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Comparative Studies on Therapeutic Potential of some Medicinal Plants against Multi-drug Resistant (MDR) *Acinetobacter baumannii* Clinical Strains

[1]Maryam Sada Abdullahi; [2]Umar Shittu and [3]*Musa Ahmed Abubakar

[1]Department of Biology, Isa Kaita College of Education Dutsin-ma, Katsina State, Nigeria
[2]Department of Science Laboratory Technology, Kano State Polytechnic, Kano State, Nigeria

**Corresponding author's e-mail:aamusakanopoly@gmail.com, 09019853667*

Abstract: *A. baumannii can frequently cause pneumonia, bacteremia, meningitis, wound infections and urinary tract infections. MDR A. baumannii is widely prevalent which has been reported and isolated from hospitals in many countries. The current study was conducted to evaluate the antibacterial efficacy of aqueous and methanol extracts of Roselle calyces of Hibiscus sabdariffa (H. sabdariffa) and leaves of Ziziphus mauritiana (Z. mauritiana) utilized in folk medicine against four clinical isolates of multidrug resistant Acinetobacter baumannii (MDR A. baumannii). Screening of Phytochemicals of H. sabdariffa and Z. mauritiana was carried out using percolation procedure. The antibacterial potential of both aqueous and methanolic extracts of H. sabdariffa and Z. mauritiana were also evaluated by agar disc diffusion assay, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) techniques. Antibiotic susceptibility of selected A. baumannii strains was examined. Based on the experimental analysis, the extracts of H. sabdariffa and Z. mauritiana revealed the presence of plants' secondary metabolites in the form of phytochemicals, vitamins and minerals in which methanolic extracts showed high yields compared to that of aqueous. The methanolic extracts of H. sabdariffa and Z. mauritiana were exhibited remarkable antibacterial activities against the non-MDR A. baumannii as well as the MDR A. baumannii strains compared to that of aqueous with a zone of inhibition ranging from (11.5 ± 0.3) to (14.2 ± 0.3) mm. The relative inhibition capacity of both plant extracts (10 mg/disc) with respect to gentamicin (10 mg/disc) had potent antibacterial activities and was much more effective than gentamicin agent. Also the values of minimum inhibitory concentration and minimum bactericidal concentration ranged from 12.5 to 25 and 25 to 50 mg/mL, respectively, revealing the potential bactericidal properties of the extracts quantitatively. For this current study, the findings showed that crude extracts of both H. sabdariffa and Z. mauritiana exhibited potent antibacterial efficacy against MDR strains of A. baumannii isolated from clinical samples in comparison to antibiotics used. Therefore, utilizing these plants may have advantageous well-being impact as natural antimicrobials for the treatment of MDR A. baumannii.*

Keywords: Antibacterial efficacy, Medicinal plants, Acinetobacter baumannii, Antibiotics, Multidrug resistant.

INTRODUCTION

Acinetobacter baumannii known as multidrug resistant (MDR) *A. baumannii* has emerged as one of the most troublesome pathogens. It is a significant nosocomial pathogen that specifically affects critically ill patients and most commonly found in intensive care units (ICU) and burn therapy units (BTU). This bacterium shows resistance to multiple antibiotics and some strains are resistant to almost all antibiotics currently in use [1, 2, 3]. *A. baumannii* can frequently cause pneumonia, bacteremia, meningitis, wound infections and urinary tract infections [4]. Plants are the main source of medications for humans, since they appear on earth and have abilities to synthesize endless secondary metabolites as phytochemical compounds which serve as plant defense mechanism against macro and microorganisms. Numerous studies are published showing a potent antibacterial activity of many medicinal plants [5].

Antibiotics, the most effective drugs against microbial infections in the 1950s, are recently losing their efficacies as most microorganisms have an acquired resistance [6]. Despite the negative side effects of antibiotics on human organs, the intensive use of antibiotics has led to emerging of what is called multidrug resistant (MDR) bacteria which are now raising remarkably all over the world and become an international public health threat [7]. Moreover, such pathogens have negative impacts on economy that infections have costed the United States 21–34 billion dollars annually [8]. *Acinetobacter baumannii* (*A. baumannii*) is a short Gram-negative nonfermentive coccobacillus belonging to genus *Acinetobacter.* It was earlier believed that this bacterium is ubiquitous in nature. But recently, it emerged as one of the most dangerous nosocomial pathogens worldwide, since it showed resistance to all known antibiotics [9].

