Phytochemical Profiling of Aqueous Extracts of Medicinal Plants and their Activities against Multi-resistant Bacteria

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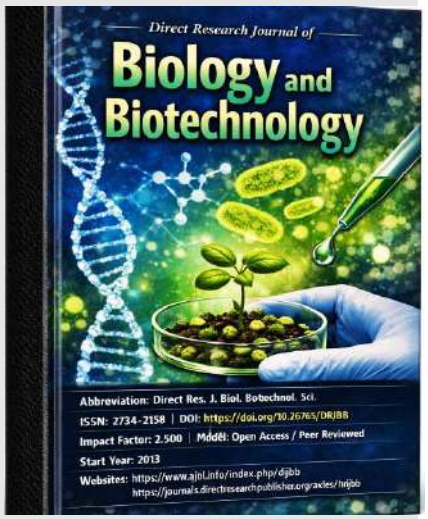
ABSTRACT

Acintobacter baumannii and Klebsiella pneumoniae are among the most critical pathogens gaining attention. These microorganisms continue to develop resistance thereby leading to various nosocomial infections which are difficult to treat. This study determined the resistance pattern of A. baumannii and K. pneumoniae and evaluates the activities of bio-molecules from selected plants against these clinical isolates. Fifty samples of A. baumannii and K. pneumoniae, which were isolated from different clinical samples were collected from different hospitals at Ogbomoso and then confirmed by their biochemical features to ascertain their identity. The antibacterial susceptibility pattern was determined by Kirky-Bauer disk diffusion method and the results were compared with the CLSI standard. Exactly, 500µL (0.5mL) of aqueous crude extract was used to evaluate the antibacterial efficacy on bacterial isolates by agar well diffusion method while gentamicin (10µg) was used as control. The qualitative phytochemical analysis of the bio-molecules in selected plant extracts was performed using standard methods and bioactive constituents were analyzed by Gas Chromatography-Mass Spectroscopy analysis. Forty-two (42) (84%) from the total of 50 bacterial isolates collected were confirmed to belong to Acinetobacter and Klebsiella genera. Total resistance (100%) was observed with Ampiclox (ACX) and cefuroxime (CXM) while ceftazidime (CAZ) showed 92% resistance to antibiotics used. Gentamicin (GEN) and nitrofurantoin (NIT) were found to be an effective antimicrobial agent with 72% susceptibility each. A. baumannii (UITH 8, UITH13 and UITH15) showed highest resistance each with 84.6% resistance to all antibiotic used, while K. pneumoniae (UITH2) showed 100% resistance. HCE and CCE were found to consistently show the strongest efficacy in a range of 4–10mm. K. pneumoniae, K. oxytoca and A. baumannii exhibited high inhibition zones up to 10mm with HCE and CCE. The aqueous hot and cold clove extracts were found to possess all metabolites at varying degree, with the exception of quinones which was absent in all samples. This study supports the potential role of medicinal plants as promising alternatives source of antimicrobials. It is recommended that the bioactive compounds present in the plant extracts should be purified and studied for their antimicrobial actions.

Keywords: *Pathogens, Antibiotics, Resistance, Phytochemicals, GC-MS*



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INTRODUCTION

Antimicrobial resistance (AMR) represents one of the biggest threats facing modern medicine.If no action is taken, it is predicted that resistance to available antimicrobials will result in 10 million fatalities by the year 2050,which will largely be attributed to infections caused by Gram-negative bacteria (GNB) (Naghavi, 2024). Multidrug resistant pathogens are among the top threat to global public health, which have evolved to resist any conventional antibiotics. Today, resistance to previously sensitive antimicrobial agents resulting into microbial infections has been the biggest challenge threatening human health and responsible for millions of deaths every year worldwide and the development of potent antimicrobial agents against this rising threat of resistance is quite challenging (Iheanacho, 2022).

The leading cause of nosocomial infections globally is largely due to group of bacteria which readily develop drug resistance, generally referred to as the ESKAPE pathogens (Santajit and Indrawattana, 2016). This group includes: *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp. Out of the members of this group, "*A. baumannii* has attracted significant attention over the last two decades due to the rapid onset of antibiotic resistance and worldwide spread of this species (Nguyen and Joshi, 2021).

Acinetobacter baumannii and *Klebsiella pneumoniae* are major clinical pathogens gaining interest (Nichols, 2019), and they are rising threat causing hospital acquired infections such as meningitis, pneumonia, urinary tract infection, wound infection and pyogenic liver abscess. *Acinetobacter baumannii* is a Gram-negative, strictly aerobic coccobacilli and opportunistic bacterial pathogen that is generally associated with nosocomial infections, causing a range of non-specific infections including pneumonia, soft tissue necrosis, and sepsis (Chen *et al.,* 2020; Lima *et al.,* 2025). Numerous medical and epidemiological reports has documented high incidence of multidrug-resistant *A. baumannii* infections among injured British and United States soldiers (Hamidian and Nigro, 2019). *Klebsiella pneumoniae* are major nosocomial pathogens producingextended spectrum beta-lactamase (ESBL) which areusually susceptible only to carbapenems, and these drugs havebeen the treatment of choice for severe infections by ESBL-producer *K. pneumoniae* (Bovo and Amadesi, 2023). Morerecently, the emergence of carbapenemase producing *K. pneumoniae* (KPC) has severely challenged antimicrobial therapy, since it confers a high level of resistance to all β-lactamsand distinct levels of resistance to carbapenems (Bovo and Amadesi, 2023; Falagas *et al.,* 2025). Carbapenem resistance among *K. pneumoniae*, which first emerged a decade ago, continues to spread (Gowsiya *et al.,* 2014; Bovo and Amadesi, 2023), and is a cause of major concern.

Recently, research on the potentials of medicinal plants has attracteda lot of attentions globally. Large body of evidence hasaccumulated to demonstrate the promising abilities of medicinal plants used in various traditional, complementary and alternate systems of treatment of human diseases (Bovo and Amadesi, 2023).Among the studied plants of antimicrobial properties are *Syzygium aromaticum, Kalanchoe pinnata, Allium sativum.* Clove contains phytochemicals such as eugenol and phenolic which exhibit great efficacy as antimicrobials against microbes (Zhao *et al.,* 2024). Bryophyllum is known for their broad pharmacological effects and has been known to show notable antibacterial activity against multidrug resistant bacteria which suggest their potential use as a source of natural antimicrobial compounds (Ojeleye *et al.,* 2024). Also, garlic (*Allium sativum*) has long been known for its antimicrobial effects due to the presence of sulfur-containing compounds such as allied thiosulfinates and allicin. Extract of *A. sativum* has shown inhibitory activity against diverse Gram-negative bacteria and fungi which suggest its potential relevance as a natural antimicrobial agent with therapeutic applications (Barbu *et al.,* 2023).
Antibiotics such as carbapenem class and polymyxin B which were previously sensitive and effective antimicrobials against these rising pathogen are increasingly less effective. These bacteria, *A. baumanii* and *K. pneumoniae* are becoming resistant even to the last line antibiotics such as imipenem and gentamycin, thus the need to find alternative therapeutic with enhanced potency against these rising pathogens. Hence, this research focused on determining the susceptibility pattern and evaluating the potentials of different plant extracts against these two clinically important rising pathogens.

MATERIALS AND METHODS

Procurement of Pathogenic Bacteria

Fifty clinical isolates of *Acinetobacter baumannii* and *Klebsiella pneumoniae* were collected from blood, urine, stool, and wound samples obtained from three tertiary hospitals including; LAUTECH Teaching Hospital, Ogbomoso, Oyo State, Bowen Teaching Hospital, Ogbomoso and University of Ilorin Teaching Hospital, Ilorin, Nigeria. The collected bacterial isolates were maintained aseptically on agar slants and transported to the laboratory for confirmation and further study.

Confirmation of Bacterial Isolates by Microscopic and Biochemical characteristics

Collected bacterial isolates were confirmed by microscopic techniques and different biochemical tests to ascertain their identity. Macroscopic and microscopic procedures such as shape, color and reaction to Gram

stain were observed. Also, various biochemical tests such as oxidase, urease, indole, citrate, catalase, MR-VP, nitrate reduction, sugar fermentation were determined to assess their metabolic potentials of collected bacterial isolates to detect enzyme activity, utilization of different specific substrates (Bergey, 2000; Bachoon *et al.,* 2008; Muhammad *et al.,*2020).

Evaluation of the Antibacterial Susceptibility of the Bacterial Isolates

This was carried out to determine the susceptibility pattern of bacterial isolates by Kirky-Bauer disk diffusion method on sterilize Mueller-Hinton agar. Standardized bacterial inoculums (McFarland = 0.5) was prepared from overnight grown bacteria culture and plated by surface spread method on the susceptibility agar plates according to the method of Ayandele *et al.* (2019). Antibiotic disc thatinclude; Ceftazidime (30µg), Cefuroxime (30µg), Cefotaxime (25µg), Gentamycin (10µg), Amoxicillin clavulanate (30µg), Ofloxacin (5µg), Ceftriaxone sulbactam (45µg), Ampiclox (10µg), Levofloxacin (5µg), Cefepime (30µg), Imipenem (10/10µg), Nalidixic acid (30µg), Nitrofurantoin (30µg) were aseptically placed on the plate and incubated for 18 – 24 hours at 37^0C. The inhibition zone were measured in diameter (mm) and results compared with CLSI standard (CLSI, 2024) to determine if the organisms are sensitive, intermediate or resistant to the antibacterial agents used.

