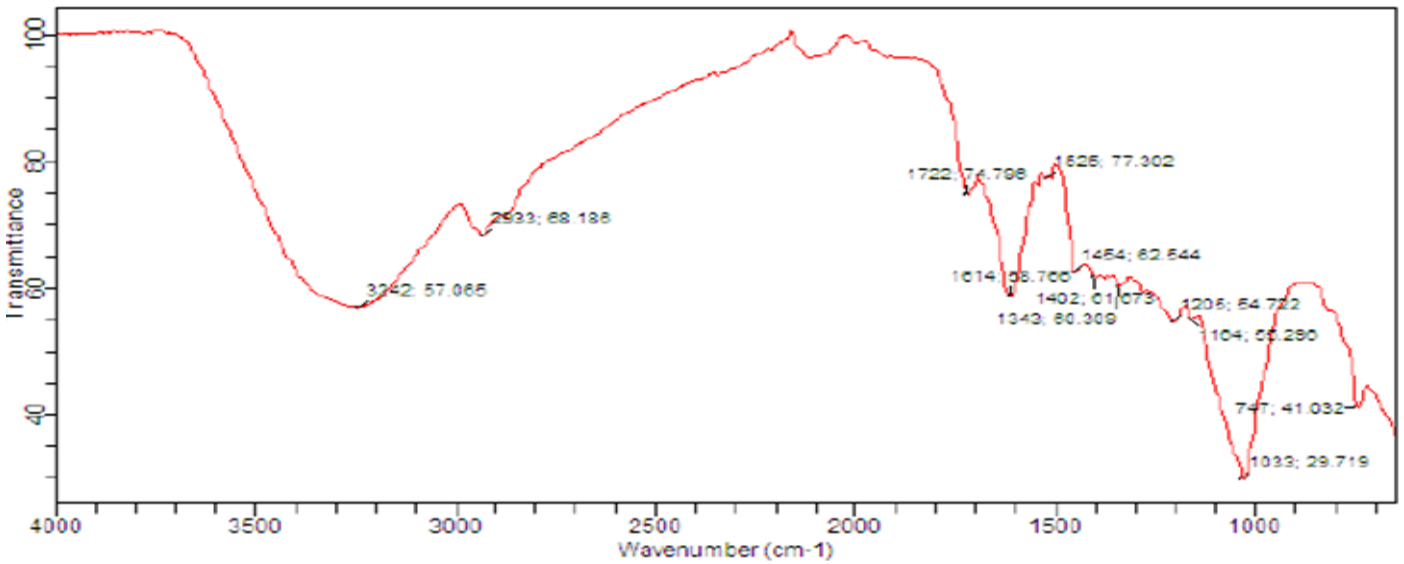

Fig 11 FTIR analysis of the n-butanol fraction of *Chrysophyllum albidum* stem bark extract