A. baumannii can frequently cause pneumonia, bacteremia, meningitis, wound infections and urinary tract infections. MDR *A. baumannii* is widely prevalent which has been reported and isolated from hospitals in many countries and areas, such as India, Turkey, Taiwan, Argentina, Korea, Japan, Iran, Saudi, Brazil, Australia, Spain, US, UK and

Africa including Nigeria [10]. Plants are the main source of medications for human, since they appear on earth and have abilities to synthesize endless secondary metabolites known as phytochemical compounds which serve as plant defense mechanism against macro and micro-organisms [5]. Alkaloids, flavonoids, phenolics and tannins are among the most important phytochemicals used in phytotherapy [11]. The World Health Organization estimated that, during the past decade, a large proportion of the population depended on traditional medicinal plants for treatment of different illnesses and preferred the modern medication, and even in developed countries, many people have begun to use medicinal plants as an alternative therapy [12]. Therefore, it is a wise approach to search for new antimicrobial agents from natural sources like plants, since most of the recent drugs are initially obtained or semi-synthesized from these sources, particularly from those which are prescribed in traditional medicine [13].

In 2014, two out of six WHO regions reported 50% or more resistance of *Klebsiella pneumoniae* to carbapenem [14]. Today *Acinetobacter baumannii* (Moraxellaceae) resists almost all known antibiotics [9]. The resistance of Staphylococcus aureus (Staphylococcaceae) to methicillin emerged in 1961. Methicillin resistant *Staphylococcus aureus* (MRSA) is now resistant to vancomycin and cefotaxime and poses a threat to human health [15, 16]. In an attempt to control bacterial resistance, WHO recommends to develop the economic case for sustainable investment that takes account of the needs of all countries and to increase investment in new medicines' [14]. Medicinal plants in Asia have the ability to synthesize a fascinating array of low molecular weight molecules with structures completely unrelated to antibiotics. One example is the alkaloid berberine produced by *Tinospora cordifolia* (Willd.), a woody climber used in Bangladesh for the treatment of tuberculosis, cough and fever [17]. This phytoconstituent not only inhibits the growth of Gram-positive cocci *Streptococcus agalactiae* (Streptococcaceae) [18], but enhances the sensitivity of *Staphylococcus strains* towards antibiotics [19]. *A. baumannii* is the well-studied of the genus, due to its notable role in human infections. Infections caused by *A. baumannii* include pneumonia, meningitis, bloodstream, and surgical site infections; it is also known to colonize patients without causing infection [20].

In addition, medicinal plants produce inhibitors of bacterial resistance. Carbapenems have been the most important agents for the treatment of infections caused by multidrug-resistant *A. baumannii* [9, 21]. Nowadays, carbapenem-resistant *A. baumannii* (CRAB) are great concerns for physicians because carbapenems are drugs of choice to treat infec-tions caused by this pathogen. In addition, therapeutic efficacy of carbapenems is mainly limited due to spread of CRAB. Carbapenem-resistant isolates of *A. baumannii* are usually resistant to all classes of antimicrobials [9, 22]. Medical practitioners can try new synthetic drug for treating these patients, the use of these synthetic drugs may subject the patient to a higher risk due to the pro-duction of more harmful side effects. Alternatively, the natural compounds/extracts showed economic benefit, availability and low rate of side effects which resulted a growing interest in the screening of extracts from medicinal plants in order to formulate new drugs since 1996 [23].

The medicinal plants used for the research are the leaves of *Ziziphus mauritiana* and Roselle calyces of *Hibiscus sabdariffa.* These plants are widely found in Australia, South-east Asia, USA, and Africa including Nigeria with noteworthy antimicrobial properties. *Ziziphus mauritiana* leaf-extracts are used in the treat-ment of diarrhoea, wounds, abscesses, swelling, gonorrhea, liver diseases, asthma and fever [24]. The different parts of the plant are used as cuts and ulcers healer, pulmonary ailments, fevers, laxative, sedative, anti-nausea, anti-rheumatic areas, anti-diarrhoeal, wounds and abscesses healer, swelling, gonorrhoea curer [24]. They are also used to treat pulmonary ailments, dysentery, fevers and skin diseases [25]. The antioxidant activity of the aqueous extract of *Z. mauritiana* leaf has been reported [26].

