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ANTIBACTERIAL EFFICACY OF SELECTED MEDICINAL PLANTS AGAINST MULTI-DRUG RESISTANT (MDR) ACINETOBACTER INFECTION ASSOCIATED WITH CLINICAL PATIENTS

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ABSTRACT

Background and Objective: *Acinetobacter baumannii* can commonly cause pneumonia, sepsis, meningitis, wound infections, and urinary tract infections. MDR *A. baumannii* is widespread and has been reported and isolated in hospitals in many countries. This study was conducted to evaluate the antibacterial effects of aqueous and methanol extracts of *Azadirachta indica (A. indica)* and *Terminalia catappa (T. catappa)* used in traditional medicine against some clinical isolates of multidrug-resistant (MDR) *A. baumannii*.

Methods: Screening of Phytochemicals of *A. indica* and *T. catappa* was carried out using percolation method. The antibacterial potential of both aqueous and methanolic extracts of *A. indica* and *T. catappa* 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.

Results: Based on the experimental analysis, the extracts of *A. indica* and *T. catappa* showed the presence of plants' bioactive metabolites in the form of phytochemicals, vitamins and minerals among which methanolic extracts gives high efficiency compared to that of aqueous. The methanolic extracts of *A. indica* and *T. catappa* 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 (10.5 ± 0.3) to (14.5 ± 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.

Conclusions: For this present study, the results showed that crude extracts of *A. indica and T. catappa* exhibited strong antibacterial effects against MDR strains of *A. baumannii* isolated from clinical samples in comparison with the antibiotics used. Therefore, the use of these plants may have a beneficial impact on health as they are natural antibacterials for the treatment of MDR *A. baumannii.*

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

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1.0 INTRODUCTION

Acinetobacter infection is well known as hospital acquired or nosocomial infection caused by multidrug resistant (MDR) *Acinetobacter baumannii (A. baumannii).* The etiological pathogen 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 (Turton *et al.,* 2006; Nowak *et al.,* 2012; Poirel *et al.,* 2003). *A. baumannii* can frequently cause pneumonia, bacteremia, meningitis, wound infections and urinary tract infections (Pasanen *et al.,* 2014). Antibiotics, the most effective drugs against microbial infections in the 1950s, are recently losing their efficacies as most microorganisms have an acquired resistance (Huh and Kwon, 2011). 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 (Magiorakos *et al.,* 2012).

Moreover, such pathogens have negative impacts on economy that infections have costed the United States 21–34 billion dollars annually (van Duin and Paterson, 2016). *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 (Peleg *et al.,* 2016). *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 (Tiwari *et al.,* 2015).

The medicinal plants used for the research were *Terminalia catappa* and *Azadirachta indica.* These plants are widely found in Australia, South-east Asia, USA, and Africa including Nigeria with noteworthy antimicrobial properties. *Terminalia catappa* (Umbrella tree)is a species of the family Combretaceae widely distributed in countries with tropical and subtropical climates. This tree is grown for its ornamental purposes and its edible nuts. The juice of its fresh leaves is used in the preparation of medicinal lotion for leprosy and scabies, and it is taken internally for stomachache and headache. In Asia and some West African countries, the leaves of this species are commonly used for the treatment of dermatitis, hepatitis, diarrhea and pyresis (Ahmad *et al.,* 2014; Baratelli *et al.,* 2012). *Azadirachta indica* (*A. indica*), commonly known as neem tree, has attracted worldwide prominence in recent years, owing to its wide range of medicinal properties. Neem has become a cynosure of modern medicine. All parts of the neem tree- leaves, flowers, seeds, fruits, roots and bark have been used conventionally for the treatment of inflammation, infections, fever, skin diseases and dental disorders (Subapriya and Nagini, 2005; Packia *et al.,* 2012).



Figure 1: The *Terminalia catappa* (Umbrella tree) and *Azadirachta indica* (Neem tree)

The use of synthetic antibiotics and their derivatives gradually creates tolerance or non-susceptibility to microorganisms, especially bacteria (Nascimento *et al.,* 2000; Cordell, 2000; Matin *et al.,* 2013). It is therefore very important to search for new sources of medicinal plants with antimicrobial properties especially susceptible to multidrug resistant (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 ineffective synthetic antibiotics used in various hospitals. The main objective of the research is to evaluate and compare the antibacterial properties of some medicinal plants against multi-drug resistant *Acinetobacter baumannii* isolated from clinical patients.

2.0 MATERIALS AND METHODS

2.1 The study area

Gumel town is situated some 127 KM Northeast of Kano State, Nigeria and lies about 26KM south of Nigeria's boarder with Niger Republic (Mudansiru *et al.,* 2016). According to National population Commission, Jigawa State is one of the developing cities in the North-west of Nigeria, and is one of the less populated states in the Northern region. The Gumel General Hospital (the study site) is also located there.