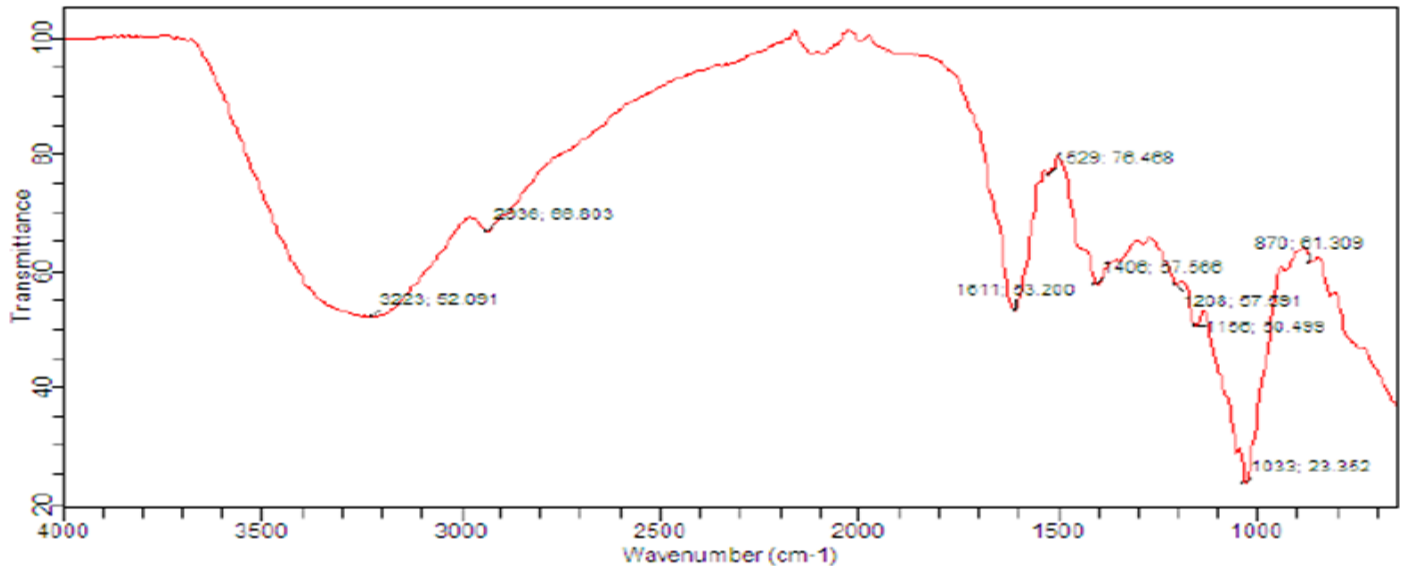

Fig 12 FTIR analysis of the aqueous fraction of *Chrysophyllum albidum* stem bark extract

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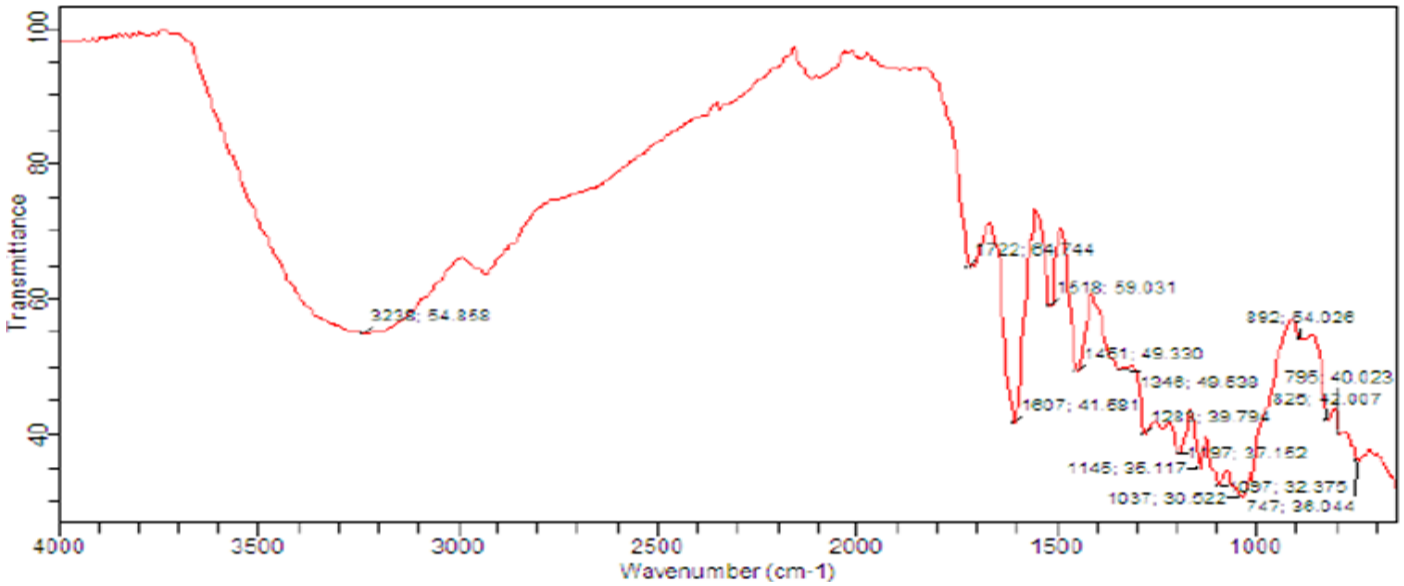

Fig 13 FTIR analysis of the ethyl acetate fraction of *Chrysophyllum albidum* stem bark extract

Gas chromatography-mass spectrometry (GC-MS) was used to analyzed the n-butanol fraction of *Chrysophyllum albidum* stem bark extract. A variety of bioactive constituents with various retention times (see Fig. 14) were discovered in the fraction. The bioactive constituents are listed in Table 4, along with their peak area (%), retention time, molecular formula and molecular weight. The predominant bioactive constituent of the fraction was n-Hexadecanoic acid.

