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Joseph Oluwaseun Odewade
Department of Microbiology, Faculty of Life Sciences, Federal University Dutsin-Ma, Dutsin-Ma, Katsina State, Nigeria

Dauda M Mukhtar
Department of Microbiology, Faculty of Life Sciences, Bayero University, Kano, Kano State, Nigeria

Ibrahim Yusuf
Department of Microbiology, Faculty of Life Sciences, Bayero University, Kano, Kano State, Nigeria

Lydia Oluwatosin Odewade
Department of Environmental Management, Faculty of Earth and Environmental Sciences, Bayero University, Kano, Kano State, Nigeria

Corresponding Author:
Joseph Oluwaseun Odewade
Department of Microbiology, Faculty of Life Sciences, Federal University Dutsin-Ma, Dutsin-Ma, Katsina State, Nigeria

Biocidal potentials and chemical composition of leaf extract of *Chrysophyllum albidum* (G. Don) against selected enterobacterial species obtained from clinical samples

Joseph Oluwaseun Odewade, Dauda M Mukhtar, Ibrahim Yusuf and Lydia Oluwatosin Odewade

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Abstract
The biocidal potentials of the fractions partitioned from the *Chrysophyllum albidum* leaf extract were assessed against selected enterobacterial species obtained from clinical samples. Sensitivity test, minimum inhibitory concentrations (MICs), minimum bactericidal concentrations (MBCs) and time-kill assay were used to evaluate antibacterial properties. The chemical constituents present in the fractions were also investigated using Fourier Transform Infrared Spectroscopy (FT-IR) and Gas Chromatography-Mass Spectrometry (GC-MS) analyses. The fractions at 10 mg/mL displayed zones of inhibition that were between (10±0.82) mm and (24±1.63) mm. The MICs ranged from 0.63 mg/mL to 5 mg/mL while the MBCs ranged from 1.25 mg/mL to 10 mg/mL. *Escherichia coli* and *Klebsiella pneumoniae* were completely eliminated by the n-butanol fraction after 120 minutes of contact time at 1 x MIC while the killing rates for these bacteria in the aqueous and ethyl acetate fractions were 95.5% and 89.4% respectively. Numerous functional groups, including alcohol, phenol, alkanes, alkenes, aliphatic ether, amide, amines, aromatics, carboxylic acids and sulfoxides were found in the sample after FT-IR analysis. One of the main constituents found in the GC-MS analysis of the plant extract was 9-octadecenoic acid. This investigation showed that leaf extract exhibited significant biocidal potentials against selected enterobacterial species isolated from clinical samples. Thus, further research is required to isolate the pure bioactive constituents from the fractions.

Keywords: *Chrysophyllum albidum*, Bactericidal, Fourier transform infrared spectroscopy (FT-IR), Gas chromatography-mass spectrometry (GC-MS)), Time-kill assay

1. Introduction
Infectious diseases are the result of the invasion of the host system by pathogens which are not repelled or destroyed by the immune system. The most frequent causes of infectious diseases are pathogenic bacterial strains such as *Staphylococcus aureus* [1], *Escherichia coli* [2], *Klebsiella pneumoniae* [3], bloodstream associated *Salmonella* spp [4], *Shigella* spp, and *Vibrio cholerae* [5]. Infectious diseases are the main causes of morbidity and mortality worldwide [6]. The ongoing spread of infectious diseases causes governments, pharmaceutical companies and health organizations all over the world great concern. Given the present trends in multi-drug resistance (MDR) among newly developing and re-emerging bacterial pathogens to accessible modern medicines or antibiotics, treatment failure bears substantial hazards [7]. The existing antibiotic therapy is seriously threatened by the appearance and spread of multidrug-resistant (MDR) enteric bacterial pathogens [8].
The most common cause of diarrheal illness with an estimated 3 million annual deaths and 4 billion infections worldwide is due to enteric pathogens [9]. Due to the high number of subclinical, unreported, and reported cases of enteric illness, it is likely to cause more morbidity than any other disease [9]. More than 30% of all isolates from nosocomial infections are gram-negative enterics [9]. *Salmonella typhi*, *Shigella dysenteriae* and various strains of *Escherichia coli* are well-known enteric pathogens that are carried by humans. Their pervasive involvement in hospital acquired infections can be attributed to their propensity for drug resistance and constant presence in the hospital environment. They result in higher mortality,

longer hospital stays and higher treatment and care costs [8, 10]. Modern medicine must ultimately prioritize the development of innovative antibiotics from natural sources to address the socioeconomic and health consequences caused by multidrug-resistant microorganisms [11].

Scientists have searched for *Chrysophyllum albidum* (G. Don) as a potential therapeutic herb. The edible tropical fruit, *Chrysophyllum albidum* (G. Don), also known as the African Star apple is a native plant. The Sapotaceae family includes the medicinal plant *Chrysophyllum albidum*. Nigeria, Uganda, Niger, Cameroon and Cote d' ivoire are among the countries where *Chrysophyllum albidum* is most common [12, 13]. In Nigeria, *Chrysophyllum albidum* is used to treat a variety of illnesses [14]. Previous studies also noted that this plant has antibacterial, antioxidant and anti-fertility properties [15–18]. It also possesses anti-inflammatory, anti-spasmodic, anti-analgesic and diuretic qualities due to its high flavonoids, steroids, glycosides and saponin content. [19]. Owing to the dearth of information regarding the biocidal effects of this plant's leaf extract against enterobacterial species isolated from clinical samples. The biocidal efficacy of the *Chrysophyllum albidum* leaf extract fractions against enterobacterial species isolated from clinical samples was assessed in this study.

2. Materials and Methods

2.1 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.

2.2 Collection of the bacterial isolates

Clinical strains and control strains were both employed in this study. *Shigella dysenteriae*, *Klebsiella pneumoniae*, *Proteus vulgaris*, *Proteus mirabilis*, *Escherichia coli* and *Salmonella typhi* were the clinical strains that were isolated from stool, urine, blood, sputum and wound samples. The control strains were obtained from the American Type Culture Collection (ATCC), Manassas, Virginia, United States of America. These include *Salmonella typhi* (ATCC 14208), *Proteus vulgaris* (ATCC 29905), *Klebsiella pneumoniae* (ATCC 13883), *Shigella dysenteriae* (ATCC 13313) and *Escherichia coli* (ATCC 25922). The identities of the bacterial isolates were verified using morphological, cultural, biochemical laboratory and API 20E tests. The bacterial isolates were confirmed, then sub-cultured in nutrient broth and incubated at 37 °C for 18 hours before being standardized to the 0.5 McFarland standard (10^6 cfu/ml) before use.

2.3 Culture media used

Mueller Hinton agar medium was used for the sensitivity testing while nutrient agar and nutrient broth media were used for sub-culturing the test isolates. The test isolates were reconfirmed using Salmonella Shigella agar, MacConkey agar, Xylose Deoxycholate agar and Eosin Methylene Blue agar.

2.4 Collection of plant sample

Fresh *Chrysophyllum albidum* (G. Don) leaves were collected in the month of December, 2020 from a farmland in Ile-Ife, Osun State, Nigeria. The plant sample was identified and authenticated in the herbarium of the Bayero University's Plant Biology Department in Kano, Kano State, Nigeria and was given the accession number BUKHAN 0522.

2.5 Plant sample preparation

The plant sample was thoroughly cleaned with tap water before being air dried in the shade, ground into a powder and sieved to obtain fine ground particles. One thousand five hundred grams of powdered leaves were cold extracted for four days with periodic agitation using a 3:2 (v/v) solution 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* using a rotary evaporator to get rid of the methanol. The next step was lyophilizing the residual aqueous residue to get 179.8 g of a dark brown crude extract.

2.6 Solvent partitioning of the leaf extract

Exactly, 250 mL of sterile distilled water were used to dissolve 100 g of leaf extract. The mixture was partitioned into n-hexane, chloroform, ethyl acetate, n-butanol and aqueous fractions in that sequence of increasing polarity. The resulting n-hexane fraction was concentrated to dryness *in vacuo* and lyophilized. The residue was left in an airtight container. A similar process was used to re-concentrate the aqueous residue *in vacuo* and extract it once more using n-butanol, ethyl acetate and chloroform. The aqueous fraction was concentrated, lyophilized and kept in freezer for further use.