2.2 Sample Collection

The leaves of the plants *(Terminalia catappa* and *Azadirachta indica)* were purchased from local herbal market in Gumel, Jigawa State, Nigeria. The plants were identified taxonomically and authenticated in the herbarium, Department of Botany, Faculty of Science, Federal University, Dutse (FUD), Jigawa State. The leaves were taken into the laboratory and air dried at room temperature. The dried leaves were ground into powder using electric blender and the bacteria stains were collected by isolation from wound swabs of burns and surgical wound patients from Hematological Department, Gumel General Hospital, Jigawa.

2.3 Extraction of plant material

About 250 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. 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.,* (2014).

2.4 Isolation and identification of *A. baumannii*

The target bacterium, *A. baumannii* was isolated from different clinical samples like wound from the Department of Pathology, Gumel General Hospital, Jigawa. 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 microscopic analysis. *A. baumannii* strains were then subjected to identification by biochemical analysis using various chemicals and reagents for confirmation.

2.5 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 (Sivaranjani *et al.,* 2013). *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.*

2.6 Susceptibility test/ Bio-assay

The modified Kirby–Bauer disc diffusion technique was utilized to evaluate the antibacterial potential of the plant extracts (Abubakar *et al.,* 2015). Prior to bio-assay, *A. baumannii* strains were sub-cultured in nutrient broth 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 experiments were repeated thrice and the mean zone of inhibition around the discs were measured and recorded in (mm).

2.7 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 (Karakoca *et al.,* 2013). 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 (Dhiman *et al.*, 2011). After incubation, the tube of the least concentration each of the plant extract that did not show any visible growth were considered as MIC. 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.

2.8 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.

2.9 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 asalkaloids, flavonoids, glycosides, saponins, tannins, terpenes, and protein contents. The phytochemical analysis was performed using standard procedure described by Rajan *et al.*, (2013) and Sofowora, (1993).

2.10 Data entry and Analysis

Data obtained was subjected to statistical analysis by mean comparison. The antibacterial efficacy of all plant-extracts was expressed as mean ± standard deviation. The results of phytochemical analysis of the plants were shown in tables after the experimental procedure.

3.0 RESULTS AND DISCUSSION

3.1 Results

The air-dried leaves of *T. catappa* and *A. indica* were extracted using percolation with two different solvents; aqueous and methanol. *A. indica* revealed high percentage yield (25.2%) in methanol compared to *T. catappa* (22.5 %), and the percentage yield of *A. indica* appears to be more in aqueous (20.1%) compared to *T. catappa* (18.2 %) as shown in Table 1. Therefore, the percentage yield of methanol was higher compared to that of aqueous. This may be due to the fact that, the type of solvent used in the extraction procedure influenced the solubility of the active compounds of the leaves (Saad *et al.*, 2014). Water and methanol or ethanol 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 (Amita and Shalini, 2014).

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

Solvent	Yield (%)	
	A. indica	*T. catappa*
Aqueous	20.1	18.2
Methanol	25.2	22.5

Table 2. Photochemical analysis of selected medicinal plants

S/N	Compound tested	Methanolic		Aqueous	
		A. indica	*T. catappa*	*A. indica*	*T. catappa*
1.	Alkaloids	++	+	+	+
2.	Tannins	++	++	+	+
3.	Glycosides	++	+	+	+
4.	Reducing sugar/ CHO	+	+	+	+
5.	Terpenoids	+	+	+	+
6.	Saponin	++	+	+	+
7.	Flavonoids	++	+	+	+
8.	Phenolics	++	+	+	++
9.	Proteins	+	+	++	+

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

Based on the experimental study, the extracts of *A. indica* and *T. catappa* revealed the presence of plants' bioactive metabolites in the form of phytochemicals, vitamins and minerals in which methanolic extracts showed high yields compared to aqueous in both plants (Table 2). This could be due to some factors that influenced the variability of the bioactive components of the plants; climate, deposition of atmosphere, soil nature, etc. (Chengyao *et al.,* 2017). Thus, the bioactive chemical components evaluated using qualitative analysis (alkaloids, tannins, saponins, glycosides, flavonoids, terpenoids, etc.) could have curative properties to be used as potential source of drugs in herbal medicine. This agrees with the findings of Khan *et al.,* (2014) and Bandyopadhyay *et al.,* (2004) who reported the presence of these phytochemicals in the leaves of *A. indica* and *T. catappa.* Furthermore, according to plant database, phytochemical components also have antibacterial properties (Perumal-samy *et al.*, 1998; Abubakar *et al.*, 2015).