Hibiscus sabdariffa (Roselle) is used traditionally for many purposes, such as hot and cold beverage, flavoring agent, food industry and traded as herbal medicine. It also holds a plentiful potential of phytochemical compounds and has antioxidant, hypotensive, hypocholesterolemic, immune-modulated, hepatoprotective, anti-obesity, antiurolithic, antidiabetic, antimicrobial and anticancer properties without any significant genotoxic effects [27]. The previous study showed that it has significant antibacterial activity against *Staphylococcus aureus, Staphylococcus epidermidis, Salmonella enterica, Klebsiella pneumonia, Pseudomonas aeruginosa, Escherichia coli, Proteus vulgaris and Bacillus cereus* [28]

Figure 1: The *Hibiscus sabdariffa* (Roselle calyces) and *Ziziphus mauritiana* (Jujube or Magarya tree)

The use of synthetic antibiotics and their derivatives gradually creates tolerance or non-susceptibility to microorganisms, especially bacteria [29, 30, 31]. It is therefore very important to search for new sources of medicinal plants with antimicrobial properties especially susceptible to MDR *A. baumannii* pathogens. Also, this alarming situation of multidrug resistance in humans paved ways to microbiologists and other medical scientists to identify potential antimicrobial compounds from various medicinal plants with novel mode of actions. Thus, the antibacterial properties of some medicinal plants against MDR *A. baumannii* isolated from clinical patients need to be evaluated so as to show the potency of medicinal plants as substitute of the current in-effective synthetic antibiotics used in various hospitals.

MATERIALS AND METHODS

Description of the study area

Dutsin-Ma is a Local Government Area (LGA) in Katsina State, Nigeria lies between latitude 120 17.00N' to 120 17.84'N and longitude 0070 26'E. Its headquarters are in the town of Dutsin Ma. Dutsinma is the administrative headquarters of the Dutsinma Local Government Area from its creation in 1976 [32] as shown in figure 1 below. Dutsin-Ma L.G.A has a land size of about 552.323km2 with a population of about 169,829 as at 2006 national population census with the people being predominantly farmers, cattle rearers and traders [33, 34]. Dutsin-ma became a Local Government in 1976. The General Hospital Dutsin-ma (the study site) is also located there.

Figure 2: Map of Katsina State showing Dutsin-Ma Local Government Area

Ethical clearance

Before the commencement of the research, permission was granted from the head of Hematological Department, General Hospital Dutsin-ma (GHTM). Approval of the study protocol by the Katsina State Ministry of Health's ethical committee was also given as well.

Sample Collection

The leaves of the plants *(Ziziphus mauritiana and Hibiscus sabdariffa)* were purchased from local herbal market in Katsina State, Nigeria. The plants were identified taxonomically and authenticated in the herbarium, Department of Botany, Faculty of Science, Federal University, Dutsin-ma, Katsina State. The leaf-samples were taken into the laboratory and air dried at room temperature. The dried leaves were ground into powder using electric blender. The test bacteria were collected by isolation from wound swabs of burns and surgical wound patients from Hematological Department, General Hospital Dutsin-ma (GHTM).

Extraction of plant material

About 200 g each of dried powder was taken and put in a sterile dark glass container and 500 mL each of methanol (Sigma–Aldrich, St. Louis, USA) and water (aqueous) were loaded gradually into the container, soaked and subjected to frequent shaking and macerated for 3 days at room temperature [(37 ± 2) _C] in a dark cabinet. The suspension was filtered using Whatman filter paper No. 1 (Sigma–Aldrich, St. Louis, USA). The filtrates were allowed to evaporate in the incubator at 45 ^{0}C for up to 10 days till sticky extracts were obtained. Before the antibacterial BIO-assay, 1 g of each extract was reconstituted in 2 mL of its extraction solvent to get 400 mg/ mL according to the method describe by Pasanen et *al*., [4].