2.7 Sensitivity test of the fractions against bacterial isolates

The agar-well diffusion method [20] was used to determine the sensitivity test of the fractions with little modifications. The standardized test isolates (0.1 mL) were inoculated into Mueller Hinton Agar that had been melted (Oxoid, UK) poured into sterile Petri dishes and then left 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 of prepared fraction solutions that had been diluted to 10 mg/mL, taking care to avoid spilling solution on the medium's surface. The plates were allowed to stand on the laboratory bench for roughly an hour before being incubated upright for 24 hours at 37 °C. Later, the millimetre rule was used to measure the diameters of the inhibitory zones. To compare the test isolates' sensitivity to the extracts, streptomycin and ampicillin, which acted as a positive control and standard antibiotics at a concentration of 1 mg/mL each, were utilized. The experiment was run in triplicates and a negative control of 10% Dimethyl sulfoxide (DMSO) was used.

2.8 Determination of the minimum inhibitory concentrations (MICs)

The minimum inhibitory concentrations of the fractions were determined by agar dilution method following standard procedure [21, 22]. Final concentrations ranging from 0.16 mg/mL to 10 mg/mL were obtained by adding 2 ml aliquots of the solution made from the fractions' two-fold dilutions to 18 mL of sterile molten Nutrient agar and allowing the medium to set. The media surfaces were allowed to dry before applying standardized bacterial cultures that had been incubated for 18 hours. The plates were incubated at 37 ^{o}C for up to 48 hours before being checked for the presence or absence of growth. A sterile agar medium plate without the extract served as the control. The lowest concentration at which the test isolates experienced growth inhibition was known as the minimum inhibitory concentrations.

2.9 Determination of the minimum bactericidal concentrations (MBCs)

Akinpelu *et al.* [22] method was used to determine the

minimum bactericidal concentrations of the fractions. Samples from the line of streaks on the MIC plates without any discernible growth were taken, sub-cultured onto recently made Nutrient agar plates and then incubated at 37 ºC for 48 hours. The minimum bactericidal concentrations of the fractions were determined to be the lowest concentration of the extract that did not produce any growth on a fresh set of plates.

2.10 Determination of the killing rate of bacterial isolates by the fractions

Odenholt *et al.* [23] with little modifications was used to determine the killing rate of the 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 h) were standardised and viable counts were counted. Exactly, 4.5 mL of various concentrations of the fractions relative to the MICs were mixed with 0.5 mL of known cell density (10^6 cfu/ml) of the bacterial suspension. The killing rate was assessed over a period of two hours after the suspension had been fully mixed and kept at room temperature. Precisely 0.5 mL of each solution was taken out at the proper intervals and added to 4.5 mL of nutrient broth recovery medium containing 3% Tween 80 to counteract the effects of the antimicrobial compounds that were carried over from the test suspensions. The resulting suspension was thoroughly agitated before being serially diluted in sterile physiological saline (0.9% NaCl) and plated for viable count. In the design of the control experiment, the extract was not utilized. Viable counts were carried out in triplicate for each sample. The decrease in the viable counts indicated killing by the extract.

2.11 Fourier Transform Infrared Spectroscopy analysis

The Fourier-transform infrared spectrometry (FT-IR) analysis was used to determine the functional groups that were present in the fractions. The spectra of each of the fractions were obtained using the FT-IR Spectrometer (Agilent technologies). As observed in the annotated spectrum, one crucial aspect of the chemical bond is the wavelength of light absorbed. Analysis of the infrared absorption spectra can be used to identify the chemical bonds that are present in a compound.

2.12 Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The GC-MS Model, QP 2010 PLUS, Shimadzu, Japan was used to analyze the n-butanol fraction (the most active fraction) using Gas Chromatography-Mass Spectrometry (GC-MS). A fused silica capillary column with a 30 m length, 0.25 mm internal diameter and 0.25 µm film thickness form part of the system. A split ratio of 10:1 was used with an injection volume of 2 µL and a carrier gas of helium (99.999% purity) set at a constant flow rate of 1.0 mL/min. The ion-source temperature was 200 °C while the injector temperature was kept at 250 °C. For two minutes, the oven's temperature was set to range from 80 °C to 280 °C. The mass range between 40 and 1000 m/z was scanned every 0.5 seconds while mass spectra were being recorded at 70 eV (ionization energy). There was a 0 to 6 min solvent delay. 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. By comparing the average peak area to the overall area, it was possible to determine the relative percentage of the fraction's constituents. Using the National Institute of Standards and Technology (NIST) database and the Fatty Acids Methyl Esters Library version 1.0 (FAME library), chemical constituents contained in the extract were identified.

2.13 Statistical analysis

The statistical analysis of the data was performed using one-way analysis of variance (ANOVA) and the results were presented as means ± SD (standard deviation) of three replicates. Values were considered significant at $p \leq 0.05$.

3. Results

The n-butanol, aqueous and ethyl acetate fractions were recovered from the plant extract, but n-hexane and chloroform did not show any affinity for the bioactive constituents of the leaf extract throughout the partitioning process. This suggests that the bioactive constituents of the plant extract are not optimally extracted using n-hexane or chloroform as organic solvents. The results of the sensitivity test revealed that different test bacterial isolates had varying degrees of sensitivity to n-butanol, aqueous and ethyl acetate fractions at 10 mg/mL (Table 1). The fractions' zones of inhibition against the bacterial isolates ranged between (10±0.82 mm) and (24±1.63 mm). The n-butanol fraction showed the largest zone of inhibition (24±1.63) mm against *Escherichia coli* (EC7) at 10 mg/mL, however none of the fractions were susceptible to *Salmonella typhi* (ST1), *Klebsiella pneumoniae* (KP6), *Proteus vulgaris* (PV3), or *Proteus mirabilis* (PM4) at this concentration. Some test isolates were sensitive at 1 mg/mL while others were resistant to the standard antibiotics used in this study. *Proteus vulgaris* (83.3%) revealed greater levels of streptomycin resistance than *Proteus mirabilis* (80%), *Salmonella typhi* (72.7%), *Escherichia coli* (36.4%), *Klebsiella pneumoniae* (36.4%), and *Shigella dysenteriae* (33.3%). *Proteus vulgaris* and *Shigella dysenteriae* both showed high rates of resistance (83.3%) to ampicillin and streptomycin. Streptomycin and ampicillin both inhibited test isolates growth by 60% and 52% at concentration of 1 mg/mL (Table 1). This study showed that almost every bacterial isolate isolated from clinical samples was susceptible to the antibacterial activity of the plant's leaf extract fractions.

Table 1: The 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 (1mg/mL)**	**Ampicillin (1 mg/mL)**	**DMSO (10%)**
EC1	12±1.63	10±0.82	0	10±0.82	0	0
EC2	14±1.63	12±0.82	12±0.00	0	18±1.63	0
EC3	12±0.00	0	0	24±0.82	22±0.00	0
EC4	16±0.82	13±1.63	12±0.00	23±0.82	0	0
EC5	12±0.82	14±0.82	0	24±1.63	12±0.00	0
EC6	14±0.82	12±0.00	0	0	20±0.82	0
EC7	24±1.63	21±1.63	18±0.82	26±0.82	28±0.82	0
EC8	20±0.82	15±0.00	12±0.82	22±0.82	26±0.00	0
EC9	16±0.82	12±0.00	14±1.63	0	0	0

