Antimicrobial Resistance Profiling of Routine Antibiotics and Mango Kernel (*Mangifera Indica* L.) Ethanolic Extract on Multidrug-Resistant *Acinetobacter* Isolates

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Abstract

Multidrug-resistant (MDR) *Acinetobacter* species are opportunistic pathogens that are clinically important, which causes severe infection during a prolonged stay in an Intensive Care Unit (ICU) of the hospital. In the present study, we screened clinical isolates of *Acinetobacter* species for its MDR nature from the ICU of a tertiary care centre at Abha, Saudi Arabia during the period of 2013-2017. Confirmed MDR *Acinetobacter* species clinical isolates were challenged against ethanolic extracts from mango kernel (*Mangifera indica* L.) by classical disc diffusion method. In total, we characterized 124 MDR *Acinetobacter* isolates, out of which 62 (50%) were *Acinetobacter baumannii* (MDR-AB), 41 (33.1%) were *Acinetobacter*

baumannii complex (MDR-ABC), 15 (12.1%) were *Acinetobacter baumannii-haemolyticus* (MDR-ABH), 3 (2.4%) were *Acinetobacter haemolyticus* (MDR-AH) and 3 (2.4%) were *Acinetobacter iwoffii* (MDR-AI). We observed that MDR-AB isolates were inhibited by ethanolic extract of *M. indica* with minimum inhibition concentration (MIC) of 0.25 mg/ml. Concentrations of 50 mg/mL, 5 mg/mL and 0.5 mg/mL exhibited average inhibition zones of 18.74±0.09, 15.51±0.08 and 9.74±0.06 mm respectively showing a concentration dependent antagonisms (R^2= 0.947). The results of *M. indica* were comparable to Colistin inhibition zone of 14.98±0.72 mm with 50 mg/mL and 5 mg/mL exceeded those of the positive control (p= 0.6721 and 0.1045, respectively). However, Colistin showed minor increase over *M. indica* at the concentration 0.5-mg/ mL (p= 0.5384). More specifically, MDR-AB strains have shown a substantial susceptibility to mango kernel extract (0.25 mg/mL) with antagonism similar to that of Colistin. Thus, the study shows that the extracts of mango kernel as a potential drug target and could be potentially used as an adjunct treatment along with the standard agents to treat *Acinetobacter* infections.

Keywords: *Acinetobacter* species, Nosocomial infection, Antimicrobial resistance, *Mangifera indica* L., Alternative medicine, Phytotherapy.

Tob Regul Sci.™ 2021;7(5-2): 5032-5047
DOI: doi.org/10.18001/TRS.7.5.2.65

1. Introduction:

Acinetobacter species are defined as an opportunistic bacterial pathogen that are ubiquitous in nature. They are aerobic, gram-negative coccobacillus comprising of more than 51 species (1). *Acinetobacter* is majorly characterized as environmentally non-pathogenic, however, under certain compromised conditions it has the potential to become a human pathogen (2). Various *Acinetobacter* species are known to cause the most common nosocomial infections which include *Acinetobacter baumannii*, *Acinetobacter calcoaceticus* and *Acinetobacter lwoffii* (3). In addition, *Acinetobacter calcoaceticus, Acinetobacter baumannii, Acinetobacter* genomic species 3, and *Acinetobacter* genomic species 13TU, have a similar shared homology and hard to distinguish by its phenotypic feature. They are collectively categorized as the *Acinetobacter calcoaceticus – Acinetobacter baumannii* complex (4). Most notably, *Acinetobacter baumannii* is the fastest emerging species worldwide and is considered as the major etiological agent associated with hospital-acquired infections (5).

The most vulnerable group for *Acinetobacter baumannii* infections are the patients who are hospitalized and admitted to the intensive care unit with the weakened immune system and are susceptible to skin breach and airway protection (6). The two major disease indications of *Acinetobacter baumannii* infection are bacteraemia and pneumonia. Additional minor clinical manifestations reported are urinary tract infection, post-surgical wound infections, post-neurosurgical meningitis and osteomyelitis. Furthermore, the spread of the infection is expedited due to its capability to thrive on dry surfaces for an extended time (7) and its mechanism to evade anti-microbial agents (5). Hence, the bacterium becomes resistant in such cases.

Resistant bacteria to antimicrobial agents are broadly categorized into multidrug-resistant

(MDR), extensively drug-resistant (XDR) and pandrug-resistant (PDR) bacteria as defined by European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC). Accordingly, MDR is non-susceptible to one, XDR is non-susceptible to two and PDR is defined to be resistant to all antimicrobial agents (8). Alarmingly, the most common cause of ICU infection like *Acinetobacter baumannii* has evolved ways by fostering antibiotic resistance mechanisms, including carbapenemases and extended-spectrum-lactamases (ESBLs) (2). Consequently, *Acinetobacter baumannii* is fast becoming global menace for the health care institutes due to its resistance to all the available antibiotics. There are only limited resources available to control the transmission and colonization of this pathogen (9). The estimated global incidence of *Acinetobacter baumannii* infection per year is in the range of 600,000-1,400,000 and out of which carbapenem-resistant isolates were reported to be 75,000 (10). Data in the literature indicates the upward trend of worldwide multiple outbreaks of MDR-AB in endemic and epidemic settings among critically ill patients. It led to the world health organization to declare *Acinetobacter baumannii* as critical in the priority list of pathogens for research and development of new antibiotics and new therapies (11).