Isolation and identification of Bacteria (*A. baumannii*)

The target bacterium, *A. baumannii* was isolated from different clinical samples like wound from the Department of Pathology, General Hospital Dutsin-ma (GHDTM). The samples were identified by sub-culturing onto blood and MacConkey agars for 24 h at 37 ^{0}C. The growing colonies were examined by Gram-staining techniques and microscopy. *A. baumannii* strains were then subjected to identification by biochemical analysis using various chemicals and reagents for confirmation.

2.6 Antibiotic sensitivity assay for classification of *A. baumannii*

The identified *A. baumannii* strains were classified as multidrug resistant (MDR) and non-MDR strains by the antibiotics sensitivity testing as described by Kirby–Bauer disk diffusion method recommended by Clinical and Laboratory Standards Institute guidelines. *A. baumannii* isolates were tested against the following standard antibiotics: amikacin (30 mg/disc), aztreonam (30 mg/disc), ciprofloxacin (5 mg/ disc), cefotaxime (30 mg/disc), ceftazidime (30 mg/disc), imipenem (10 mg/disc) and piperacillin (100 mg/disc) that were purchased from Oxoid Limited, Basingstoke, UK. Any isolate that shows resistance to antibiotics mentioned was considered as MDR *A. baumannii.*

Bio-assay (susceptibility) test

The modified Kirby–Bauer disc diffusion technique was utilized to evaluate the antibacterial potential of the plant extracts [35]. Prior to bio-assay, *A. baumannii* strains were sub-cultured in nutrient broth (Riyadh, Saudi Arabia) and incubated for 18 h at 37 ^{0}C in order to reach the exponential phase, then adjusted by adding normal saline to be equivalent to 0.5 McFarland standard solution, which comprised 1.0 × 108 CFU/mL. About 100 mL from each adjusted strain was loaded separately sterile disposable Petri dishes and 20 mL sterile warm Mueller–Hinton agar was poured onto each plate and left at room temperature (35– 37 ^{0}C) until solidified. Then, 20 mL each of both plant extracts *(H. sabdariffa* and *Z. mauritiana)* at a concentration of 400 mg/mL (10 mg/disc) was loaded to the sterile disc and put over the seeded Mueller–Hinton agar plate. Afterwards, the sterilized 6 mm in diameter of punched disc-papers containing 0.02ml of each of the plant extracts (methanol and water) were placed with the aid of a pair of forceps onto the agar plates at an equidistant position and left for few minutes at room temperature for pre-diffusion. 10 mg/disc gentamicin (Oxoid Limited, Basingstoke, UK) was used as positive control. All plates were incubated for 24 h at 37 ^{0}C. The experiment were repeated thrice and the mean zone of inhibition around the discs were measured and recorded in (mm).

Determination of Minimum Inhibitory Concentration (MIC)

MIC was evaluated as the lower concentration of the extracts at which no visible macroscopic growth or turbidity was observed on the well plate's bottom. The MICs of both test-extracts of all plant samples were investigated by broth dilution technique in mg/ml using 96-well micro-plate [36]. 100 µL from each sample stock (200 mg/ml) was taken and two-fold serial dilutions were carried-out to obtain six different concentrations; 400, 200, 100, 50, 25, 12.5 and 6.25 mg/mL. While a tube containing 1.0 mL sterile distilled water and methanol and 1.0 mL broth was served as negative control. All tubes were seeded with 10 mL tested bacterial strains adjusted to 0.5 Mac- Farland. Another tube containing only 1.0 mL absolute methanol and 1.0 mL broth was used as

negative control. All tubes were incubated for 18 h at 37 ^{0}C. After incubation, the tube of the least concentration each of the plant extract that did not show any visible growth were considered as MIC [37]. The least concentration at which no visible microscopic growth or turbidity was observed on the well plate bottom was taken as the MIC level measurement. Gentamycin antibiotic was used as positive control.

Determination of Minimum Bactericidal Concentration (MBC)

Based on the results of MICs obtained, 10 µL of solution from the last-clear well of each of the test samples and their controls, respectively was pipetted on to the surface of nutrient agar plates and spread gently with a sterile glass rod to obtain uniformity on the surface. The inoculated plates were incubated in an upright position for 24 hours at 37 ^{0}C. The MBC was measured immediately after incubation as the dilution at which there was no visible growth observed on the plates.