EC10	12±0.82	10±0.00	10±1.63	16±0.82	0	0
EC11	16±1.63	14±0.82	11±0.82	24±0.82	22±0.00	0
ST1	0	0	0	13±0.82	0	0
ST2	10±0.00	12±0.82	0	20±0.82	0	0
ST3	12±0.82	0	0	10±0.00	20±0.82	0
ST4	17±0.82	14±0.82	12±0.82	13±0.82	22±0.82	0
ST5	10±0.82	0	12±0.00	0	0	0
ST6	14±0.82	14±0.00	10±0.00	12±0.00	20±1.63	0
ST7	16±0.82	13±0.82	11±0.82	24±0.82	26±0.00	0
ST8	18±0.82	16±1.63	12±1.63	0	22±0.82	0
ST9	17±1.63	14±0.00	13±0.82	0	0	0
ST10	14±1.63	10±0.82	10±1.63	23±0.82	21±0.82	0
ST11	16±1.63	14±0.82	13±1.63	0	0	0
KP1	16±0.82	14±0.82	14±0.00	19±0.00	12±0.82	0
KP2	0	11±1.63	0	16±0.82	0	0
KP3	11±1.63	13±0.00	13±0.82	0	13±0.82	0
KP4	12±1.63	14±1.63	10±0.82	21±0.00	19±0.82	0
KP5	16±0.00	14±0.82	12±1.63	24±0.82	22±0.00	0
KP6	0	0	0	0	18±0.82	0
KP7	12±0.82	0	0	20±0.82	0	0
KP8	15±0.82	18±1.63	12±0.82	22±0.82	20±0.82	0
KP9	12±0.82	0	0	13±0.82	12±0.00	0
KP10	17±0.82	14±0.00	12±0.82	10±0.82	12±0.00	0
KP11	12±0.82	10±0.82	11±0.00	18±0.00	0	0
SDI	10±0.00	0	0	0	0	0
SD2	12±1.63	12±0.82	0	21±0.82	19±0.82	0
SD3	14±0.82	12±0.00	0	25±0.00	0	0
SD4	12±0.00	0	0	18±0.00	0	0
SD5	18±0.82	14±1.63	11±0.82	26±0.00	0	0
SD6	16±0.82	12±0.00	12±0.82	0	0	0
PV1	12±0.00	0	12±0.82	0	0	0
PV2	15±0.82	13±1.63	11±1.63	0	23±0.00	0
PV3	0	0	0	0	0	0
PV4	10±0.82	0	10±0.00	21±0.82	0	0
PV5	14±0.82	0	12±1.63	0	24±0.82	0
PV6	18±1.63	14±1.63	12±0.82	0	0	0
PM1	14±0.00	12±0.82	10±0.82	18±0.82	20±0.00	0
PM2	12±0.00	0	0	0	0	0
PM3	16±1.63	10±0.00	11±0.82	0	0	0
PM4	0	0	0	0	0	0
PM5	17±0.00	13±0.82	15±1.63	0	18±0.82	0

Key: EC1-EC10 = Strains of *Escherichia coli*, EC11=*Escherichia coli* (ATCC 25922), ST1-ST10 = Strains of *Salmonella typhi*, ST11= *Salmonella typhi* (ATCC 14028), KP1-KP10 = Strains of *Klebsiella pneumoniae*, KP11= *Klebsiella pneumoniae* (ATCC 13883), SD1-SD5=Strains of *Shigella dysenteriae*, SD6 = *Shigella dysenteriae* (ATCC 13313), PV1-PV5 = Strains of *Proteus vulgaris*, PV6 = *Proteus vulgaris* (ATCC 29905), PM1-PM5 = Strains of *Proteus mirabilis*, ATCC = American type culture collection, 0 =Not sensitive, mm* = mean of three replicates, P≤ 0.05, DMSO = Dimethyl sulfoxide

The minimum inhibitory and minimum bactericidal concentrations were also tested against the bacterial isolates. As demonstrated in Table 2, the fractions displayed a range of MICs and MBCs against the bacterial isolates employed in this study. The n-butanol fraction's MICs ranged from 0.63 mg/mL to 5 mg/mL while the aqueous and ethyl acetate fractions ranged from 1.25 mg/mL to 5 mg/mL respectively against the bacterial isolates. The butanol fraction displayed the lowest MIC (0.63 mg/mL) against *Escherichia coli*. The n-butanol fraction showed MBCs against the bacterial isolates that varied from 1.25 mg/mL to 10 mg/mL while the aqueous and ethyl acetate fractions showed MBCs that ranged from 2.5 mg/mL to 10 mg/mL respectively. The lowest MBC of 1.25 mg/mL was expressed by n-butanol fraction against *Escherichia coli* **(**Table 2).

Table 2: The minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of the leaf fractions of *Chrysophyllum albidum* against bacterial isolates

	Butanol (mg/mL)		**Aqueous (mg/mL)**		**Ethyl acetate (mg/mL)**	
Strains code	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
EC1	5	10	5	10	ND	ND
EC2	2.5	5	5	10	5	10
EC3	5	10	ND	ND	ND	ND
EC4	2.5	5	5	10	5	10
EC5	5	10	2.5	5	ND	ND
EC6	2.5	5	5	10	ND	ND
EC7	0.63	1.25	2.5	5	2.5	5
EC8	1.25	1.25	2.5	5	5	10
EC9	2.5	5	5	5	5	10
EC10	5	10	5	10	5	10
EC11	2.5	5	2.5	5	5	10
ST1	ND	ND	ND	ND	ND	ND
ST2	5	10	5	10	ND	ND

ST3	5	10	ND	ND	ND	ND
ST4	2.5	5	2.5	5	5	10
ST5	5	10	ND	ND	5	10
ST6	2.5	5	2.5	5	10	ND
ST7	2.5	5	5	10	5	10
ST8	1.25	2.5	2.5	5	5	10
ST9	2.5	5	5	10	5	10
ST10	2.5	5	5	10	5	10
ST11	2.5	5	5	10	5	10
KPI	2.5	5	2.5	2.5	2.5	5
KP2	ND	ND	5	5	ND	ND
KP3	5	10	5	10	5	10
KP4	5	10	2.5	5	5	10
KP5	2.5	5	2.5	5	5	5
KP6	ND	ND	ND	ND	ND	ND
KP7	5	10	ND	ND	ND	ND
KP8	2.5	5	1.25	2.5	5	10
KP9	5	10	ND	ND	ND	ND
KP10	2.5	5	5	10	5	10
KP11	5	5	5	10	5	10
SD1	5	10	ND	ND	ND	ND
SD2	5	10	5	5	ND	ND
SD3	2.5	5	5	10	ND	ND
SD4	5	5	ND	ND	ND	ND
SD5	2.5	2.5	5	10	5	10
SD6	2.5	5	5	10	5	10
PVI	5	10	ND	ND	5	10
PV2	2.5	5	5	10	5	10
PV3	ND	ND	ND	ND	ND	ND
PV4	10	ND	ND	ND	5	10
PV5	2.5	5	ND	ND	5	10
PV6	1.25	1.25	2.5	5	5	10
PM1	2.5	5	5	10	5	5
PM2	5	10	ND	ND	ND	ND
PM3	2.5	5	5	10	5	10
PM4	ND	ND	ND	ND	ND	ND
PM5	1.25	2.5	5	10	2.5	2.5

Key: EC1-EC10 = Strains of *Escherichia coli*, EC11 = *Escherichia coli* (ATCC 25922), ST1-ST10 = Strains of *Salmonella typhi*, ST11 = *Salmonella typhi* (ATCC 14028), KP1-KP10 = Strains of *Klebsiella pneumoniae*, KP11= *Klebsiella pneumoniae* (ATCC 13883), SD1-SD5 = Strains of *Shigella dysenteriae*, SD6 = *Shigella dysenteriae* (ATCC 13313), PV1-PV5 = Strains of *Proteus vulgaris*, PV6 = *Proteus vulgaris* (ATCC 29905), PM1-PM5 = Strains of *Proteus mirabilis*, ATCC = American type culture collection, ND = Not determined

The biocidal potentials of the fractions partitioned from the *Chrysophyllum albidum* leaf extract was also examined using time-kill assay. *Escherichia coli* and *Klebsiella pneumoniae* were used as representative isolates for the killing rate experiment. At 1 x MIC after 15 min of contact time, the killing rate of *Escherichia coli* by the fractions partitioned into n-butanol, aqueous and ethyl acetate were 58.1%, 50.5% and 44.1% respectively (Figure 1, Figure 2 and Figure 3). The percentage of test cells killed by the n-butanol, aqueous and ethyl acetate fractions rose to 86.6%, 77.4% and 77.4% respectively after 60 min of contact time. The rate at which these test cells were killed increased with a contact time of these test cells with the fractions. The percentage of test cells killed by n-butanol, aqueous, and ethyl acetate fractions at 1 x MIC increased to 100%, 94.1%, and 91.9% respectively when the contact period was extended to 120 min. When the fractions' concentrations were increased to 2 x MIC and 3 x MIC, the similar pattern of responses was seen. Similarly, at 1 x MIC and after 15 min of contact time, the fractions partitioned into n-butanol, aqueous and ethyl acetate killed *Klebsiella pneumoniae* at rates of 57.1%, 58.1% and 40.4% respectively (Figure 4, Figure 5 and Figure 6). The percentage of test cells killed by the n-butanol, aqueous and ethyl acetate fractions rose to 85.9%, 81.8%, and 68.2% respectively after 60 min of contact. The rate at which these test cells were killed increased with a contact time of these test cells with the fractions. The percentage of test cells killed by n-butanol, aqueous and ethyl acetate fractions at 1 x MIC rose to 100%, 95.5% and 89.4% respectively, when the contact time was extended to 120 min. When the fractions' concentrations were increased to 2 x MIC and 3 x MIC, the similar pattern of responses was seen.