Collection, Preparation and Preliminary Screening of Plants Extracts

Six (6) different plants that include fresh tumeric (*Curcuma longa*) rhizomes, Clove (*Syzygium aromaticum*) buds, Garlic (*Allium sativum* L.), Moringa (*Moringa oleifera*) leaves, Miracle leave (*Bryophyllum pinnatum*), and Sour sop (*Annona muricata*) leaves were procured from local markets and environments in Ogbomoso, Oyo State, Nigeria. Collected plants were authenticated by a taxonomist. The plants/parts were prepared accordingly by standard method of preparation (Malini *et al.,* 2023). The dried plant samples were then blended to powder and sieved with a mesh of size 0.2 mm in diameter. Fifty grams (50.0 g) each of the powdered plants were dissolved in 250 mL sterile distilled water (cold and hot water extraction) and allowed to soak for 2days. Mixtures are then filtered through whatman's filter paper to obtain crude plant extract and concentrated and then kept for the analysis. Sterility testing for all extracts were done by inoculating on nutrient agar media and incubated overnight at 37 ^{0}C.

Evaluation of the Antibacterial Potency of Plant Extracts

Each pure bacteria colonies were suspended in sterile normal saline and standardized to turbidity of McFarland tube number 0.5 (1.5×10^8 CFU/ ml) is attained. A loopful of standardized bacterial isolates was swabbed onto prepared Muller Hinton agar (HiMEDIA).Six wells of 6 mm were bored in the inoculated media using a sterile cork-borer. Each well was then filled with 500*µ*l (0.5mL) of 100 % crude extracts from different plants. Gentamicin (10µg) was used for positive control. It was then be allowed to diffuse for about 30 minutes at room temperature and incubated for 18-24 hours at 37°C. After incubation, plates were evaluated for antibacterial activity by observing and recording the formation of a clear zone around the well and measured in mm.

Qualitative Phytochemical Screening in the Plant Extracts

The qualitative analysis of the bio-constituents present in the selected plant extracts was performed using the methods of Trease and Evans (1989) and Adepoju *et al.,* (2020).

Test for Alkaloid (Mayer's)

A few milliliters of the filtrate are treated with 2% hydrochloric acid and mixed. Subsequently, 1– 2 drops of Wagner's reagent are added along the test tube's inner surface. The appearance of a brown or reddish precipitate indicates the presence of alkaloids.

Test for Tannins (Braymer's test)

One milliliter of the filtrate was mixed with three milliliters of distilled water, followed by the addition of three drops of 10% ferric chloride solution. The development of a blue-green coloration indicated the presence of tannins in the sample.

Test for Saponins (Foam test)

Two milliliters of water were added to a measuring cylinder containing 5 milliliters of the extract. The mixture was vigorously shaken for 15 minutes. The formation of a stable foam layer approximately 2 cm in thickness indicated the presence of saponins in the extract.

Test for Flavonoids (Alkaline reagent test)

One milliliter of the extract was mixed with two milliliters of 2% sodium hydroxide solution, resulting in the development of an intense yellow color. Upon the addition of a few drops of 2 % hydrochloric acid, the yellow color disappeared, confirming the presence of flavonoids in the sample.

Test for Quinones (Alcoholic KOH test)

The presence of quinones was identified using the

alcoholic potassium hydroxide test. One milliliter of the plant extract was mixed with a few milliliters of alcoholic potassium hydroxide solution. The development of a red to blue coloration indicated the presence of quinones in the sample.

Test for Glycoside (Borntrager's test)

To test for glycosides, 2 milliliters of the filtrated hydrolysate were mixed with 3 milliliters of chloroform and shaken thoroughly. The chloroform layer was then separated and treated with 10% ammonia solution. The development of a pink or reddish coloration in the ammonia layer indicated the presence of glycosides.

Test for Cardiac Glycosides (Keller-Killani test)

One milliliter of the filtrate was mixed with 1.5 milliliters of glacial acetic acid. A single drop of 5% ferric chloride solution was added, followed by the careful addition of concentrated sulfuric acid along the side of the test tube. A blue coloration in the acetic acid layer confirmed the presence of phenolic compounds.

Test for Terpenoids (Liebermann-test)

Half (0.5) milliliters of the extract were mixed with 2 milliliters of chloroform. Concentrated sulfuric acid was then added carefully along the side of the test tube. The formation of a red-brown color at the interface indicated the presence of terpenoids.

Test for Phenolic compounds (Ferric chloride test)

The extract filtrate was mixed with a few drops of 5% ferric chloride solution. The development of a dark green or bluish-black coloration indicated the presence of phenolic compounds.

Test for Steroids (Salkowski test)

Two (2) mL of plant extract was mixed with 2 mL of chloroform, followed by the addition of 10 drops of acetic anhydride and 3 drops of concentrated sulfuric acid. The emergence of a red-rose color indicates the presence of steroids.

Test for Anthraquinones (Borntrager's test)

To test for anthraquinones, 10 milliliters of 10% ammonia solution were added to a few milliliters of the filtrate, followed by vigorous shaking for 30 seconds. A pink, violet, or red-colored solution indicated the presence of anthraquinones.

Test for Anthocyanins (Hydrochloric acid test)

To test for flavonoids, 2 milliliters of the plant extract was mixed with 2 milliliters of 2N hydrochloric acid. A pink-red solution was formed, which turned blue-violet upon the addition of ammonia, indicating the presence of flavonoids.

Test for Coumarins (Sodium hydroxide test)

To test for coumarin, a plant extract was mixed with 10% sodium hydroxide and chloroform. The formation of a yellow color indicated the presence of coumarins.

Elucidation of Bioactive Metabolites in the Plant Extract by GC-MS

The GC-MS analysis of the leaf extracts was carried out using Agilent 19091S Gas chromatograph (GC) interfaced to a mass spectrometer 433HP-5MS instrument employing the following conditions: silica capillary column fused with 100% phenyl methyl silox, (length; 30 m × 250 µm; film thickness 0.25 µm). For GC-MS detection, an electron ionization system with ionization energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1.5 ml/min and an injection volume of 1 µl was employed (Split ratio of 50:1) injector temperature-300°C; average velocity of 45.67 cm/s. The oven temperature was programmed from 100°C (Isothermal for 4 min) with an increase of 4°C min-1 to 240°C. Total GC running time was 49 min (Adepoju *et al.,* 2020).

RESULTS

Bacterial Isolates collected from Hospitals

From the total of 50 clinical isolates which were previously isolated from different clinical sites that include blood, urine, wound and stool, 42 (84%) were confirmed to belong to the genera of *Acinetobacter* and *Klebsiella.* Among these, twenty 17 (40.5%) were identified as *Acinetobacter* sp. while 25 (59.5%) were *Klebsiella* sp. In this study, *Klebsiella pneumoniae* accounted for 17 isolates (34%) and being the most prevalent genera identified. Other *Klebsiella* species included *K. oxytoca* with 4 isolates (8%), *K. ornithinolytica* with 3 isolates (6%), and *Klebsiella* sp with 1 isolate (2%). *Acinetobacter baumannii* was the second most prevalent with 14 isolates representing 28%. Others are *Acinetobacter* sp. with 3 isolate (6%) and *Pseudomonas* sp. 3 isolates (6%), *Enterobacter* sp. with 2 isolates (4%) and *E. coli* with 2 isolates (4%) and *Raoultella* sp. with only 1 isolate (2%).The biochemical features of the 50 bacterial isolates showed that all isolates are Gram negative and are predominantly negative for catalase and citrate tests which were primarily identified as *K. pneumoniae*, *K.oxytoca*, and *K. ornithinolytica*. The colonial, microscopic and biochermical features of the bacterial isolates is represented in (Table 1).

Table 1: Colonial, Microscopic and Biochemical Characteristics of the Collected Bacterial Isolates.

S/N	Site of Isolation	Colour	Shape	Gram reaction	Catalase	Citrate	Starch hydrolysis	Indole	Oxidase	Urease	Motility	Nitrate	MR	VP	H_2S	Glucose	Sucrose	Mannitol	Isolate Identity
1	LTH1 (Urine)	Pink	Short rod	-	+	+	-	-	-	+	-	+	+	+	-	+	+	+	*Klebsiella pneumoniae*
2	LTH2 (blood)	Pink	Rod	-	+	+	-	-	-	+	-	+	+	+	-	+	+	+	*Klebsiella pneumoniae*
3	LTH3 (blood)	Pink	Rod, short	-	+	+	-	-	-	+	-	+	+	+	-	+	+	+	*Klebsiella pneumoniae*
4	LTH4 (blood)	Pink	Large, rod-like	-	+	+	-	-	-	+	-	+	-	-	-	+	+	+	*Klebsiella oxytoca*
5	LTH5 (blood)	Pink	Large rod	-	+	+	-	+	-	+	-	+	+	+	-	+	+	+	*Klebsiella ornithinolytica*
6	LTH6 (Urine)	Pink	Clustered chain	-	+	+	-	-	+	-	+	+	-	+	-	+	+	+	*Enterobacter* sp
7	LTH7(wound)	Pink	Rod	-	+	+	-	-	-	+	-	+	-	-	-	+	+	+	*Klebsiellaoxytoca*
8	UITH1 (Wound)	white	Circular, chain	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	*Acinetobacter baumannii*
9	UITH2 (Wound)	pink	Rod, large	-	+	+	-	-	-	+	-	+	+	+	-	+	+	+	*Klebsiella pneumoniae*
10	UITH3 (Blood)	white	Chain, circular	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	*Acinetobacter baumannii*
11	UITH4 (Wound)	pink	Short rod	-	+	+	-	-	-	+	-	+	+	+	-	+	+	+	*Klebsiella* sp
12	UITH5 (Blood)	white	Chain, circular	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	*Acinetobacter johnsonii*
13	UITH6 (Urine)	white	Circular chain	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	*Pseudomonas anguilliseptica*
14	UITH7 (Wound)	pink	Short rod	-	+	+	-	-	-	+	-	+	+	+	-	+	+	+	*Klebsiella pneumoniae*
15	UITH8 (Blood)	white	Circular, rod-like	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	*Acinetobacter baumannii*
16	UITH9 (Blood)	white	Small, circular chain	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	*Acinetobacter iwofii*
17	UITH10 (Wound)	pink	Rod, long, large	-	+	+	-	-	-	+	-	+	-	-	-	+	+	+	*Klebsiella oxytoca*
18	UITH11 (Wound)	white	Circular chain	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	*Acinetobacter baumannii*