Phytochemical Analysis

Qualitative phytochemical tests of the test extracts (methanol and aqueous) was determined to detect possibly the different '*bioactive or phytoconstituents'* of the plants; such as alkaloids, flavonoids, glycosides, saponins, tannins, terpenes, and protein contents. The phytochemical analysis was performed using standard procedure described by Rajan *et al.,* [38] and Sofowora [39].

Data entry and Analysis

Data obtained was subjected to statistical analysis by mean comparison using one way analysis of variance (ANOVA) test and values of $p \leq 0.05$ was considered significantly different, while p values > 0.05 was considered statistically non-significant. The antibacterial efficacy of all plant-extracts was expressed as mean ± standard deviation. The results of phytochemistry of the plants were shown in tables after the experimental analysis.

RESULTS AND DISCUSSION

Results

The air-dried leaves of *Z. mauritiana* and *H. sabdariffa* were extracted using two different solvents; aqueous and methanol. *Z. mauritiana* revealed high percentage yield (22.7%) in methanol compared to *H. sabdariffa* (22.3%), and the percentage yield of *H. sabdariffa* appears to be more in aqueous (19.2%) compared to *Z. mauritiana* (18.10%) as shown in Table 1. Therefore, the percentage yield of methanol was higher compared to that of aqueous. This is likely in light of the fact that, the type of solvent used in the extraction procedure influenced the solubility of the active compounds of the leaves [40]. Water and methanol are widely used for plant extraction due to their low toxicity and high extraction yield. The extraction yield of plants depends highly on the solvent polarity [41].

Table 1. Shows percentage (%) yield of selected medicinal plants using aqueous and methanol extraction

Solvent	**Yield (%)**	
	Z. mauritiana	*H. sabdariffa*
Aqueous	18.10	19.2
Methanol	22.7	22.3

Table 2. Results of Photochemical analysis of selected medicinal plants' extracts

S/N	**Compound tested**	**Methanolic**		**Aqueous**	
		Z. mauritiana	*H. sabdariffa*	*Z. mauritiana*	*H. sabdariffa*
1.	Alkaloids	+	+	+	+
2.	Tannins	++	++	+	+
3.	Glycosides	++	+	+	+
4.	Reducing sugar/ CHO	++	++	+	+
5.	Terpenoids	+	+	+	+
6.	Saponin	++	+	+	+
7.	Flavonoids	++	+	+	+
8.	Phenolics	++	+	+	++
9.	Proteins	++	+	++	+

Key:- + = moderately present, ++ = indicates highly present, - = Indicates absent

Based on the experimental analysis, the extracts of *Z. mauritiana* and *H. sabdariffa* revealed the presence of plants' secondary metabolites in the form of phytochemicals, vitamins and minerals in which methanolic extracts showed high yields compared to aqueous in both plants (see Table 2). This could be due to some factors that influence the variability of the bioactive components of the plants such factors include; climate, deposition of atmosphere, soil nature, etc. [42]. Hence, the bioactive active chemical constituents evaluated qualitatively (alkaloids, tannins, saponins, glycosides, flavonoids, terpenoids, etc.) have curative properties to be used as potential source of drugs in herbal medicine. This agrees with the work of Dahiru *et al.,* [43] and Da-Costa-Rocha *et al.,* [27] who reported the presence of these phytochemicals in the leaves of *Z. mauritiana* and *H. sabdariffa*. Furthermore, according to plant database, phytochemical components also have antibacterial properties [44].

The presence of tannins and glycosides in both extracts agrees with the report of Evans [45] that, tannins are important in herbal medicine and they are applied to bleeding and wound healing. Traditionally saponins have been extensively used as detergents, pesticides as well as mollucides, in addition to their industrial application such as foaming, surface active agents etc. and also found to have beneficial health effects [46]. One phytochemical constituent may contain hundreds of species which are extracted differently based on the polarity of solvent used [47]. In addition to medicinal uses of *Z. mauritiana* and *H. sabdariffa,* the phytochemical components of the plants have been reported to inhibit the growth of various microbes of human pathogen namely; Gram positive (*Staphylococcus aureus, Bacillus subtilis, etc.*) and Gram negative (*Escherichia coli, Pseudomonas aeruginosa, etc.*) bacteria [43, 48, 49].