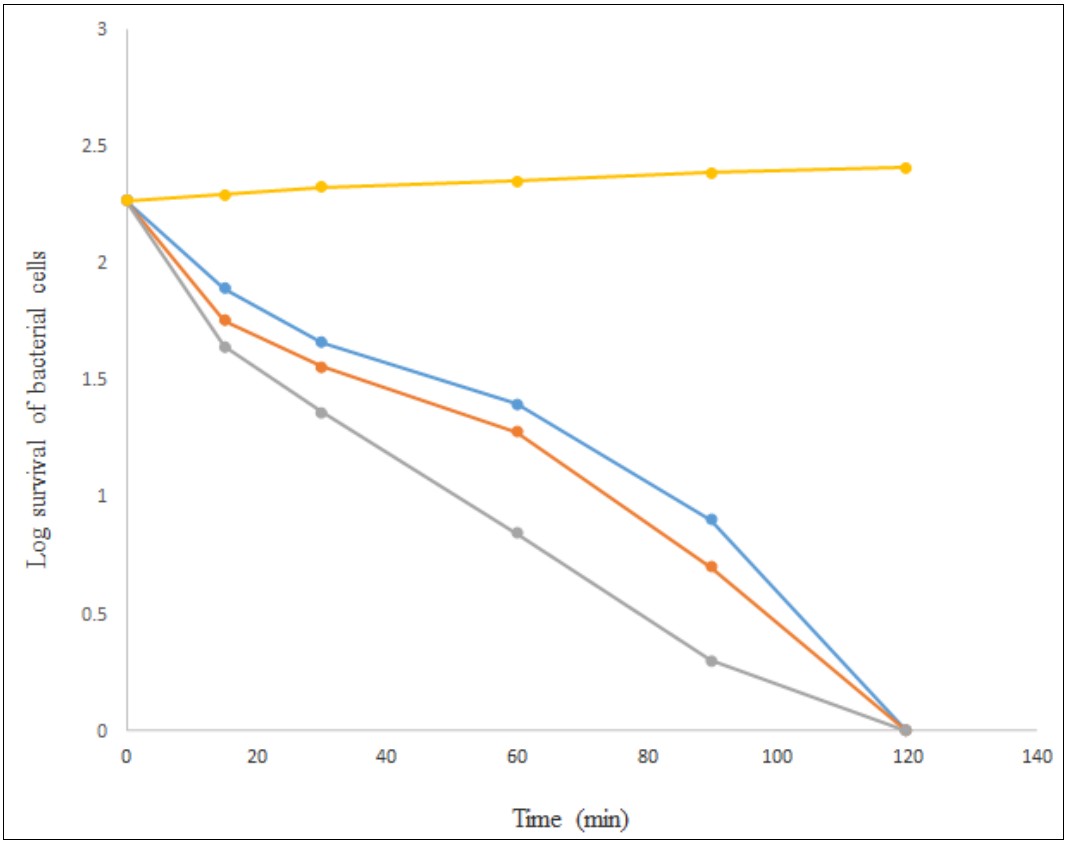

Fig 1: The killing rate of *Escherichia coli* by n-butanol fraction of *Chrysophyllum albidum* leaf extract at 1 x MIC ( ), 2 x MIC ( ), 3 x MIC ( ) and control ( ). Each point displays the $\log_{10}$ of the mean survival of bacterial cells at a specific period in the presence of the fraction

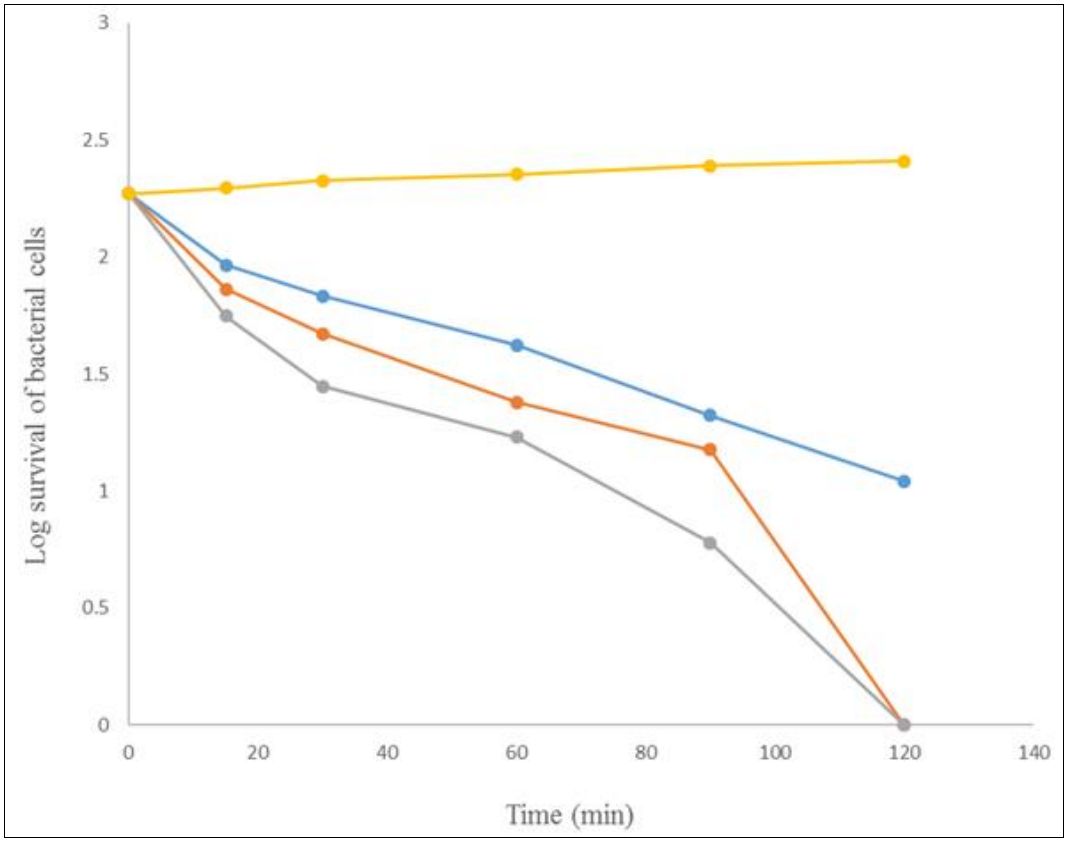

Fig 2: The killing rate of *Escherichia coli* by aqueous fraction of *Chrysophyllum albidum* leaf extract at 1 x MIC ( ), 2 x MIC ( ), 3x MIC ( ) and control ( ). Each point displays the $\log_{10}$ of the mean survival of bacterial cells at a specific period in the presence of the fraction