S/N6	Site of Isolation	Colour	Shape	Gram reaction	Catalase	Citrate	Starch hydrolysis	Indole	Oxidase	Urease	Motility	Nitrate	MR	VP	H_2S	Glucose	Sucrose	Mannitol	Isolate Identity
19	Bowen1 (urine)	pink	Short	-	+	+	-	-	-	+	-	+	+	+	-	+	+	+	*Klebsiella pneumoniae*
20	Bowen2 (urine)	pink	Short rod	-	+	+	-	-	-	+	-	+	+	+	-	+	+	+	*Klebsiella pneumoniae*
21	Bowen3 (urine)	pink	Short, large	-	+	+	-	-	-	+	-	+	+	+	-	+	+	+	*Klebsiella pneumoniae*
22	Bowen4(wound)	pink	Clustered chain	-	+	+	-	-	-	+	-	+	+	+	-	+	+	+	*Klebsiella pneumoniae*
23	UITH12 (wound)	Grayish white	Circular, clustered	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	*Pseudomonas* sp.
24	UITH13 (blood)	Grayish white	Circular round	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	*Acinetobacter baumannii*
25	UITH14 (blood)	white	Chain, rod	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	*Acinetobacter* sp.
26	UITH15 (blood)	Grayish white	Circular and rod-like	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	*Acinetobacter baumannii*
27	UITH16 (wound)	white	Rod-like	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	*Acinetobacter baumannii*
28	LTH8 (wound)	pink	Short rod	-	+	+	-	-	-	+	-	+	-	-	-	+	+	+	*Klebsiella oxytoca*
29	LTH9(wound)	Pink	Rod	-	+	+	-	-	-	+	-	+	+	+	-	+	+	+	*Klebsiella pneumoniae*
30	LTH10 (stool)	pink	Large	-	+	-	-	+	-	-	+	+	+	-	-	+	+	+	*Escherichia coli*
31	LTH11 (stool)	pink	Short, rod	-	+	+	-	-	-	+	-	+	+	+	-	+	+	+	*Klebsiella pneumoniae*
32	LTH12 (urine)	pink	Clustered, rod	-	+	+	-	-	-	+	-	+	+	+	-	+	+	+	*Klebsiella pneumoniae*
33	UITH17(stool)	Pink	Large	-	+	+	-	+	-	+	-	+	+	+	-	+	+	+	*Raoultella* sp.
34	UITH18 (stool)	pink	Short rod	-	+	+	-	-	-	+	-	+	+	+	-	+	+	+	*Klebsiella pneumoniae*
35	UITH19 (stool)	Pink	Short rod	-	+	+	-	+	-	+	-	+	+	+	-	+	+	+	*Klebsiella ornithinolytica*
36	UITH20 (blood)	Pink	Short rod	-	+	+	-	+	-	+	-	+	+	+	-	+	+	+	*Klebsiella ornithinolytica*
37	UITH21 (blood)	pink	Chain	-	+	+	-	-	+	-	+	+	-	+	-	+	+	+	*Enterobacter* sp.

S/N	Site of Isolation	Colour	Shape	Gram reaction	Catalase	Citrate	Starch hydrolysis	Indole	Oxidase	Urease	Motility	Nitrate	MR	VP	H_2S	Glucose	Sucrose	Mannitol	Isolate Identity
38	UITH22 (urine)	pink	Rod	-	+	+	-	-	-	+	-	+	+	+	-	+	+	+	*Klebsiella pneumoniae*
39	UITH23 (blood)	Creamy white	Round	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	*Acinetobacter baumannii*
40	UITH24 (blood)	white	Convex edge	-	-	+	-	-	+	-	+	-	-	-	-	-	-	-	*Pseudomonas aeruginosa*
41	UITH25 (wound)	white	Small size, round	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	*Acinetobacter baumannii*
42	UITH26 (blood)	white	Round, smooth	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	*Acinetobacter baumannii*
43	UITH27 (wound)	pink	Large	-	+	+	-	-	-	+	-	+	+	+	-	+	+	+	*Klebsiella pneumoniae*
44	UITH28 (wound)	Pink	Chain	-	+	+	-	-	-	+	-	+	+	+	-	+	+	+	*Klebsiella pneumoniae*
45	UITH29 (stool)	Pink	Large chain	-	+	-	-	+	-	-	+	+	+	-	-	+	+	+	*Escherichia coli*
46	UITH30 (urine)	White	Smooth	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	*Acinetobacter baumannii*
47	UITH31 (wound)	Creamy	Convex edge	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	*Acinetobacter baumannii*
48	UITH32 (urine)	Pink	Clustered	-	+	+	-	-	-	+	-	+	+	+	-	+	+	+	*Klebsiella pneumoniae*
49	UITH33 (wound)	Whitish cream	Round, smooth	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	*Acinetobacter baumannii*
50	UITH34 (wound)	White	Smooth surface	-	-	+	-	-	-	-	-	-	-	-	-	+	-	-	*Acinetobacter baumannii*

Susceptibility Pattern of Bacterial Isolates to Antibacterial Agents

The antimicrobial susceptibility result showed that Ampiclox (ACX) and cefuroxime (CXM) exhibited total resistance with **0%** susceptibility, while ceftazidime (CAZ) demonstrated minimal activity with 8% susceptibility**.** Ceftriaxone (CRO) and nalidixic acid (NA) showed 12% effectiveness while cefotaxime (CTX) with 20**%** which indicate a widespread production of beta lactamase among the bacterial isolates. Gentamicin (GEN) and nitrofurantoin (NIT) were found to be effective with 72% susceptibility respectively, while Cefepime (FEP) and Imipenem (IMP) showed moderate activity with 58% and 44% susceptibility respectively against resistant Enterobacterales.

Most bacterial isolates are resistant to 8–11 antibiotic used. *K. pneumoniae*, (from wound source, UITH2) showed total resistance (100%) leaving no active agent among those tested.Most *A. baumannii* isolates showed 69.2–84.6% resistance which indicate their extensive drug resistance (XDR).*A. baumannii* (UITH 8, UITH13 and UITH15) showed highest resistance each with 84.6% resistance to all antibiotic used; followed by sample UITH3 with 76.9% resistance. *K. pneumoniae* (UITH2) showed 100% resistance; LTH3 with 84.6%; LTH9, LTH11, UITH27 and UITH32 showed equal resistance with 76.9% resistance (Table 2).

From the 42 isolates confirmed to belong to both the *Acinetobacter* and *Klebsiella* genera, 41 of them representing 97.6% was found to be multidrug resistant bacteria (MDR) since they confer resistance to three or more antimicrobial classes used. Only 1 isolate representing 2.4%, *K. pneuminiae* isolated from wound site (UITH2) was Pan Drug Resistant (PDR) showing resistance to all agents while no isolate was found to be Extensively Drug Resistant.

Antibacterial activities of Plant Extracts against bacterial Isolates

The results of antimicrobial potency of cold and hot extracts in the six selected plant is represented in the Table 4 below. Hot extracts generally produced larger inhibition zones than cold extracts**.** Across all the bacterial isolates, hot and cold clove extract were found to consistently show the strongest efficacy with clearance zones in a range of 4–10mm. *K. pneumoniae*, *K. oxytoca* and *A. baumannii* exhibited high inhibition zones of up to 10mm with HCE and CCE. *K. pneumoniae* (UITH27 and UITH32) showed 10 mm inhibition with HCE (Table 3).

Qualitative Phytochemical Screening of Selected Plant Extracts

The qualitative screening of the phytochemicals in the selected plant extracts showed the presence of different metabolites in the samples. The aqueous clove extracts (hot and cold) were found to possess all metabolites at varying degree with the exception of quinones which was not detected in all samples except in CBE. Alkaloids, tannins, flavonoids, glycosides and coumarin were detected in all the plant extracts while terpenoids, anthraquinones, anthocyanin and cardiac glycoside were not detected in the HBE and CBE. Tannin was detectedin highly abundance in the clove extract (Table 4).