The presence of tannins and glycosides in both extracts agrees with the findings of Evans (1998) 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 (Arunasalam, *et al.*, 2004). One phytochemical constituent may contain hundreds of species which are extracted differently based on the polarity of solvent used (Silver *et al.,* 1998). In addition to medicinal uses of *A. indica* and *T. catappa*, the phytochemical contituents 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 (Dahiru *et al.,* 2006; Hussain *et al.,* 2008; Abdallah, 2016).

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

S/N	Bacterial strain	Source
1.	Ab1	Wound
2.	Ab2	Wound
3.	Ab3	Wound
4.	Ab4	Wound

Key; Ab: *Acinetobacter baumannii strain (A.baumannii*)

Acinetobacter baumannii strains were isolated from different samples of wound associated with clinical patients at the Department of Pathology General Hospital Gumel, Jigawa 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	Antibioticsused						
		AK	**ATM**	**CAZ**	**CIP**	**CTX**	**IMI**	**PRL**
1.	**Ab1**	S	S	S	S	R	S	R
2.	**Ab2**	R	R	R	R	R	S	R
3.	**Ab3**	R	R	R	R	R	S	R
4.	**Ab4**	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 (Ab1) was susceptible to all antibiotics tested except for CTX and PRL; Cefotaxime and piperacillin which were found resistant to antibiotics used. Ab2, Ab3 and Ab4 strains were resistant to six categories of antibiotics and sensitive only to IMI; Imipenem. The antibacterial potential of *A. indica* and *T. catappa* leaves 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 properties against bacterial pathogens tested. Table 5 shows the mean zone of inhibition of *A. baumannii* strains (Ab1–Ab4) relative to *A. indica* leaf-extracts at concentration 400 mg/mL. The non-MDR *A. baumannii* (Ab1) showed sensitivity against all the test extracts of *A. indica* in which methanolic extract (14.5 ± 0.3) mm was found more potent compared to that of aqueous (11.8 ± 0.3) mm, but still less than the positive control gentamicin (25.0 ± 0.2) mm. Surprisingly, other MDR *A. baumannii* strains (Ab2–Ab4) revealed significant sensitivity to the extracts and were higher than gentamicin antibiotic, especially Ab4 which was almost resistant to gentamicin (7.0 ± 0.1) mm and sensitive to the extracts of *Z. mauritiana*.

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

Extract/standard agent used	Mean zone of inhibition of *A. baumannii* (mm)			
	Ab1	**Ab2**	**Ab3**	**Ab4**
Methanol Extract (10 mg/disc)	14.5 ± 0.3	11.2 ± 0.3	10.9 ± 0.3	12.5 ± 0.3
Aqueous Extract (10 mg/disc)	11.8 ± 0.3	10.5 ± 0.3	10.5 ± 0.3	11.5 ± 0.3
Gentamycin (10 µg/mL)	25.0 ± 0.2	8.22 ± 0.2	7.90 ± 0.1	7.10 ± 0.2

Table 6 summarizes the mean zone of inhibition of *A. baumannii* strains (Ab1–Ab4) against *A. indica* leaf-extracts at concentration of 400 mg/mL. The non-MDR *A. baumannii* (Ab1) showed sensitivity against all the test extracts in which methanolic extracts (14.5 ± 0.3) mm was found more sensitive compared to that of aqueous (11.8 ± 0.3) mm, but still less than the

gentamicin agent (25.0 ± 0.2) mm. However, other MDR *A. baumannii* strains (Ab2–Ab4) showed tremendous sensitivity to the extracts and were higher than gentamicin standard control agent, particularly Ab3 and Ab4 which were completely resistant to gentamicin agent (6.0 ± 0.1) mm and sensitive to the extracts of *T. catappa*. Methanol extracts were more sensitive compared to that aqueous. Hence, the most susceptible strains for methanol extraction were Ab1 (15.9± 0.3) and Ab4 (14.6± 0.3), followed by Ab2 (14.6± 0.3) mm as shown in Table 6.

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

Extract/standard agent used	Mean zone of inhibition of *A. baumannii* (mm)			
	Ab1	**Ab2**	**Ab3**	**Ab4**
Methanol Extract (10 mg/disc)	15.9 ± 0.3	14.6 ± 0.3	12.5 ± 0.3	14.8 ± 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	6.0 ± 0.2