Table 4 GC-MS properties of n-butanol fraction of *Chrysophyllum albidum* stem bark extract

S/NO	Retention Time	Peak Area %	Compound Name	Molecular Formula	Molecular Weight(G/Mol)
1	12.016	53.36	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256
2	14.138	46.64	Bicyclo [3.1.1] heptane, 2,6,6,trimethyl-,[1S (1.alpha,2.beta.,5.alpha.)]-	$C_{10}H_{38}$	138.25

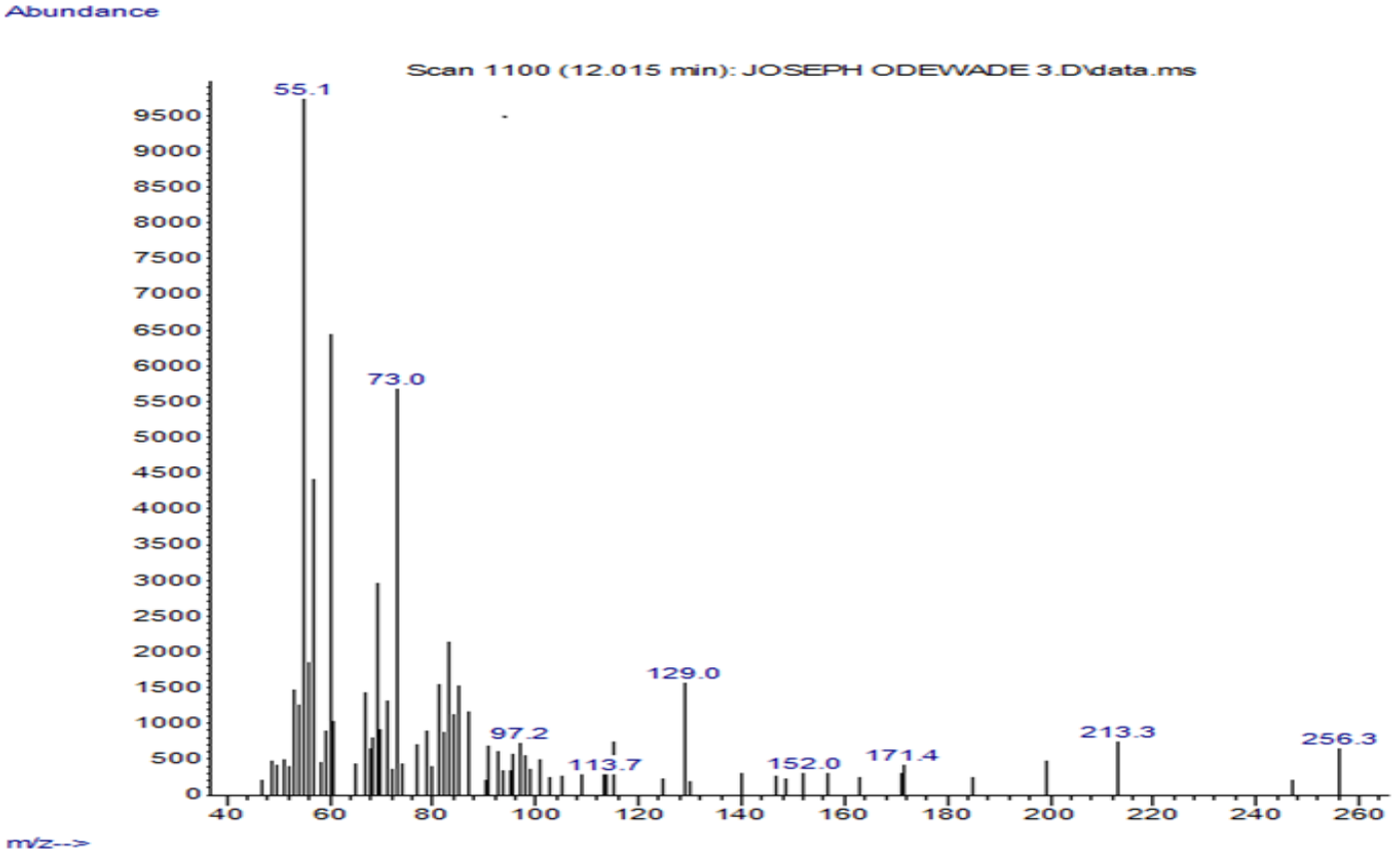

Fig 14 Gas Chromatogram of n-butanol faction of *Chrysophyllum albidum* stem bark extract

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IV. DISCUSSION

The fractions of *C. albidum* stem bark extract were evaluated against a panel of bacterial strains implicated in gastrointestinal infections. *Escherichia coli*, *Salmonella typhi* and *Shigella dysenteriae* were employed in this study. Significant bioactivity was shown by the fractions against the bacterial isolates in Table 1. The n-butanol fraction was the most active fraction, displaying a high level of antibacterial activity against the bacterial isolates at 10 mg/mL with various zones of inhibition. At the same concentration, this fraction inhibited 96% of the bacterial isolates used in the investigation as opposed to 93% and 89% for the aqueous and ethyl acetate fractions respectively (Table 1). Zones of inhibition for the fractions against the bacterial isolates ranged from 10±0.82 mm to 27±0.00 mm. Contrarily, streptomycin and ampicillin inhibited the growth of the test isolates by 68% and 50% respectively at 1 mg/mL. The fractions compared favorably with the positive control, streptomycin and ampicillin employed in this study. This shows that the stem bark extract from *C. albidum* may be helpful in the development of drugs of natural origin for the treatment of gastrointestinal infections. The fractions were effective against the gastrointestinal pathogens; *Escherichia coli* and *Shigella dysenteriae* employed in this study. Thus, this study confirms Florence and Adiaha (2015) earlier findings that this plant can be used to treat diarrhea and dysentery in Nigeria.