Analysis of Organic Compound Contents in the Plant Extracts by GC-MS

The presence of various organic compound in the HCE were detected by the GCMS analysis; the presence of eugenol and phenol, 2-methoxy-3-(2-propenyl) were found to be dominant with percentage peak of 85.44% at retention time of 8.145 minutes (Table 5). Twenty-two (22) different organic compounds were detected in the HCE at different retention time, peak area and peak height. The lowest peak area (0.88%) with a retention time 13.507 minutes was observed for dodecane. heptadecane, hexadecane. In the CCE, 36 different organic compounds were detected at 13 different peak, retention time and peak height. The organic compounds eugenol and 3-Allyl-6-methoxyphenol were detected at peak area 85.11%, retention time of 11.94 minutes and

Table 2: Negative Reaction Antibiotic Susceptibility Testing of Bacterial Isolates.

Site of Isolation	Isolate Identity	ACX (10 µg)	CAZ (30µg)	CXM (30µg)	GEN (10µg)	IMP (10/10 µg)	CRO (45 µg)	NA (30 µg)	CTX (25 µg)	FEP (30 µg)	OFX (5µg)	AUG (30µg)	NIT (30 µg)	LBC (5 µg)	%Resistance
LTH1 (Urine)	*Klebsiella pneumoniae*	R	R	R	I	R	R	R	R	R	R	S	I	I	69.2
LTH2 (blood)	*Klebsiella pneumoniae*	R	R	R	S	S	R	R	R	R	R	S	S	R	69.2
LTH3 (blood)	*Klebsiella pneumoniae*	R	R	R	S	R	R	R	R	R	R	R	R	I	84.6
LTH4 (blood)	*Klebsiella oxytoca*	R	R	R	R	R	R	R	R	I	R	R	R	R	92.3
LTH5 (blood)	*Klebsiella ornithinolytica*	R	R	R	R	I	R	R	R	R	S	R	R	R	84.6
LTH6 (Urine)	*Enterobacter* sp.	R	R	R	S	I	R	R	R	I	R	I	I	R	61.5
LTH7 (wound)	*Klebsiella oxytoca*	R	R	R	S	R	R	R	R	R	I	R	R	R	84.6
UITH1 (Wound)	*Acinetobacter baumannii*	R	R	R	I	R	I	R	R	S	R	I	R	R	69.2
UITH2 (Wound)	*Klebsiella pneumoniae*	R	R	R	R	R	R	R	R	R	R	R	R	R	100
UITH3 (Blood)	*Acinetobacter baumannii*	R	R	R	R	R	R	R	R	R	S	I	S	R	76.9
UITH4 (Wound)	*Klebsiella* sp.	R	R	R	I	I	R	I	R	I	R	R	R	R	69.2
UITH5 (Blood)	*Acinetobacter johnsonii*	R	R	R	S	R	R	R	R	I	R	R	I	R	76.9
UITH6 (Urine)	*Pseudomonas anguilliseptica*	R	R	R	I	R	R	R	R	R	S	R	R	R	84.6
UITH7 (Wound)	*Klebsiella pneumoniae*	R	R	R	I	S	R	R	R	S	R	R	I	I	61.5
UITH8 (Blood)	*Acinetobacter baumannii*	R	R	R	R	R	R	R	R	R	S	R	I	R	84.6
UITH9 (Blood)	*Acinetobacter iwofii*	R	R	R	I	I	R	R	R	R	S	R	I	R	69.2
UITH10 (Wound)	*Klebsiella oxytoca*	R	R	R	S	R	R	I	R	R	S	I	R	I	61.5
UITH11 (Wound)	*Acinetobacter baumannii*	R	R	R	S	I	R	R	R	I	R	R	I	R	69.2
Bowen1 (urine)	*Klebsiella pneumoniae*	R	R	R	I	S	R	R	R	S	R	S	S	R	61.5
Bowen2 (urine)	*Klebsiella pneumoniae*	R	R	R	R	R	R	I	R	R	R	R	S	R	84.6
Bowen 3 (urine)	*Klebsiella pneumoniae*	R	I	R	I	S	R	R	R	I	R	S	I	R	53.8
Bowen4(wound)	*Klebsiella pneumoniae*	R	I	R	S	R	R	R	S	I	R	R	R	R	69.2
UITH12 (wound)	*Pseudomonas* sp.	R	I	R	S	R	S	R	R	I	R	I	R	R	61.5
UITH13 (blood)	*Acinetobacter baumannii*	R	R	R	R	R	R	R	R	I	R	R	S	R	84.6
UITH14 (blood)	*Acinetobacter* sp.	R	R	R	I	R	R	R	I	R	I	R	S	R	69.2
UITH15 (blood)	*Acinetobacter baumannii*	R	R	R	R	R	R	R	R	I	R	R	S	R	84.6
UITH16 (wound)	*Acinetobacter baumannii*	R	R	R	R	I	R	R	I	R	I	R	S	R	69.2
LTH8 (wound)	*Klebsiella oxytoca*	R	R	R	S	R	R	R	S	I	R	R	S	R	69.2
LTH9 (wound)	*Klebsiella pneumoniae*	R	R	R	S	R	R	R	R	R	R	I	S	R	76.9
LTH 10 (stool)	*Escherichia coli*	R	R	R	S	I	R	R	R	I	R	R	R	R	76.9
LTH11 (stool)	*Klebsiella pneumoniae*	R	R	R	R	I	R	R	R	I	R	R	I	R	76.9
LTH12 (urine)	*Klebsiella pneumoniae*	R	R	R	S	R	R	R	R	R	S	R	S	I	69.2
UITH17 (stool)	*Raoultella* sp.	R	I	R	R	S	I	R	R	I	S	R	I	S	46.2
UITH18 (stool)	*Klebsiella pneumoniae*	R	R	R	S	I	R	I	I	S	R	S	R	S	46.2
UITH19 (stool)	*Klebsiella ornithinolytica*	R	R	R	R	R	R	R	I	I	R	R	S	R	76.9
UITH20 (blood)	*Klebsiella ornithinolytica*	R	R	R	S	R	I	R	R	R	S	R	S	R	69.2
UITH21 (blood)	*Enterobacter* sp.	R	R	R	I	I	R	R	R	I	S	R	S	R	61.5
UITH22 (urine)	*Klebsiella pneumoniae*	R	R	R	S	S	R	R	R	S	R	R	S	I	61.5
UITH23 (blood)	*Acinetobacter baumannii*	R	R	R	I	S	R	R	R	I	R	R	I	R	69.2
UITH24 (blood)	*Pseudomonas aeruginosa*	R	R	R	S	S	R	R	R	S	R	R	I	I	61.5
UITH25 (wound)	*Acinetobacter baumannii*	R	R	R	S	I	R	R	R	R	R	R	S	I	69.2
UITH26 (blood)	*Acinetobacter baumannii*	R	R	R	I	R	R	R	I	R	R	R	I	I	69.2
UITH27 (wound)	*Klebsiella pneumoniae*	R	R	R	R	I	R	R	S	R	R	R	I	R	76.9
UITH28 (wound)	*Klebsiella pneumoniae*	R	R	R	I	I	R	R	R	I	I	I	R	R	61.5
UITH29 (stool)	*E. coli*	R	R	R	S	R	R	R	R	I	R	R	I	I	69.2
UITH30 (urine)	*Acinetobacter baumannii*	R	R	R	R	R	R	R	I	I	R	R	S	I	69.2
UITH31 (wound)	*Acinetobacter baumannii*	R	R	R	S	R	S	R	R	I	R	R	S	I	61.5
UITH32 (urine)	*Klebsiella pneumoniae*	R	R	R	S	R	R	I	R	R	R	R	S	R	76.9
UITH33 (wound)	*Acinetobacter baumannii*	R	R	R	S	R	R	R	I	I	S	R	S	R	61.5
UITH34 (wound)	*Acinetobacter baumannii*	R	R	R	S	I	I	I	R	I	S	R	S	R	46.2
Percentage Sensitivity (% Sens.)		0.0	8.0	0.0	72	44	12	12	20	58	30	24	72	28	

Keys: CAZ; Ceftazidime; CXM: Cefuroxime; CTX: Cefotaxime; GEN: Gentamycin; AUG: Amoxicillin clavulanate; OFX: Ofloxacin; CRO: Ceftriaxone sulbactam; ACX: Ampiclox; LBC: Levofloxacin; FEP: Cefepime; IMI: Imipenem; NA: Nalidixic acid; NIT: Nitrofurantoin; R: resistant; S: Sensitive; I: Intermediate

peak height 38381364 (Table 6). In the hot bryophyllum extract (HBE), 113 different organic compounds were detected at 39 different retention time, peak area and peak height.Bis(2-ethylhexyl) phthalate and Phthalic acid, di(hex-3-yl) ester has the highest percentage peak area at 9.46% with peak height of 1026671 (Table 7). In the cold bryophyllum extract (CBE), 123 different organic compounds were detected at 44 different retention time, peak area and peak height.Bis(2-ethylhexyl) phthalate and Phthalic acid, cyclohexyl 2-pentyl ester had the highest peak area 6.60 at retention time 23.864 minutes

Table 3: Antibacterial Activities of Different Plant Extracts against Bacterial Isolates.