Table 3. Sources of *Acinetobacter baumannii* from clinical swabs

S/N	Bacterial strain	Source
1.	Abn1	Wound
2.	Abn2	Pus
3.	Abn3	Wound
4.	Abn4	Wound

Key; Abn: *Acinetobacter baumannii (A. baumannii*)

Acinetobacter baumannii strains were isolated from different samples of wound associated with clinical patients at the Department of Pathology General Hospital Dutsin-ma, Katsina Sate, Nigeria (Table 3). The samples were identified by sub-culturing onto blood agar and MacConkey agar for 24 h at 37 C. The growing colonies were examined with Gram-staining techniques, which were Gram-negative coccobacilli under the microscope confirmed as *A. baumannii* by further biochemical tests and then classified as MDR and non-MDR strains by the antibiotics susceptibility testing.

Table 4. Antibiotic sensitivity profile of the test organisms /bacterial strains

S/N	Acinetobacter strains	Antibiotics used						
		AK	**ATM**	**CAZ**	**CIP**	**CTX**	**IMI**	**PRL**
1.	**Abn1**	S	S	S	S	S	S	R
2.	**Abn2**	R	R	R	R	R	S	R
3.	**Abn3**	R	R	R	R	R	S	R
4.	**Abn4**	R	R	R	R	R	S	R

Key; S: Sensitive; **R:** Resistant; **AK:** Amikacin; **CAZ:** Ceftazidime; **ATM:** Aztreonam; **PRL:** Piperacillin; **IMI:** Imipenem; **CIP:** Ciprofloxacin; **CTX:** Cefotaxime

As shown in Table 4, non-MDR *A. baumannii* strain (Abn1) was susceptible to all antibiotics tested except for PRL; piperacillin which was found resistant to antibiotics used. Abn2, Abn3 and Ab4 strains were resistant to six categories of antibiotics and sensitive only to IMI; Imipenem. The antibacterial potential of *Z. mauritiana* and *H. sabdariffa* calyces against the isolated *A. baumannii* strains of clinical origin have been evaluated in the present study. The results revealed that the test extracts exhibited significant antibacterial property against these bacterial pathogens. Table 5 shows the mean zone of inhibition of *A. baumannii* strains (Abn1–Abn4) relative to *Z. mauritiana* leaf-extracts at concentration 400 mg/mL. The non-MDR *A. baumannii* (Abn1) showed sensitivity against all the test extracts of *Z. mauritiana* in which methanolic extract (13.2 ± 0.3) mm was found more potent compared to that of aqueous (11.5 ± 0.3) mm, but still less than the positive control gentamicin (23.5 ± 0.2) mm. Surprisingly, other MDR *A. baumannii* strains (Abn2–Abn4) revealed significant sensitivity to the extracts and were higher than gentamicin antibiotic, especially Abn3 which was almost resistant to gentamicin (7.0 ± 0.1) mm and sensitive to the extracts of *Z. mauritiana*.

Table 5. Antibacterial evaluation of *Z. mauritiana* against isolated strains of *A. baumannii* at 400 mg/mL

Extract/standard agent used	Mean zone of inhibition of *A. baumannii* (mm)			
	Abn1	**Abn2**	**Abn3**	**Abn4**
Methanol Extract (10 mg/disc)	13.2 ± 0.3	14.2 ± 0.3	11.6 ± 0.3	13.5 ± 0.3
Aqueous Extract (10 mg/disc)	11.5 ± 0.3	13.5 ± 0.3	12.2 ± 0.3	12.9 ± 0.3
Gentamycin (10 µg/mL)	23.5 ± 0.2	8.2 ± 0.2	7.0 ± 0.1	8.5 ± 0.2

Table 6 summarizes the mean zone of inhibition of *A. baumannii* strains (Abn1–Abn4) against calyces of *H. sabdariffa* extracts at concentration 400 mg/mL. The non-MDR *A. baumannii* (Abn1) showed sensitivity against all the test extracts in which methanolic extract (15.2 ± 0.3) mm was found more sensitive compared to that of aqueous (11.5 ± 0.3) mm, but still less than the gentamicin agent (23.8 ± 0.2) mm. However, other MDR *A. baumannii* strains (Abn2–Abn4) showed tremendous sensitivity to the extracts and were higher than gentamicin positive control agent, particularly Abn3 which was totally resistant to gentamicin (6.0 ± 0.1) mm and sensitive to the extracts of calyces of *H. sabdariffa*. Methanol extracts were more sensitive compared to that aqueous. Hence, the most susceptible strains for methanol extraction were Abn2 (16.5 ± 0.3) and Abn4 (14.5 ± 0.3), followed by Abn3 (12.5 ± 0.3) mm as shown in Table 6.