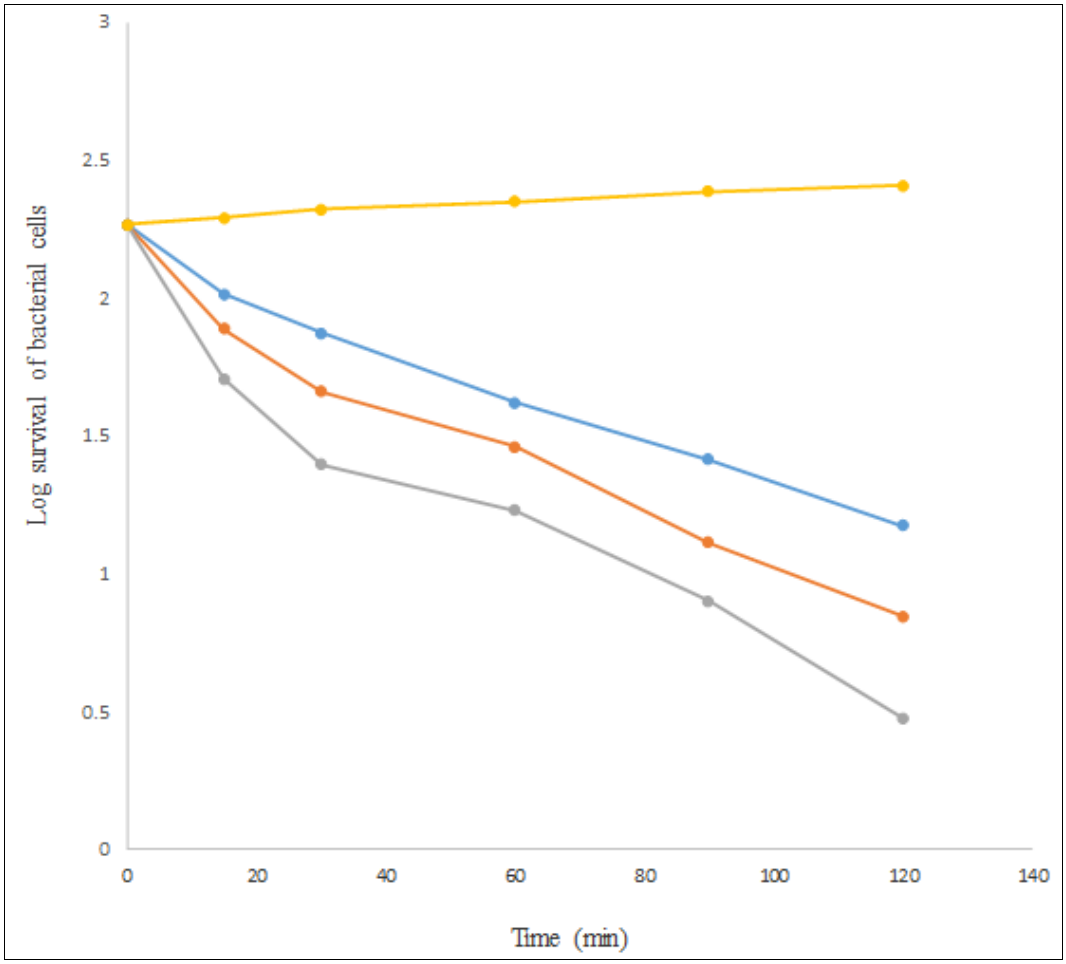

Fig 3: The killing rate of *Escherichia coli* by ethyl acetate fraction of *Chrysophyllum albidum* leaf extract at 1 x MIC ( ), 2 x MIC ( ), 3 x MIC ( ) and control ( ). Each point displays the $\log_{10}$ of the mean survival of bacterial cells at a specific period in the presence of the fraction.

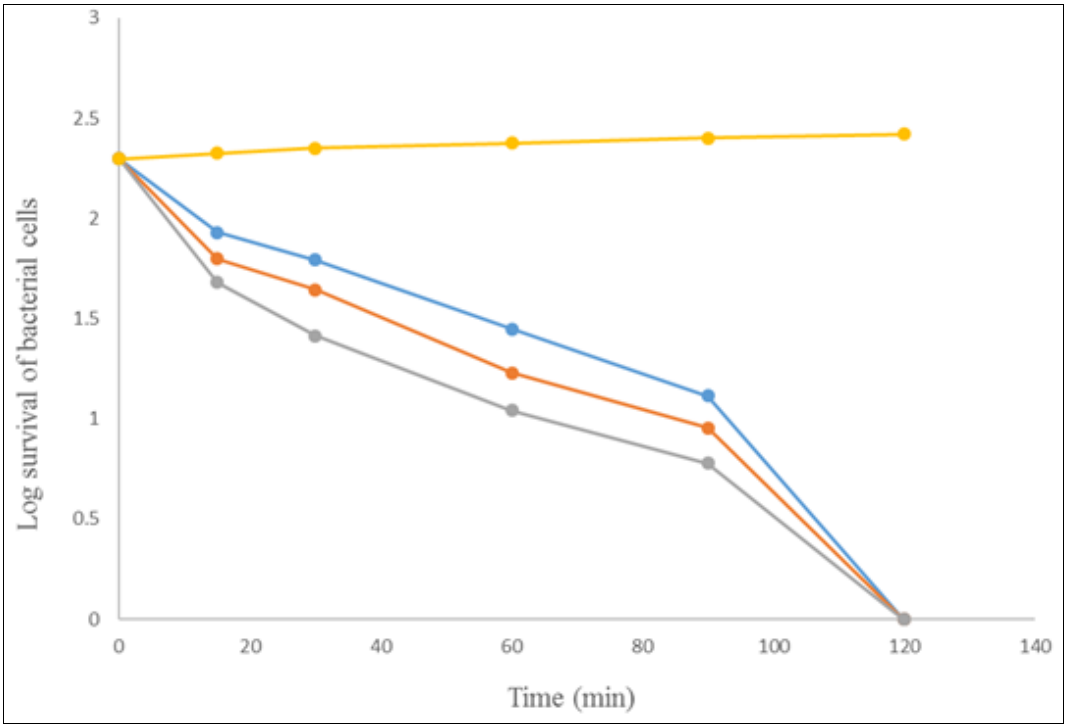

Fig 4: The killing rate of *Klebsiella pneumoniae* by n-butanol fraction of *Chrysophyllum albidum* leaf extract at 1 x MIC ( ), 2 x MIC ( ), 3 x MIC ( ) and control ( ). Each point displays the $\log_{10}$ of the mean survival of bacterial cells at a specific period in the presence of the fraction.

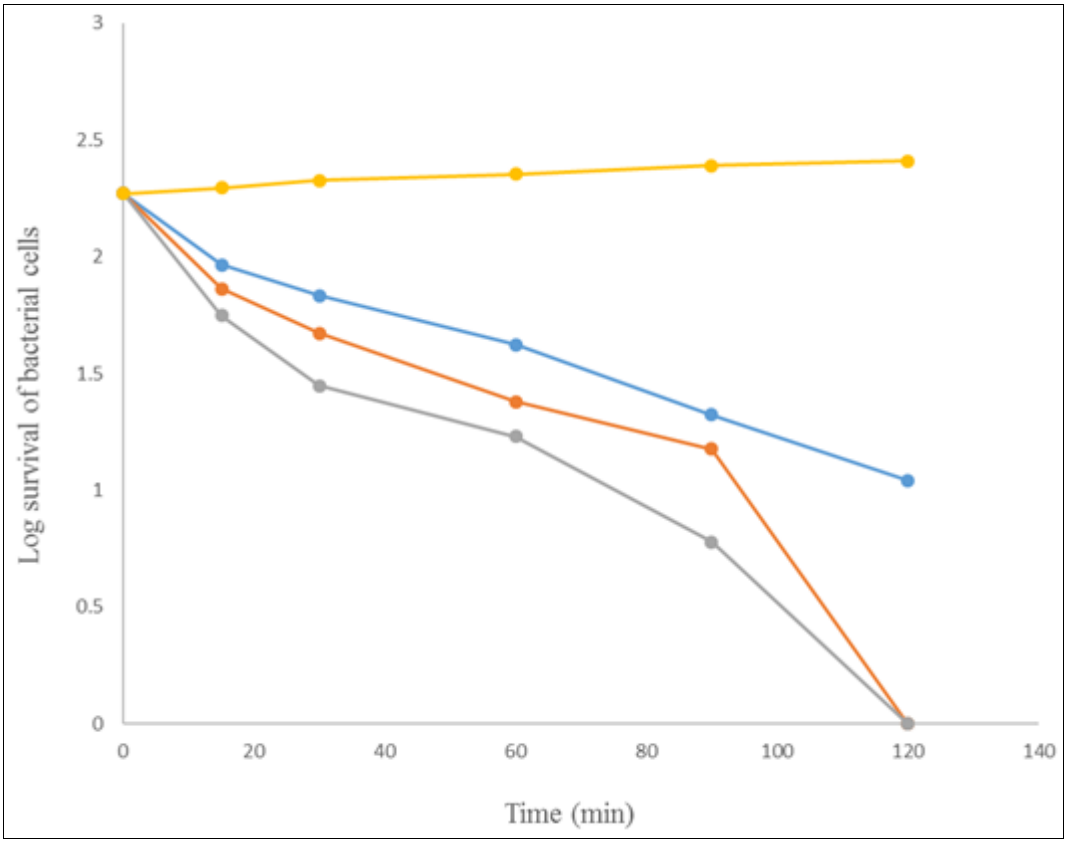

Fig 5: The killing rate of *Klebsiella pneumoniae* by aqueous fraction of *Chrysophyllum albidum* leaf extract at 1 x MIC ( ), 2 x MIC ( ), 3 x MIC ( ) and control ( ). Each point displays the $\log_{10}$ of the mean survival of bacterial cells at a specific period in the presence of the fraction.