		Hot and Cold Extracts from Plants											
Site of Isolation	**Isolate Identity**	**HME**	**CME**	**HTE**	**CTE**	**HCE**	**CCE**	**HGE**	**CGE**	**HBE**	**CBE**	**HSE**	**CSE**
LTH1 (Urine)	*Klebsiella pneumoniae*	0	0	0	0	2	4	0	0	0	0	0	0
LTH2 (blood)	*Klebsiella pneumoniae*	2	0	3	1	10	5	0	0	6	3	2	0
LTH3 (blood)	*Klebsiella pneumoniae*	0	0	2	0	6	5	0	0	6	4	0	0
LTH4 (blood)	*Klebsiella oxytoca*	0	0	0	0	8	6	0	0	8	5	0	0
LTH5 (blood)	*Klebsiella ornithinolytica*	4	2	8	6	8	8	0	0	4	2	0	0
LTH6 (Urine)	*Enterobacter* sp.	0	0	3	0	4	2	0	0	0	0	0	0
LTH7 (wound)	*Klebsiella oxytoca*	0	0	0	0	0	0	0	0	2	2	0	0
UITH1 (Wound)	*Acinetobacter baumannii*	0	0	2	0	6	5	0	0	4	2	0	0
UITH2 (Wound)	*Klebsiella pneumoniae*	2	2	0	0	4	2	0	0	4	0	0	0
UITH3 (Blood)	*Acinetobacter baumannii*	4	4	6	2	2	0	0	0	8	3	0	2
UITH4 (Wound)	*Klebsiella* sp.	0	0	2	2	2	2	0	0	0	0	0	0
UITH5 (Blood)	*Acinetobacter johnsonii*	2	0	0	0	8	6	0	0	2	0	0	0
UITH6 (Urine)	*Pseudomonas anguilliseptica*	0	0	0	0	2	2	0	0	0	2	0	4
UITH7 (Wound)	*Klebsiella pneumoniae*	0	0	12	11	4	4	0	0	0	0	2	0
UITH8 (Blood)	*Acinetobacter baumannii*	4	4	4	2	8	7	0	0	4	4	0	0
UITH9 (Blood)	*Acinetobacter iwofii*	0	0	6	6	8	6	0	0	2	0	0	0
UITH10(Wound)	*Klebsiella oxytoca*	0	0	8	4	8	8	0	0	7	8	0	0
UITH11(Wound)	*Acinetobacter baumannii*	3	0	4	4	6	3	0	0	4	2	2	2
Bowen1 (urine)	*Klebsiella pneumoniae*	2	2	2	2	6	4	0	0	4	4	0	2
Bowen2 (urine)	*Klebsiella pneumoniae*	1	0	0	0	3	2	0	0	0	0	0	0
Bowen 3 (urine)	*Klebsiella pneumoniae*	0	0	0	0	0	0	0	0	0	0	0	0
Bowen 4(wound)	*Klebsiella pneumoniae*	4	3	3	0	3	1	0	0	2	2	2	2
UITH12(wound)	*Pseudomonas* sp.	2	0	5	2	6	6	0	0	0	2	2	0
UITH13 (blood)	*Acinetobacter baumannii*	2	0	7	4	8	8	0	0	6	3	0	0
UITH14 (blood)	*Acinetobacter* sp.	4	4	3	1	6	8	0	0	4	4	2	2
UITH15 (blood)	*Acinetobacter baumannii*	0	1	2	0	9	6	0	0	9	7	0	0
UITH16(wound)	*Acinetobacter baumannii*	2	2	4	5	7	4	0	0	0	0	0	0
LTH8 (wound)	*Klebsiella oxytoca*	0	2	0	0	4	4	0	0	2	2	2	2
LTH9 (wound)	*Klebsiella pneumoniae*	0	0	2	2	6	6	0	0	4	2	0	0
LTH 10 (stool)	*Escherichia coli*	2	0	3	3	5	3	0	0	1	0	0	0
LTH11 (stool)	*Klebsiella pneumoniae*	4	3	0	0	4	4	0	0	3	1	0	0
LTH12 (urine)	*Klebsiella pneumoniae*	0	0	2	0	4	0	0	0	2	0	0	0
UITH17 (stool)	*Raoultella* sp.	5	4	0	0	6	4	0	0	4	2	2	2
UITH18 (stool)	*Klebsiella pneumoniae*	0	0	2	2	8	6	0	0	4	4	0	0
UITH19 (stool)	*Klebsiella ornithinolytica*	0	0	2	0	4	0	0	0	6	0	2	2
UITH20 (blood)	*Klebsiella ornithinolytica*	0	0	1	0	2	0	0	0	2	2	0	0
UITH21 (blood)	*Enterobacter* sp.	2	1	3	5	5	2	0	0	0	0	0	0
UITH22 (urine)	*Klebsiella pneumoniae*	0	0	0	0	2	0	0	0	2	2	2	2
UITH23 (blood)	*Acinetobacter baumannii*	4	3	3	2	4	4	0	0	0	0	0	0
UITH24 (blood)	*Pseudomonas aeruginosa*	2	0	0	0	6	4	0	0	5	3	0	2
UITH25(wound)	*Acinetobacter baumannii*	0	0	0	0	2	1	0	0	0	0	0	0
UITH26 (blood)	*Acinetobacter baumannii*	0	0	2	0	3	2	0	0	2	4	2	1
UITH27(wound)	*Klebsiella pneumoniae*	4	2	4	4	10	8	0	0	8	6	0	0
UITH28(wound)	*Klebsiella pneumoniae*	2	1	5	6	8	7	0	0	5	5	1	0
UITH29 (stool)	*E. coli*	4	2	0	0	0	0	0	0	2	2	0	0
UITH30 (urine)	*Acinetobacter baumannii*	0	0	0	0	2	2	0	0	2	2	2	2
UITH31(wound)	*Acinetobacter baumannii*	2	2	0	0	4	8	0	0	0	0	0	0
UITH32 (urine)	*Klebsiella pneumoniae*	4	4	3	4	10	8	0	0	0	1	1	0
UITH33(wound)	*Acinetobacter baumannii*	0	0	4	4	8	4	0	0	0	0	0	0
UITH34(wound)	*Acinetobacter baumannii*	0	0	0	2	3	5	0	0	2	2	0	0

Keys: HME: Hot Moringa leaf extract; CME: Cold Moringa leaf extract; HTE: Hot turmeric extract; CTE: cold turmeric extract; HCE: hot clove extract; CCE: colc clove extract; HGE: hot garlic extract; CGE: cold garlic extract; HBE: hot bryophylum leaf extract; CBE: cold bryophylum leaf extract; HSE: Hot sour sopleave extract; CSE: cold sour sop leave extract

Table 4: Qualitative Phytochemical Screening of the Selected Plant Extracts.

Phytochemicals	**HCE**	**CCE**	**HBE**	**CBE**
Alkaloid	++	++	+	+
Tannin	+++	+++	++	++
Saponin	++	+	+	-
Flavonoid	++	++	++	++
Quinone	-	-	-	+
Glycoside	++	++	+	+
Cardiac glycoside	+	+	-	-
Terpenoid	+	+++	-	-
Steroid	+	+	-	-
Anthraquinone	+	+	-	-
Anthocyanin	+	+	-	-
Coumarin	+	+	+	+

Keys: HCE: Hot clove extract; CCE: Cold clove extract; HBE: Hot bryophyllum extract; CBE: Cold bryophyllum extract; +: present; ++: Moderately present; +++: highly present; -: absent

Table 5: Analysis of the Organic compounds in the hot clove plant extract by GC-MS.

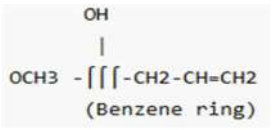
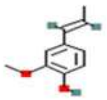
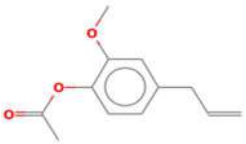
S/N	Retention time (min)	Peak Area (%)	Organic compounds	Peak height	Fragmentation pattern Peak	Molecular weight	Chemical Structure
1	8.145	85.44	Eugenol Phenol, 2-methoxy-3-(2-propenyl)-	27450428	164, 149, 137, 121	164.20 g/mol	
2	9.198	1.78	2-Methoxy-4-propenylphenol (Isoeugenol)	734996	149, 131, 121	164.20 g/mol	
3	9.845	3.37	Phenol, 2-methoxy-4-(2-propenyl)-, acetate	2234219	164, 149, 137, 121, 91	206.24 g/mol	
4	11.613	1.04	Heneicosane Methylheptacosane Methylnonadecane	440710	394, 379, 197, 85	394.77 g/mol	
5	13.507	0.88	Dodecane Heptadecane Hexadecane	504501	155, 141, 127, 57	170.33 g/mol	
6	16.007	4.22	2-Piperidinone, N-[4-bromo-n-butyl]- Oxalic acid, isobutyl hexadecyl ester	347667	227, 213, 187, 170, 127	244.14 g/mol	

Table 6: Analysis of the Organic compounds in the cold clove extract by GC-MS.