Table 6. Antibacterial evaluation of *H. sabdariffa* against isolated strains of *A. baumannii* at 400 mg/mL

Extract/standard agent used	Mean zone of inhibition of *A. baumannii* (mm)			
	Abn1	**Abn2**	**Abn3**	**Abn4**
Methanol Extract (10 mg/disc)	15.2 ± 0.3	16.5 ± 0.3	12.5 ± 0.3	14.5 ± 0.3
Aqueous Extract (10 mg/disc)	11.5 ± 0.3	12.7 ± 0.3	10.7 ± 0.3	13.0 ± 0.3
Gentamycin (10 µg/mL)	23.8 ± 0.2	8.5 ± 0.2	6.0 ± 0.0	10.0 ± 0.2

The antibacterial efficacy of the calyces of *H. sabdariffa* and *Z. mauritiana* was also evaluated using MIC and MBC tests to measure the bacteriostatic and bactericidal potential of the plant extracts (Table 7). The results showed that the MIC values ranged between 12.5 to 50 mg/mL, while the MBC values ranged between 25 and 50 mg/mL. The MIC was evaluated as the lowest concentration of the extracts at which no visible macroscopic growth or turbidity was observed on the well plate's bottom. Therefore, the extraction of *H. sabdariffa* and *Z. mauritiana* using methanol exhibited the most remarkable antimicrobial activities as compared to aqueous with MICs of 12.5 mg/mL for both Abn1, for Abn2 and Abn4 25 mg/mL, while 50 mg/mL for Abn3. Whereas, aqueous extract shows 50 mg/mL for all the four bacterial strains (Table 7). However, in the case of gentamycin agent, the MIC values for both *H. sabdariffa* and *Z. mauritiana* extracts were very low against the three bacteria strains but in contrast, Abn3 was totally resistant to gentamicin (-) in both extracts because, a visible macroscopic growth or turbidity was observed on the well plate's bottom.

Table 7. Minimum Inhibitory Concentration (mg/mL) of the selected plant extracts obtained from Micro-dilution techniques

Extract/ agent used	Bacterial Isolates used for the study							
	H. sabdariffa				***Z. mauritiana***			
	Abn1	**Abn2**	**Abn3**	**Abn4**	**Abn1**	**Abn2**	**Abn3**	**Abn4**
Methanol	12.5	25	50	25	12.5	50	50	50
Aqueous (H_2O)	50	50	50	50	50	50	50	50
Gentamycin agent	200	400	-	200	200	400	-	200

Note: the higher the MIC value (400 mg/mL) the lower the activity of the extract thus, methanolic extracts for both plants have better activities against Abn1, Abn2, Abn3 and Abn4 than aqueous extracts in which the MIC values against all the bacteria strains were the same (50 mg/mL), except for Abn1(12.5 mg/mL).

The MBC was also determined by sub-culturing the test dilution (used in MIC) on to a fresh agar medium and incubated again for 18-24 hours at 37 °C. The concentrations of *H. sabdariffa* and *Z. mauritiana* extracts in mg/mL that completely killed the bacterial strains have been taken as MBC. Therefore, based on MBC results, no visible growth of all four bacteria was observed on the plates after incubation period of 1 day except for Abn3 plates where growth was observed in the case of gentamycin antibiotic for both methanol and aqueous extracts at all concentrations. This shows that MBC value is the same as MIC and thus, confirms the antibacterial efficacy of the plant extracts quantitatively.