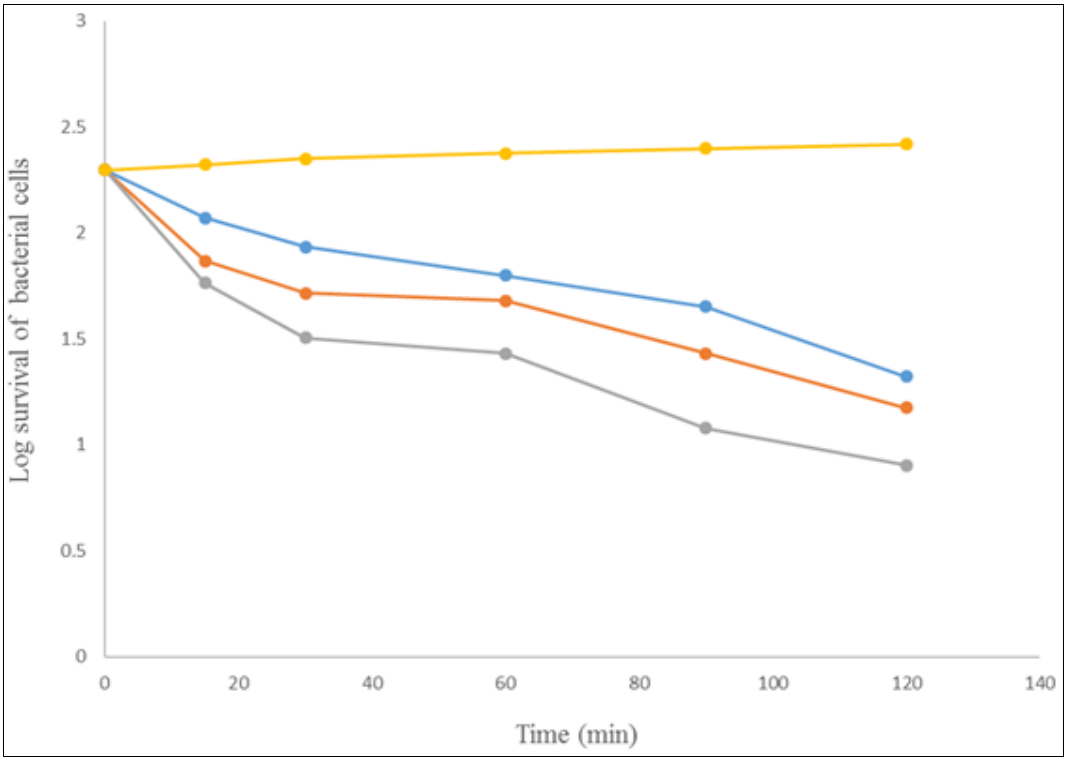

Fig 6: The killing rate of *Klebsiella pneumoniae* by ethyl acetate fraction of *Chrysophyllum albidum* leaf extract at 1 x MIC ( ), 2 x MIC ( ), 3 x MIC ( ) and control ( ). Each point displays the $\log_{10}$ of the mean survival of bacterial cells at a specific period in the presence of the fraction.

Based on the peak values in the infrared radiation region, the functional groups present in the fractions were identified using the FT-IR analysis. Figure 7 to Figure 9 depict the spectra from the FT-IR investigation of the fractions. Alcohol, phenol, alkanes, alkenes, alkynes, aliphatic ether, amines, amide, aromatics, carboxylic acid, esters and sulfoxides were among the functional groups identified by the findings of the FT-IR analysis of the fractions (Table 3).

Table 3: FTIR analysis of the potent fractions of the *Chrysophyllum albidum* leaf extract

Functional groups	Functional groups description	BUT	ETH	AQU
		Absorption frequency (cm-1)		
Alcohol, Phenol	C-O stretching	1201	1238	1212
	O-H bending	1343	-	1402
	O-H stretching	-	-	3227
Alkanes	C-H stretching	2933	2933	-
	CH_3 bending	1454	1451	-
Alkenes	C-H out of plane bend	747	721	-
	C-H out of plane bend	821	817	933
	C=C stretching	1607	1607	1603
Aliphatic ether	C-O stretching	1149	1145	-
Amide	C=O stretching	-	1689	-
Amines	C-N stretching	-	1026	-
	C-N stretching	1149	1145	-
	N-H bending	1514	1514	-
Aromatics	C-H out of plane bend	747	721	-
	C-H out of plane bend	821	817	-
	C=C stretching	1514	1514	-
	C=C stretching	1607	1607	
Carboxylic acids	O-H stretching	3193	3178	-
Sulfoxides	S=O stretching	1030	-	1037

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

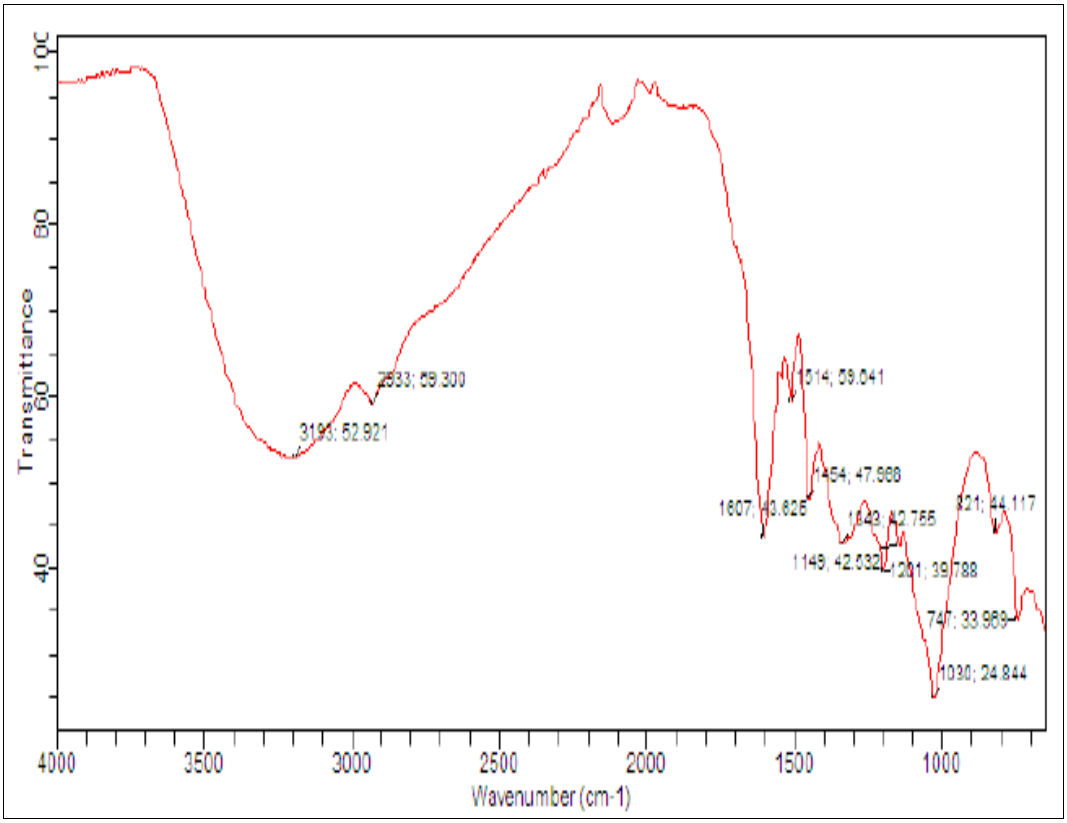

Fig 7: FT-IR analysis of the n-butanol fraction of *Chrysophyllum albidum* leaf extract.