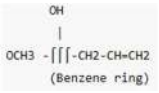
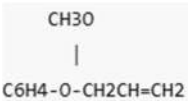
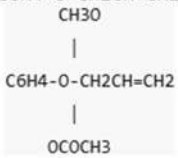
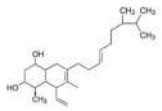
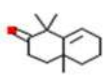
S/N	Retention time (min)	Area (%)	Organic compounds	Peak height	Fragmentation pattern peak	Mol. Weight	Chemical structure
1	11.934	85.11	Eugenol 3-Allyl-6-methoxyphenol	38381364	164, 149, 137, 121	164.20g/mol	
2	12.706	0.80	Caryophyllene Bicycloundec-4-ene, 4,11,11-trimethyl-8-methylene-	1394864	189, 174, 161, 147	204.0 g/mol	
3	13.107	2.14	Phenol, 2-methoxy-4-(1-propenyl)- (Z)-trans-Isoeugenol	2110671	149, 134, 119, 91	164.21 g/mol	
4	13.890	2.45	Phenol, 2-methoxy-4-(2-propenyl)acetate	3737733	165, 150, 120, 107	180.20 g/mol	
5	25.563	2.21	17-(1,5-Dimethylhexyl)-10,13-dimethyl-4-vinylhexadecahydrocyclopenta[a]phenanthren-3-ol Ergost-5-en-3-ol, (3.beta.)- 2,2-Dimethylpropanoic acid, 2,6-dimethylnon-1-en-3-yn-5-yl ester	721091	396, 367, 320, 290	400.7 g/mol	
6	25.964	0.61	Supraene 2(1H)-Naphthalenone, octahydro-4a-methyl-7-(1-methylethyl)-,(4a.alpha.,7.beta.,8a.beta.)- 2,6,10-Dodecatrien-1-ol, 3,7,11-trimethyl-	590901	203, 170, 48	218.3346 g/mol	

Table 7: Analysis of the Organic compounds in the hot bryophyllum extract by GC-MS.

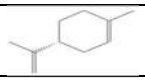
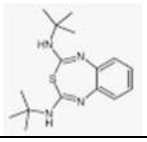
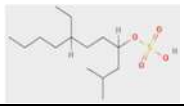
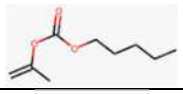
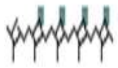
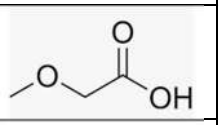
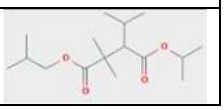
S/N	Retention time (min)	Area (%)	Organic compounds	Peak height	Fragmentation pattern		Chemical structure
					Peak	Mol. Weight (g/mol)	
1	6.852	1.82	D-Limonene Cyclohexene, 1-methyl-4-(1-methylethenyl)-	290588	121, 107, 93, 68	136.23	
2	13.839	1.21	Phenol, 2,6-bis(1,1-dimethylethyl) Phenol, 3,5-bis(1,1-dimethylethyl)-2,4-Di-tert-butylphenol	137211	206, 153, 105	220.35	
3	20.139	1.30	Carbonic acid, eicosyl vinyl ester 2-Piperidinone, N-[4-bromo-n-butyl]- Oxalic acid, allyl tetradecyl ester	177145	170, 112,84,70, 56	249.16 g/mol	
4	20.774	1.45	Sulfurous acid, 2-propyl tetradecy l ester Sulfurous acid, octadecyl 2-propyl ester	106047	229, 215, 201	272.46 g/mol	
5	25.472	5.06	Carbonic acid, 2-ethylhexyl nonyl ester Carbonic acid, bis(2-ethylhexyl) ester	375930	171, 113, 85, 57	300.47 g/mol	
6	25.964	4.87	2,6,10,14,18-Pentamethyl-2,6,10,14,18-eicosapentaene trans-Farnesol Squalene	488949	337, 99, 57	352.67 g/mol	
7	27.749	1.06	Methoxyacetic acid, heptadecyl ester Behenic alcohol Docosyl isobutyl ether	33890	73, 58, 46	90.08 g/mol	
8	29.363	1.47	17-Pentatriacontene Docosyl isobutyl ether Isobutyl tetracosyl ether	45948	699, 685, 156, 85, 57	713.31 g/mol	
9	31.131	2.14	Propanamide, N-(3-methoxyphenyl)-2,2-dimethyl- 2,2-Dimethylpropanoic acid	135770	333, 72, 64	390.63 g/mol	

Table 8: Analysis of the Organic compounds in the cold bryophyllum extract by GC-MS.

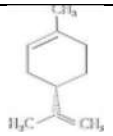
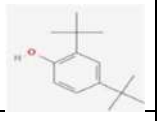
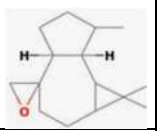
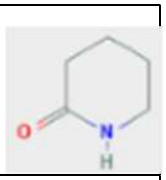
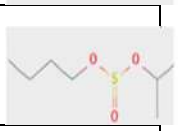
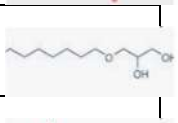
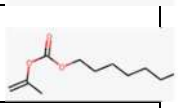
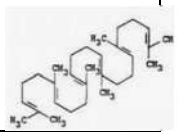
S/N	Retention time (min)	Area (%)	Organic compounds	Peak height	Fragmentation pattern		Chemical Structure
					Peak	Mol. Weight (g/mol)	
1	6.852	2.13	D-Limonene	275334	121, 93,68, 41	136.24	
2	13.839	1.19	2,4-Di-tert-butylphenol Phenol, 2,6-bis(1,1-dimethylethyl)	143246	191, 177, 161, 135, 57	206.32	
3	15.649	1.27	Alloaromadendrene oxide-(1) N,N'-di-sec-Butyl-p-phenylenediamine 1,2-dimethyl-3-methylene-cyclopropane	52255	221, 191, 161, 135, 121	236.37	

Table 8

4	19.401	2.06	2-Piperidinone, N-[4-bromo-n-butyl]- n-Hexadecanoic acid 1-Decanol, 2-hexyl-	258340	82, 70, 57, 42, 29	99.13	
5	20.299	3.98	Octacosane Tridecane Eicosane	419123	216, 201, 186, 171, 156	396.79 g/mol	$CH3-[CH2]_{26}-CH3$
6	20.585	1.18	Carbonic acid, decylhexadecyl ester Octacosyltrifluoroacetate Sulfurous acid, octadecyl 2-propylester	138778	230, 215, 200, 185, 170	410.72 g/mol	
7	20.774	1.37	Sulfurous acid, 2-propyl tetradecyl ester Sulfurous acid, pentadecyl 2-propyl ester	73812	181, 121, 91	316.54	
8	23.320	1.03	Batilol Hentriacontane Isopropyl tetradecyl ether	64275	150, 135, 105	344.58	
9	25.466	3.69	Carbonic acid, 2-ethylhexyl nonyl ester 4-Methyldocosane	301779	222, 207, 192	312.53	
10	25.958	4.69	Squalene	406088	310,280,165	410.80	