Discussion

For this current study, the Acinetobacter strains used in this determination were three representing MDRs (Abn2, Abn3 and Abn4) and one representing non-MDR (Abn1) in order to compare the susceptibility of these bacteria towards the plant extracts of calyces of *H. sabdariffa* and leaves of *Z. mauritiana*. As presented in Table 4, most of the examined strains showed resistance against various antibiotics used. These antibiotics were recently launched, which revealed that *A. baumannii* is a virulent pathogenic bacteria and able to develop resistance quickly against antibiotics. This observation was in agreement with Lowings *et al.,* [50] and Emad, [51] who stated that *A. baumannii* evolves resistance to all known antibiotics and has rapid developing mechanisms and no effective treatment options observed in the near future. As represented in Table 3, the MDR A. *baumannii* strains (Abn2–Abn4) showed significant sensitivity to both methanol and aqueous extracts of the plants used and were found higher than gentamicin standard positive control. However, the methanol extracts of both *H. sabdariffa* calyces and leaves of *Z. mauritiana* have potent antibacterial potential against MDR *A. baumannii*. Thus, agrees with the findings of Jaroni and Ravishankar [52] and Abdallah [49] that, the calyces of *H. sabdariffa* have antibacterial activities on different pathogens, especially foodborne pathogens, methicillin-resistant *Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *A. baumannii*. These potent antibacterial activities of the calyces of *H. sabdariffa* leaves of *Z. mauritiana* could be attributed to their availability of bioactive phytochemicals. The previous study on Sudanese roselle and various medicinal plants including *Z. mauritiana* revealed the presence of alkaloids, phenolic compounds, flavonoids and saponins, glycosides and tannins [53, 49]. These bioactive components are considered to be the major groups of antimicrobial compounds in plants generally [54].

The results of the antibacterial potential of the both extracts were compared with the positive control (gentamicin agent). Recently it was reported that, *A. baumannii* strains developed high resistance to gentamicin [55]. Table 7 summarizes MIC and MBC of both *H. sabdariffa* and Z. *mauritiana* methanol and aqueous extracts against different *A. baumannii* strains isolated. Both extracts were indicated to be bacteriostatic and bactericidal because, their MIC values were found to be significant for Abn1, Abn2, Abn3 and Abn4. Moreso, MIC value same as MBC and thus, confirms the antibacterial efficacy of the plant extracts quantitatively. According to previous literature, the calyces of *H. sabdariffa* possess effective bactericidal property against all tested strains of *A. baumannii*. These results are similar to what Liu *et al.*, [54] reported in that, based on MIC values, *A. baumannii* was remarkably inhibited. Several studies on medicinal plants were also documented antibacterial efficacy against MDR clinical strains of *A. baumannii* including *Magnolia officinalis, Mahonia bealei, Rabdosia rubescens, Rosa rugosa, Rubus chingii, Scutellaria baicalensis, Terminalia chebula and Ziziphus mauritiana* [56].

CONCLUSION AND RECOMMENDATIONS

Conclusion

For this current research, the findings showed that the methanol crude extracts of both *H. sabdariffa* and *Z. mauritiana* exhibited potent antibacterial property against MDR strains of *A. baumannii* isolated from clinical samples in comparison to antibiotics and these properties were considered to be bactericidal. The antimicrobial properties of the tested plants are attributed to the presence of bioactive metabolites contained in the plants. Therefore, utilizing *H. sabdariffa* and *Z. mauritiana* may have advantageous wellbeing impact if consumed routinely especially as diet in nutrition or as natural antimicrobials for bacterial diseases and infections including MDR *A. baumannii.*

Recommendations

1. Further future studies are recommended for the fractionation of the compound(s) responsible for their antibacterial potential in addition to other pharmacological studies, which may lead to the discovery of new natural antibiotic from H. sabdariffa and Z. mauritiana plant species.

2. Given the lack of new antibiotics and the spread of MDR A. baumannii, there will be an essential need for new antibacterial drugs with a different mode of action. This study suggested that roselle calyces *(H. sabdariffa) and leaves of Z. mauritiana* possess potent antibacterial activity against MDR A. baumannii which could be used as an antibacterial drug.

3. It is worthy for the research scholars and pharmaceutical industries/ or companies to carry out further investigations on natural products and medicinal plants for isolation and identification of new antibacterial components which can be formulated as effective antibiotics to be used as

alternative agents in the treatment of various multidrug resistant bacteria and other microbes.

4. Further research on scientific proofs of safety and toxicity of the plants are guaranteed.

CONFLICT OF INTEREST STATEMENT

The authors declare no known competing interests or personal relationships that could have appeared to influence the work reported in this research paper.

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