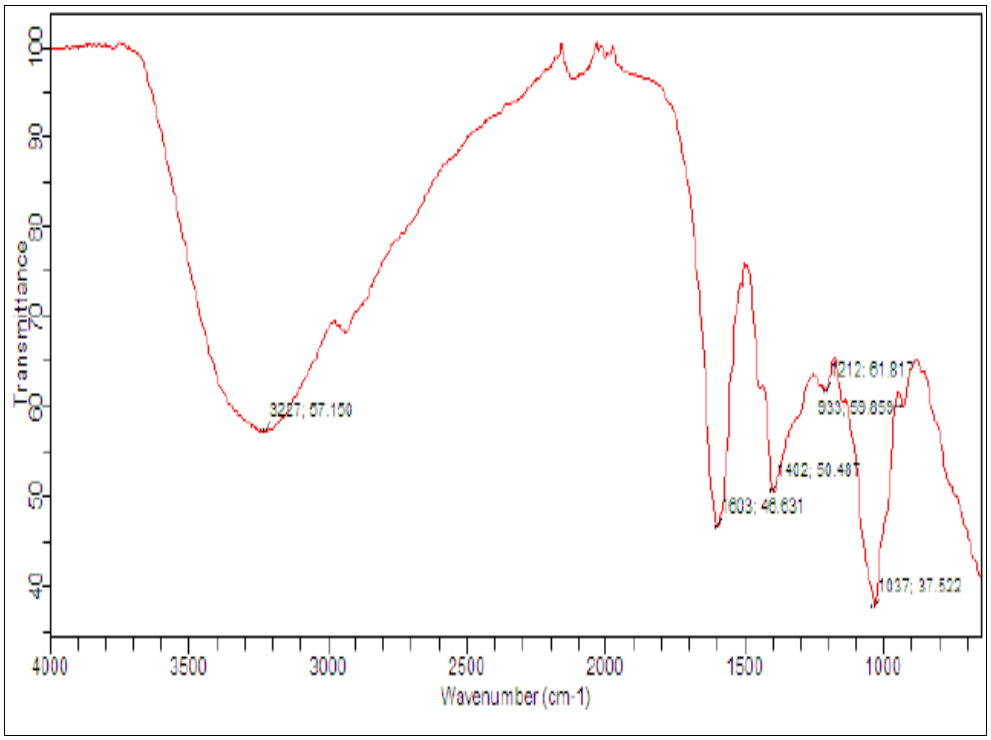

Fig 8: FT-IR analysis of the aqueous fraction of *Chrysophyllum albidum* leaf extract.

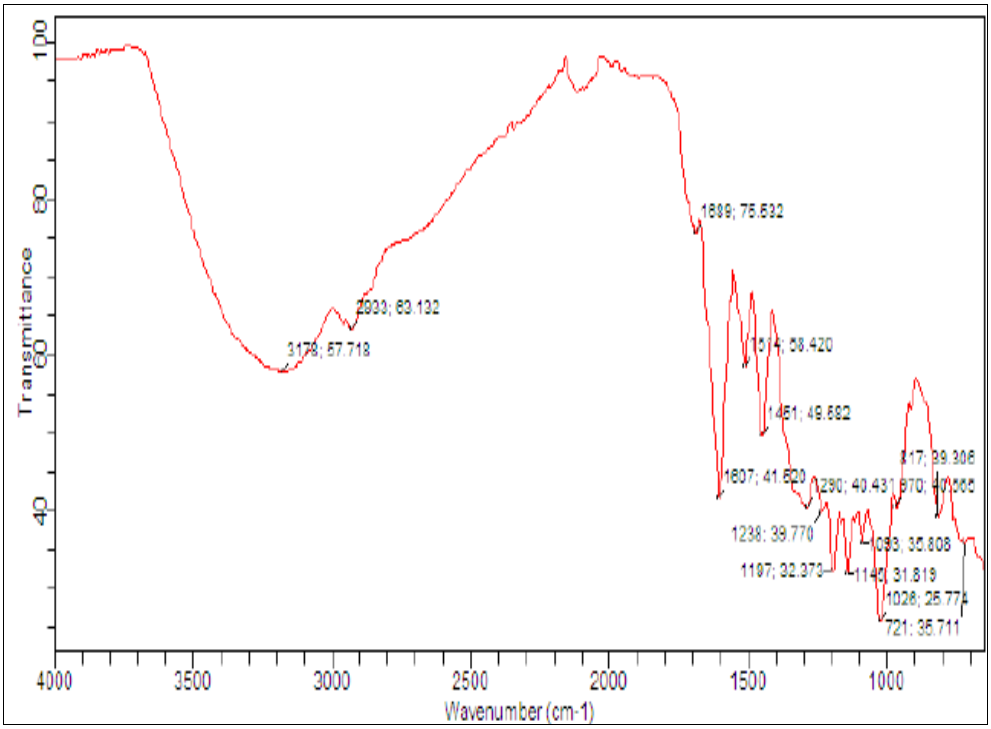

Fig 9: FT-IR analysis of the ethyl acetate fraction of *Chrysophyllum albidum* leaf extract.

As shown in Figure 10, the analysis of the butanol fraction's Gas chromatograms indicated the presence of many bioactive constituents of various kinds and retention times. These bioactive constituents were found using gas chromatography and mass spectrometry. Table 4 showed the detected bioactive constituents along with their peak area (%), retention time, molecular formula and molecular weight. The n-butanol fraction of leaf extract contained 18 bioactive constituents. 9-Octadecenoic acid was one of the main constituents in the n-butanol fraction of the *C. albidum* leaf extract.

Table 4: GC-MS properties of n-butanol fraction of *Chrysophyllum albidum* leaf extract

S/NO	Retention time	Peak Area %	Compound name	Molecular formula	Molecular weight (g/mol)
1	5.219	2.75	Z, Z-6, 13-Octadedien-1-ol acetate	$C_{20}H_{36}O_2$	308.5
2	11.400	6.37	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	270.4507
3	11.731	0.73	9-Hexadecenoic acid	$C_{16}H_{30}O_2$	254.41
4	11.780	0.63	9, 12-Octadecadienoyl chloride, (Z, Z)-	$C_{18}H_{31}ClO$	298.9
5	11.960	23.08	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42

6	13.447	1.63	9, 12-Octadecadienoic acid (Z, Z)-, methyl ester	$C_{19}H_{34}O_2$	294.4721
7	13.518	4.19	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296.4879
8	13.845	0.52	Methyl stearate	$C_{19}H_{38}O_2$	298.5038
9	14.161	42.53	9-Octadecenoic acid	$C_{18}H_{34}O_2$	282.4614
10	14.438	6.62	n-Hexadecanoic acid	$C16_6H_{32}O_2$	256.4241
11	16.136	1.57	9-Hexadecenoic acid	$C_{16}H_{30}O_2$	254.4082
12	18.641	2.65	9-Hexadecenoic acid	$C_{16}H_{30}O_2$	254.4082
13	19.192	1.07	Glycerol 1-palmitate	$C_{19}H_{38}O_4$	330.5026
14	19.692	0.99	Di-n-octyl phthalate	$C_{24}H_{38}O_4$	390.5561
15	21.764	2.89	9-Oxabicyclo[6.1.0] nonane	$C_8H_{14}O$	126.1962
16	22.981	0.48	Oleic acid	$C_{18}H_{34}O_2$	282.4614
17	23.034	0.12	3-Propionyloxypentadecane	$C_{18}H_{36}O_2$	284.5
18	23.512	1.17	Cyclooctane carboxaldehyde	$C_{19}H_{16}O$	140.2227