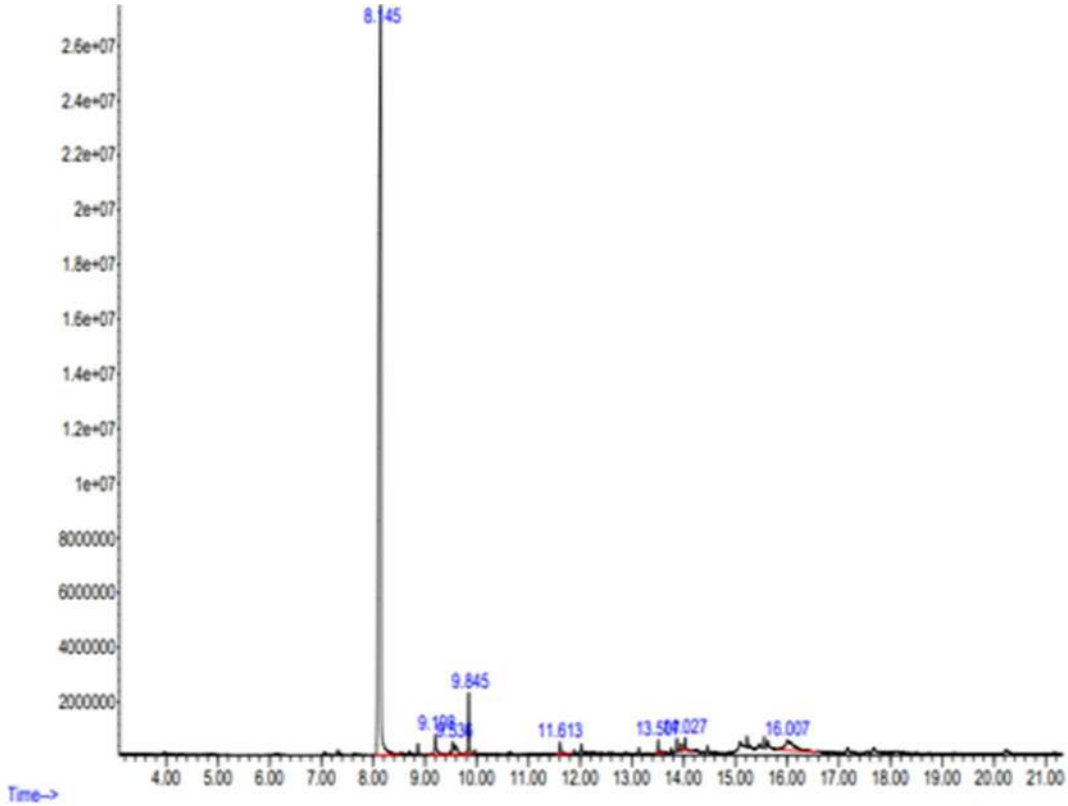


Figure 1: Gas chromatograph of organic compounds in the hot clove extract

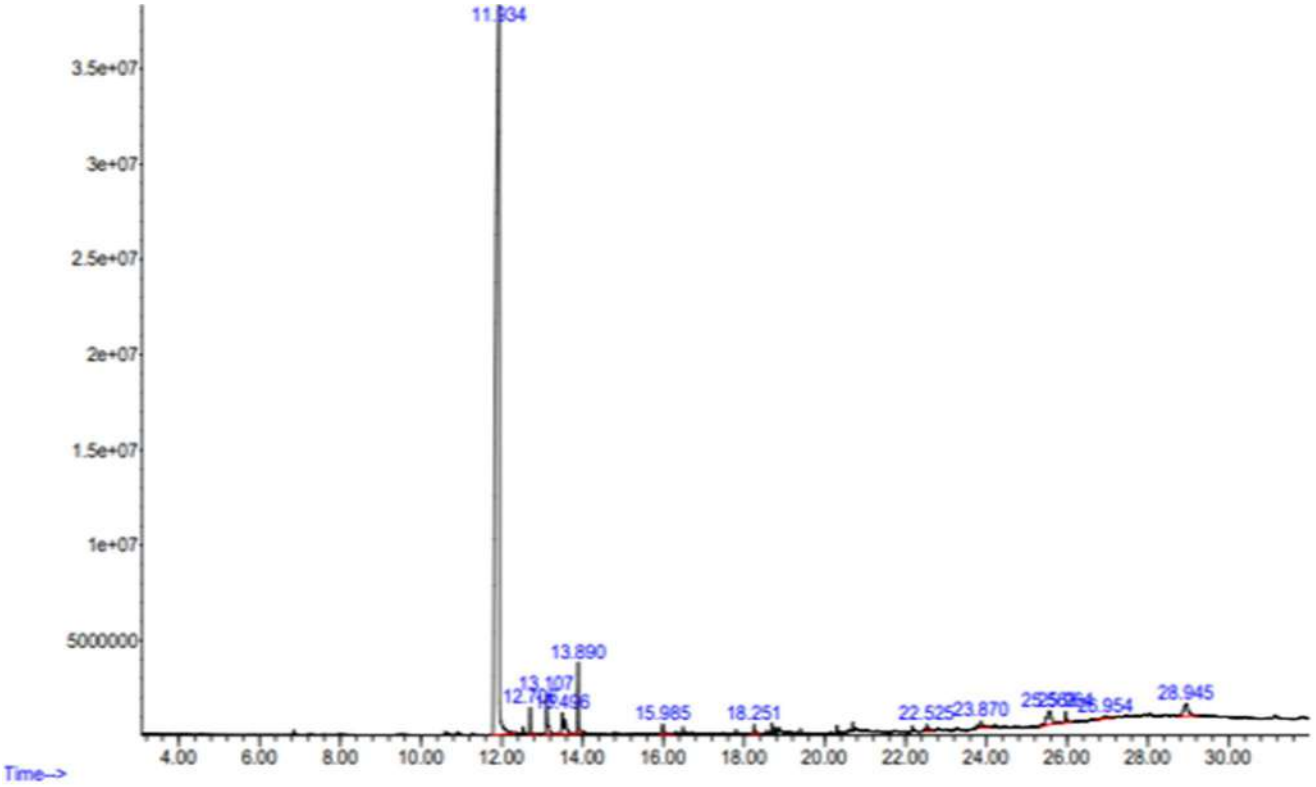


Figure 2: Gas chromatograph of organic compounds in the cold clove extract

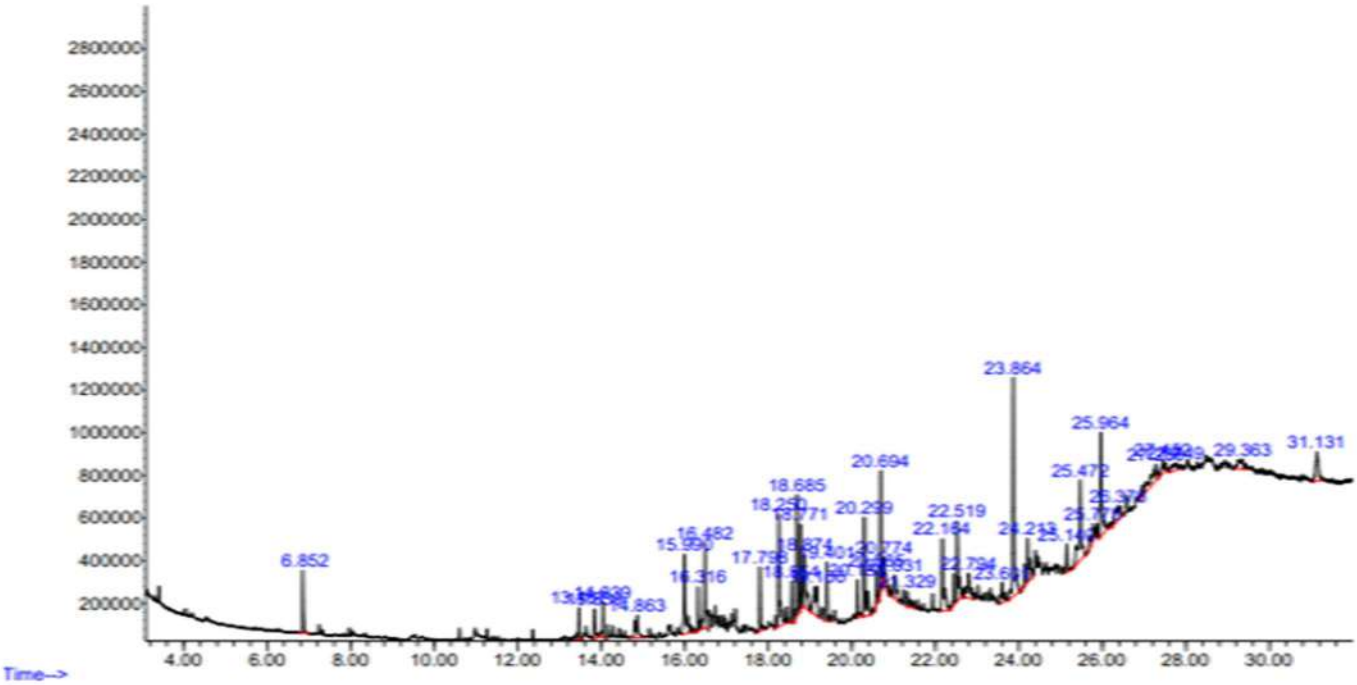


Figure 3: Gas chromatograph of organic compounds in the hot bryophyllum extract

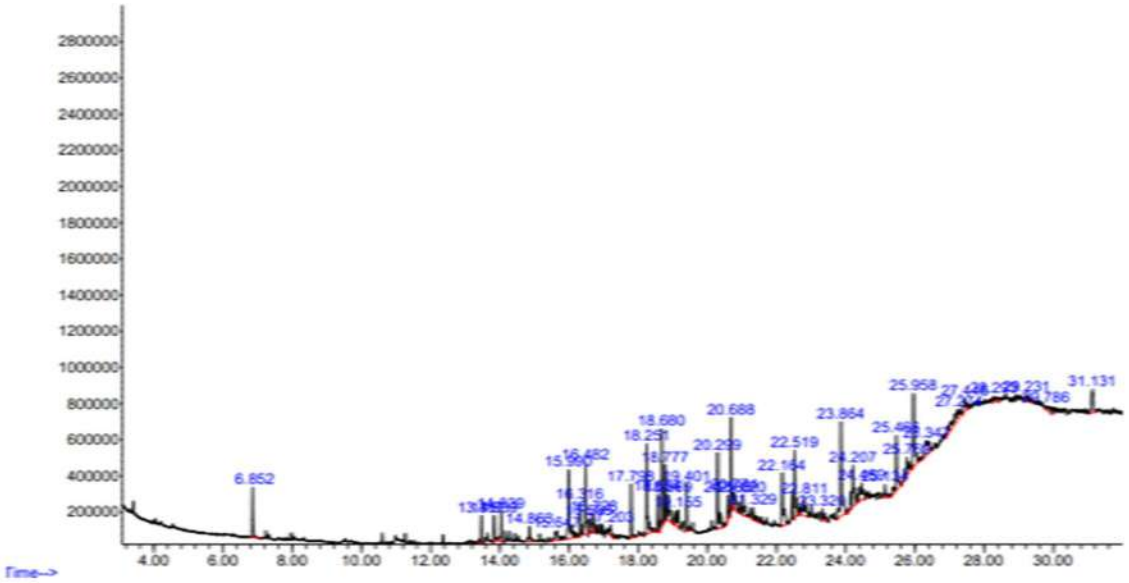


Figure 4: Gas chromatograph of organic compounds in the cold bryophyllum extract

(Table 8). The Gas Chromatographs of the different plant extracts showing the separation of components in the plant extracts were represented in (Figures 1-4).