The antibacterial efficacy of the calyces of *A. indica* and *T. catappa* was also evaluated using MIC and MBC tests to measure the bacteriostatic and bactericidal potentials 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 *A. indica* and *T. catappa* using methanol exhibited the most remarkable antimicrobial activities when compared to aqueous with MICs of 12.5 mg/mL for both Ab1, for Ab2 were 25 mg/mL, while 50 mg/mL for Ab3 and Ab4. Whereas, aqueous extracts showed 50 mg/mL for all the four bacterial strains (Table 7). However, in the case of gentamycin agent, the MIC values for both *A. indica* and *T. catappa* extracts were very low against the three bacteria strains but in contrast, Ab3 and Ab4 were 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***			
	Ab1	**Ab2**	**Ab3**	**Ab4**	**Ab1**	**Ab2**	**Ab3**	**Ab4**
Methanol	12.5	25	50	50	12.5	25	50	50
Aqueous (H_2O)	50	50	50	50	50	50	50	50
Gentamycin agent	200	400	-	-	200	400	-	-

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 Ab1, Ab2, Ab3 and Ab4 than aqueous extracts in which the MIC values against all the bacteria strains were the same (50 mg/mL), except for Ab1(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 *A. indica*

and *T. catappa* 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 Ab3 and Ab4 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.

4.2 Discussion

For the present study, the Acinetobacter strains utilized in this determination were three representing MDRs (Ab2, Ab3 and Ab4) and one representing non-MDR (Ab1) in order to compare the susceptibility of these bacteria towards the plant extracts of *A. indica* and *T. catappa*. 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.,* (2015) and Emad, (2016) 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 (Ab2–Ab4) 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 *A. indica* and *T. catappa*have potent antibacterial potential against MDR *A. baumannii*. Thus, agrees with the findings of (Altayb *et al.,* 2022) that, the leaves of *A. indica* have antibacterial activities on different pathogens, especially foodborne pathogens, methicillin-resistant *Staphylococcus aureus, P. aeruginosa, E. coli, Proteus* spp., *S. epidermidis, Citrobacter* spp., *K. pneumoniae, E. faecalis, S. aureus* ATCC 25,923, and *E. coli* ATCC 25,922 and *MDR S. aureus*. These potent antibacterial activities of leaves of *A. indica* and *T. catappa*could be attributed to their availability of bioactive phytochemicals. The previous study on *A. indica* and *T. catappa*and various medicinal plants revealed the presence of alkaloids, phenolic compounds, flavonoids and saponins, glycosides and tannins (Liu *et al.,* 2005; Abdallah, 2016). These bioactive components are considered to be the major groups of antimicrobial compounds in plants generally (Edwards, 2013).

The results of the antibacterial potential of both extracts were compared with the positive control (gentamicin agent). Recently it was reported that, *A. baumannii* strains developed high resistance to gentamicin (Henwood *et al.,* 2002). Table 7 summarizes MIC and MBC of both *A. indica* and *T. catappa* 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 significant for Ab1, Ab2, Ab3 and Ab4. Moreso, MIC value same as MBC and thus, confirms the antibacterial efficacy of the plant extracts quantitatively. According to previous literature, the *T. catappa* extracts possess effective bactericidal property against multidrug resistant (MDR) *Staphylococcus aureus*. These results are also similar to what Liu *et al.,* (2005) 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* (Miyasaki *et al.,* 2013).

Also according research documented by Faujdar *et al.,* (2020), it was shown that maximum zone of inhibitions and lowest minimum inhibitory concentration and minimum bactericidal concentration values were revealed by *Acinetobacter baumannii* strains using extracts of *A.*

indica.A. baumannii showed 15-08 mm zones. Kavitha M.*et al.,* stated 12 mm inhibitory zones at 250 µg/ml against Acinetobacter (Kavitha *et al.,* 2017). Another author also demonstrated 14.56 mm zone with methonolic of neem which is also in concordance to our study (Bhattacharjee *et al.,* 2019). On the other hand Autade *et al.,* (2015) did not observe any antibacterial activity against *A. baumannii.*

4.0 CONCLUSION AND RECOMMENDATIONS

4.1 Conclusion

For this current research, the findings showed that the methanol crude extracts of both *A. indica* and *T. catappa* 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 results therefore highlighted the significant inhibition of multidrug-resistant (MDR) *A. baumannii* strains. The antimicrobial properties of the tested plants also attributed to the presence of bioactive components contained in the plants. However, the mechanism behind antibacterial properties of the test plant remains unclear. Further, well-designed in vivo clinical trials are needed to identify and characterize newer bio-active molecules which can be used in alternative to antibiotics. Therefore, utilizing *A. indica* and *T. catappa* may have advantageous well-being impact when utilized as natural antimicrobials for bacterial diseases and infections including MDR *A. baumannii.*

4.2` Recommendations

1. Further future studies are recommended for the fractionation of the compounds responsible for their antibacterial potential in addition to other pharmacological studies, which may lead to the discovery of new natural antibiotic from *A. indica* and *T. catappa* 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 *A. indica* and *T. catappa* 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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