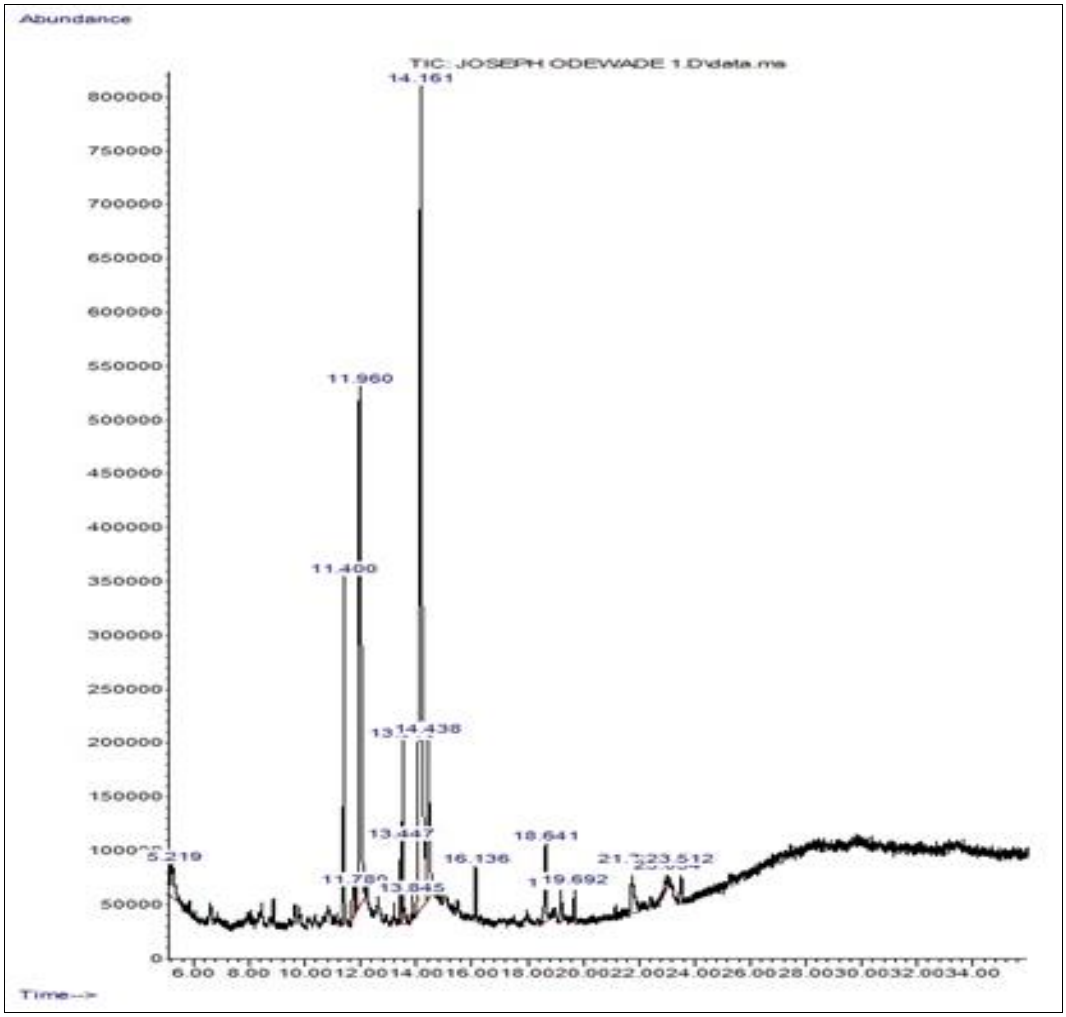

Fig 10: Gas chromatogram of n-butanol faction of *Chrysophyllum albidum* leaf extract.

4. Discussion

The biocidal potentials of leaf extract fractionated into n-butanol, aqueous and ethyl acetate fractions were evaluated against a panel of selected enterobacterial species obtained from clinical samples. *Escherichia coli*, *Salmonella typhi*, *Klebsiella pneumoniae*, *Shigella dysenteriae*, *Proteus mirabilis* and *Proteus vulgaris* are some of the bacterial isolates that make up this group. The fractions exhibited significantly bioactivity against the test bacterial isolates employed in this study. With distinct zones of inhibition and a significant level of bioactivity against the test isolates at 10 mg/mL, the n-butanol fraction was the most active fraction. This fraction inhibited bacterial isolates by 90% while aqueous and ethyl acetate fractions employed in this study inhibited bacterial isolates by 70% and 64% respectively (Table 1). The fractions' zones of inhibition against the bacterial isolates ranged from (10±0.82) mm to (24±1.63) mm. Streptomycin and ampicillin, on the other hand, inhibited test isolates growth by 60% and 52%, respectively at 1 mg/mL. The fractions compared favorably to the two standard antibiotics employed as positive control, streptomycin and ampicillin. This suggests that the *Chrysophyllum albidum* leaf extract could be utilized as a guide for the creation of all-natural drugs to treat diseases caused by these pathogens. The extract was effective against urinary tract infection-causing *Proteus mirabilis* and *Klebsiella pneumoniae*, and the diarrhea-causing *Escherichia coli* and *Shigella dysenteriae*. Thus, this study confirms Florence and Adiaha's earlier findings [24] that this plant is effective in treating diarrhea and urinary tract infections in Southern part of Nigeria.

Investigations were also conducted into the fractions' MICs and MBCs. The fractions displayed low MICs and MBCs in contrast to the test isolates (Table 2). For instance, the fractions' lowest MIC was 0.63 mg/mL and their lowest MBC

was 1.25 mg/mL. The medicinal plant extracts with extremely low MIC and MBC are known to have significant antibacterial potentials [25] thus supported this observation. Additionally, plant extracts are considered to be bactericidal if their MIC are less than 2 mg/mL and bacteriostatic if their MIC are greater than 2 mg/mL but less than 16 mg/mL [26]. This finding showed that fractions of *C. albidum* leaf extract have bactericidal potentials and this plant could be used to make potent natural antimicrobial agents to fight the enteric bacterial pathogens that are currently impeding the effective treatment of infectious diseases in clinical settings.

The killing rates of the test isolates by the fractions were investigated to further examine the biocidal potentials. The fractions' ability to kill bacterial isolates within the shortest period of time was shown by this finding, which is evidence of more potent bactericidal action. This was in line with Pankey and Sabath's findings [27]. With increasing concentrations and time intervals, the fractions' killing rate of the bacterial isolates rose (Figure 1 to Figure 6). For instance, at 1 x MIC, n-butanol, aqueous and ethyl fractions each killed 85.9%, 81.8%, and 68.2% of *Klebsiella pneumoniae* within 60 min of contact time. Exactly, 100%, 95.5%, and 89.4% of the test cells were killed when the contact time was extended to 120 min. The rate at which the fractions cause the number of test strains to decrease therefore suggested a significant medicinal potential of this plant when the fractions' concentrations were increased to 2 x MIC and 3 x MIC respectively.

Alcohol, phenol, alkanes, alkenes, alkynes, aldehydes, aliphatic ether, amines, amide, aromatics, carboxylic acid, esters, aromatic ester and sulfoxides were among the functional groups found in the fractions according to the results of the FT-IR analysis of the fractions (Table 3). Similar functional groups were also found in several plant constituents according to earlier investigations [28, 29, 30].

9-Octadecenoic acid was discovered to be the main bioactive constituent in the n-butanol fraction (most active fraction) (Table 4). The presence of these bioactive constituents justifies traditional medical practitioners' use of various plant parts for the treatment of various ailments. The biocidal effects that these plant fractions demonstrated were significantly influenced by the bioactive constituents. Such constituents can be isolated from this plant in order to make an effective natural antibacterial drug for the treatment of microbial illnesses caused by these pathogens.

Conclusion

This finding showed that the plant's fractions have significant biocidal potentials against the selected enterobacterial species isolated from clinical samples. Due to its ability to kill the test strains used for this study at low concentrations and short contact times, *Chrysophyllum albidum* leaf extract has been identified as a potential future natural remedy for treating infections caused by these pathogens. The drugs produced from this plant will greatly enhance the delivery of healthcare. To purify and isolate the bioactive constituents that give this plant its biocidal characteristics, further research is still being conducted.

Conflicts of interest

The authors declare no conflict of interest.

Author's funding

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