DISCUSSION

Infections due to *A. baumannii* and *K. pneumoniae* remain a global concern and much is needed to be understood about their resistance patterns especially in Nigeria where they are understudied. In the present study, *A. baumannii* and *K. pneumonia*e isolated from clinical samples were collected from major government and private hospitals in Ogbomoso, Oyo State. Out of the total of 50 bacterial isolates collected, 42 (84%) were confirmed to belong to the genera of *Acinetobacter* and *Klebsiella* by their morphology and biochemical features. Odih*et al.*(2022) also reported high prevalence of *Acinetobacter* on rectal colonization and nosocomial transmission of carbapenem-resistant *Acinetobacter baumannii* in an intensive care unit, Southwest Nigeria' reported similar trend in the isolation of *A. baumannii* from ICU patients (Odih*et al.,*2022). The rising emergence of these isolates is due to the possession of carbapenem resistance determinants with genetic context like transfer of resistant genes among isolates.

Another study by Adebanjo and Thonda, (2025), also reported high prevalence of resistant *A. baumannii* and *K. pneumoniae* with 55.6% resistance across the six geo-political zones in Nigeria with varying prevalence from the South-Western zone of Nigeria. Dada-Adegbola *et al.* (2020) isolated 87 *A.baumannii* from different clinical samples such as blood, tracheal, wound, and confirmed 43 using Microbact with 37 further identified using Vitek 2. Suwaiba *et al.* (2020) in their study also isolated 46 (13.1%) *K. pneumoniae* from 300 urine samples. Generally, *Klebsiella* species and *A. baumannii* were isolated using similar technique and culture media although they had varying morphology and biochemical characteristics.

Multi-drug resistance (MDR) is a concern in the management of uropathogens. This MDR bacteria maintain resistance dueto thepossession ofmobile genetic elements such as plasmids which are extra-chromosomal DNA that can carry multiple antibiotic resistance genes (ARGs)transferring them between bacteria via conjugation as observed for $bla_{KPC,}$ bla_{NDM} genes on plasmids in *K. pneumoniae*. Multidrug Resistance (MDR) in *K. pneunomiae* is currently on the increase throughout the world (Innocent *et al.,* 2023). Susceptibility result in this study showed a high prevalence of multidrug resistance among these bacterial isolates. The complete resistance (100%) observed in ACX and CXM showed there ineffectiveness against the isolates. Ceftazidime (8%), Ceftriaxone (12%), and Cefotaxime (20%) showed reduced activity which indicates a widespread production of β-lactamase enzymes, particularly ESBLs. A similar study by Ayeni*et al.* (2025) on ESBL-producing *K. pneumoniae* isolated from urinary tract infections in Keffi also demonstrated 100% resistance to Ceftazidime, and 95% each to Ceftriaxone and Cefuroxime, and high MDR prevalence, thus confirming widespread β-lactam resistance patterns similar to what was observed in the present finding which these antibiotics were largely ineffective. Also, in a study carried out by Nwadike *et al.* (2013) in a Nigeria ICU facility, they reported total resistance (100%) to

Amoxicillin-clavulanate, Ceftriaxone, Ciprofloxacin, and Ofloxacin which further support that *Acinetobacter* in Nigeria commonly resists first-line β-lactam similar to this study.

The effectiveness of gentamicin (GEN) and nitrofuratoin (NIT) at 72% each may be attributed to their less frequent misuse due to its restricted use and peculiar mechanism of action, especially in UTI which decreases resistant development. Cefepime (FEP) showed moderate susceptibility with 58% which reflect increased stability against some beta-lactamases. Imipenem (IMP) usually considered as last resort antibiotic also showed partial susceptibility with 44%. This suggests the likely presence of carbapenem-resistant mechanisms. The reports from this study correlate with the report of Ashefo *et al.* (2023) which also demonstrated higher susceptibility rate of *K. pneumoniae* in their study. The prevalence of Carbapenem resistant *K. pneumoniae* and Carbapenem resistant *A. baumannii* in a study by Adebanjo and Thonda, (2025) was reported in their published articles from 2014-2024.

Higher antibacterial susceptibility up to 10 mm was observed against*A. baumannii* and *K. pneumoniae* from extract with hot extraction process. This suggests that the plant extracts may exhibit antibacterial activity against these bacterial isolates due to the presence of bioactive phytochemicals such as phenolic acids, tannins, and flavonoids, which are capable of disrupting bacterial cell membranes, leading to the death of the organisms.Both hot and cold extract of HCE and CCE exert thestrongest inhibition zones ranging from 4-10mm. Heat or hot extraction can improve extraction yield to release bioactive compounds like the alkaloids, polyphenols, flavonoids and improve the potency of antibacterial especially of the heat-stable compounds thereby enhancing solubility of phytochemicals (Bitwell *et al.,* 2023). Phytochemicals can also reduce the formation of biofilm in resistant organisms like *A. baumannii* thereby enhancing their susceptibility (Gonelimali *et al.,* 2018; Majid *et al.,* 2025). Alkaloids are nitrogen-containing secondary metabolites common in many plants. The high presence in HCE and CCE suggests antimicrobial and potential therapeutic effects, while lower levels (+) in HBE and CBE might confer milder bioactivity (Kumar *et al.,* 2023). Many alkaloids show broad antimicrobial and anticancer activities through DNA intercalation, enzyme inhibition, and disruption of cell membranes (Rodríguez-Negrete *et al.,* 2024). Significantly high levels can cause poisoning or cytotoxicity due to their potent effects on nervous systems. The presence of tannins in the hot and cold extracts indicates strong antimicrobial, antioxidant properties while moderate activity were observed in hot and cold extract of bryophyllum. Tannin from plant extract is known to inhibit bacterial growth and neutralize free radicals (Kumar *et al.,* 2023). The presence of saponin which is glycosides with soap-like properties interacts with the cell membrane to exert their biological effects in membrane-disruptive antimicrobial action. Their absence in cold bryophyllum extract might reduce this effect. Arabski *et al.* (2009) suggested that saponins bind to lipids, which may lead to an increase in the permeability of the outer membrane of the bacterial cell wall and thereby facilitate the penetration of antibiotics into bacterial cells (Arabski *et al.,* 2009; Horie *et al.,* 2018). Flavonoids are prevalent in plant extracts which is consistent with this study and support the general antioxidant and protective roles against pathogens (Ullah *et al.,* 2020). Quinone which is only present in cold bryophyllum extract has been reported to have antioxidant property which may contribute to their antioxidant effects (Rodriguez-Negrete *et al.,* 2024). Cardiac glycosides, which help in improving cardiac muscle contraction is found only in both extracts of clove (Kumar *et al.,* 2023). A review by Isibor (2026) reported that flavonoids, alkaloids, saponins, terpenoids contributes to the antioxidant, antimicrobial, anti-inflammatory, antidiabetic and wound-healing properties of plants. Different organic compounds reported in the GC-MS analysis in this study was also reported by Batiha *et al.* (2020), where they determined the biological activities of clove extracts and its essential oil with GC-MS. The synergistic interaction between phytochemical compounds could enhance antimicrobial effects than individual components as documented in other related study (Wijerathna *et al.,* 2025).

The analysis of components by GC-MS in the hot and cold plant extract showed diverse profile of the organic compounds. Eugenol and/or its derivatives like 3-ally-6-methoxyphenol provide biochemical basis for the antimicrobial potency against the bacterial isolates (Marchese *et al.,* 2017). This compound or its derivatives are the most abundant component in HCE and CCE with 85.44% and 85.11% respectively of the total peak area. The high abundance is a signal as the principal contributors to the efficacy observed especially on *A. baumannii* and *K. pneumoniae*. The detection of 113 organic compounds in the hot bryophyllum extract (HBE) suggests the complexity of the plant chemicals (Zhang *et al.,* 2018). Eugenol from *S.aromaticum* extract is a major phenolic compound which has been widely studied for their pharmacological potentials as antimicrobial, antioxidant, anti-inflammatory. Eugenol dominates the biological activities of the clove extracts particularly as antimicrobial. The mechanism of eugenol as antimicrobial occurs when eugenol interacts with microbial membranes causing leakage of cellular contents and growth inhibition (Awojide *et al.,* 2024). β-Caryophyllene observed at retention time 12.706 min contributes to the synergistic activity with phenolic compounds such as eugenol to enhance their antioxidant activity (Awojide *et al.,* 2024). Though, it is present in small amount in this study, but it has been reported that it can enhance bioactivity through their additive effects with flavonoids and eugenol. Ergost-5-en-3-ol is a sterol (similar to ergosterol/plant

sterols) that can influence membrane integrity and fluidity, particularly in microbial cells. These compounds may have health-related effects in human such as the modulation of cholesterol metabolism and anti-inflammatory responses (Huang *et al.,* 2021; Xu *et al.*, 2025).

CONCLUSION

The findings from this study demonstrated **the** burden of antimicrobial resistance and the need for effective therapeutic agents against these resistant bacteria isolates particularly *Klebsiella pneumoniae* and *Acinetobacter baumannii*. Susceptibility to antibiotics used revealed high multidrug resistance ≥70**%** and the worrying emergence of carbapenem resistance, particularly as observed in *A. baumannii*. The evaluation of plant extracts on bacterial isolates showed slightly measurable antibacterial efficacy even against highly resistant pathogens. Hot and cold water clove extractsproduced the largest zones of inhibition (up to 10 mm)**.** The observed slight/moderate activity compared with conventional antibiotics is biologically significant given the resistance profiles of these bacterial isolates. Therefore, it is recommended that the specific bioactive compounds present in the plant extracts should be purified and further studied for their antimicrobial actions.

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Conflict of Interest

No conflicting interest is declared by the authors of this manuscript

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