The minimum inhibitory concentrations and minimum bactericidal concentrations were also evaluated. Low MICs and MBCs were observed in this study (Table 2). For instance, the fractions' lowest MIC and MBC were 0.31 mg/mL and 0.63 mg/mL respectively. This observation was backed by the fact that plant extracts with exceptionally low MIC and MBC are known to have appreciable antibacterial potentials (Achinto and Munirrudin, 2009). According to Shanmughapriya *et al*. (2008), plant extracts with MIC indices of more than 2 mg/mL but less than 16 mg/mL are considered to be bacteriostatic and those with MIC indices of less than 2 mg/mL are said to be bactericidal. This finding demonstrates the fraction's bactericidal activity. Therefore, this plant may be a source of potent natural antibacterial drugs that can be employed to treat gastrointestinal infections, a deadly disease, particularly in children under the age of five.

The biocidal potentials were assessed using the time kill assay, potassium ions leakage, and nucleotides leakage from the bacterial isolates by the fractions. The result obtained in this study demonstrated that the fractions exhibited the biocidal potential against the bacterial isolates associated with gastrointestinal infections. This finding corroborated those of Pankey and Sabath (2004). As contact time and fraction concentrations increased, a correlation between the amounts of cellular constituents leaked from the bacterial isolates and the percentage of bacterial isolates killed was also observed. For instance, 66.7% of the bacterial isolates were killed and the quantities of nucleotides and potassium ions leaked from *Escherichia coli* at 1 x MIC were 0.16 µg/mL and 0.22 µg/mL respectively within 15 minutes. The amounts of nucleotides and potassium ions leaked from *Klebsiella pneumoniae* at 1 x MIC within 15 minutes were 0.33 µg/mL and 0.51µ g/mL respectively, the percentage of test cells killed was 66.2% (Figure 1 to Figure 10). When the fraction concentrations were increased to 2 x MIC and 3 x MIC, similar trends in the reactions were observed.

Figures 8 to Figure 10 depict leakages of nucleotides and potassium ions caused by the fraction at various concentrations, demonstrating that this fraction disrupts the test cells' cytoplasmic membrane as its mode of action. This finding is further supported by the results of Abidoye *et al*. (2020), which indicated that leakages of cytoplasmic constituents arise from considerable and irreversible cytoplasmic membrane damage. Therefore, the biocidal activity of the n-butanol fraction (the most active fraction) against the cytoplasmic membrane of the bacterial isolates is based on the availability of the bioactive constituents identified in the plant extract. For example, phenolic compounds are well known for their capacity to damage and subsequently enhance membrane permeability, resulting in leakages of certain cellular components such as proteins, nucleotides and inorganic ions (Campos *et al*., 2009).

The fractions contained alcohol, phenol, alkanes, alkenes, aliphatic ether, amines, aromatics, carboxylic acids, esters and sulfoxides according to an analysis using Fourier transform infrared spectroscopy (Table 3). Previous research (Florence and Jeeva, 2015; Rajiv *et al*., 2017; Bashir *et al*., 2020) revealed that related functional groups were found in various plant parts. The therapeutic properties of *C. albidum* stem bark extract may be a result of functional groups. For instance, the O-H group in the extract indicates the presence of phenolic compounds which confer the antibacterial potential on the bacterial isolates. Lack of cyanide groups indicates that there is no absorbance between the 2200 and 2260 cm^{-1} band in the fractions of the *C. albidum* stem bark extract, which raises the possibility that the plant is not harmful (Gomathi *et al*., 2014).

N-Hexadecanoic acid was detected to be the main bioactive constituent in the n-butanol fraction (Table 4). The presence of these bioactive constituents justifies traditional medical practitioners' use of various plant parts for treating a variety of diseases. In order to produce an antibacterial drug of natural origin for the treatment of gastrointestinal infections caused by these pathogens, such constituents can thus be isolated from this plant extract.

V. CONCLUSION

The fractions of *Chrysophyllum albidum* stem bark extract exhibited significant bioactivity against bacterial strains implicated in gastrointestinal infections. The plant's potential as a drug is validated by the fact that its extract can effectively eradicate a wide range of bacterial isolates. The drugs derived from this plant may provide an affordable and accessible type of treatment for gastrointestinal infections that have long-term consequences on the gastrointestinal tract and other organ systems in the human body.

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