Multidrug-resistant bacteria have managed to elude even the most potent antibiotic combinations. Interestingly, traditional healers have commonly used plants to prevent and treat infectious diseases. Concomitantly, phytotherapy has been shown to have curative effect against diseases worldwide. It is reported around 50% of the current pharmaceuticals are of plant origin, but none of them are used as antimicrobials (12-14). Additionally, the use of phytotherapy is proven to be inexpensive. Thus, there is an opportunity to explore and study medicinal plants and their activity as an antimicrobial agent.

There is a vast majority of data available on the extract and crude materials from *M. indica* for its antimicrobial property (15-19). Published results have proven that the extracts from *M. indica* are potential candidates against many species of pathogenic bacteria, which includes both gram positive and gram negative bacteria (20). It is worth exploring the use of plant extracts against MDR-AB and offers a viable economic alternative. A recent study which was carried out at the Aseer region, Kingdom of Saudi Arabia has found that the 69% of the bacterial isolates were MDR-AB from the hospitalized patients (21). We believe that further assessment is required to identify the risk factors affiliated with MDR-AB in Aseer region, Saudi Arabia. In addition, an alternative therapeutic approach is required to combat the resistant strains.

In the current study, we want to understand the clinical feature of MDR-AB. Further, we want to determine their antimicrobial characteristic against routine antimicrobial agents and ethanolic extracts of mango kernel.

2. Materials and Methods

2.1 Study design

The study was carried-out at the Department of Microbiology and Clinical Parasitology, College of Medicine, King Khalid University, Abha, Saudi Arabia. The clinical samples were collected from the patients who attended Aseer Central Hospital between 2013 and 2017 with different clinical indications. In this study, both males and females are included and the patient information was saved in a spreadsheet for analysis.

2.2 Ethical Approval

The Ethical Committee of the College of Medicine, King Khalid University, Abha, Saudi Arabia approved has the research and the reference letter number is REC # 2017-02-17.

2.3 Bacterial isolates

Acinetobacter strains were isolated from various clinical samples from the Intensive Care Unit, a tertiary care Centre, Abha, Saudi Arabia between 2013 and 2017. Majority of the samples were collected from respiratory tract. Samples were also isolated from sputum, end tracheal tube, throat swab, tracheal secretion, tracheostomal discharge and from the tracheal tube. Samples isolated from other specimens include wound, cerebrospinal fluid (CSF), urine, bedsore, swab, abscess and tissue biopsy. A total of 124 strains were isolated from all the samples. Isolation and preliminary nomenclature was performed as per routine laboratory methods following standard protocols (7). Identification of the isolates was confirmed using the VITEK 2 automated system according to the manufacturer procedures (bioMérieux Inc., Durham, NC 27712, USA). and were used for the *in vitro* assay with *M. indica* kernel extract. The isolates included different species of the genus *Acinetobacter. Acinetobacter baumannii* ATCC19606 was used as a positive control. Defining Multidrug resistant (MDR), Extensively Drug Resistant (XDR), and Pan Drug Resistant (PDR) in *Acinetobacter* species was done according to the standardized international terminology criteria (22). An MDR *Acinetobacter* species is the one that is non-susceptible to ≥1 agent in ≥3 antimicrobial categories (22).

2.4 Plant collection and identification

Fresh kernel (seeds) of *M. indica* were collected from different local markets in Abha and were identified as well as authenticated in the Department of Biological Sciences, College of Science, King Khalid University. The collected kernels were stored in plastic bags at room temperature until further use.

2.5 Extraction of mango kernels

1000 grams of *M. indica* kernels were air-dried and roughly powdered followed by extraction in Soxhlet apparatus to obtain ethanolic extracts. Further, the extract was filtered, and the filtrates were vaporized to dryness, and weighed to determine the % yield of the extracts. The following formula was used in order to calculate: % yield = (weight of the extract/ weight of ground plant material) ×100 as stated (23). The stock solutions of the crude ethanolic extract were prepared by dilution with 50% methanol to obtain the desired final concentrations of 0.05 mg/mL, 0.25mg/mL, 0.5 mg/mL, 5 mg/mL and 50 mg/mL.

2.6 Assays for antimicrobial activity of plant extracts

The assay for antimicrobial activity of plants extract was done by the disk impregnation method following standard methods (24). One to three loopful of 24-hour old culture from each test strain was used to prepare 0.5 McFarland standard suspensions. A loopful of 0.5 McFarland suspensions was used to streak Mueller Hinton's agar plates (Difco). Disks impregnated filter paper (5.5 mm diameters, Whatman) with the desired concentrations of ethanolic extract was placed on the inoculated agar. For negative control, filter paper disc which was impregnated with 50% ethanol was used, while Colistin disk (10µg; Bio-Rad) was used as a positive control.

All the discs were placed on the inoculated agar and the inoculated plates were incubated at 37°C and examined after 24, 48 and 72 hours for

inhibition zones under and around the impregnated disc. Inhibition zones were measured in mm using a ruler for each bacterial strain under and around the impregnated disc.

2.7 The minimal inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC)

Inhibitory activities (mean ± SE) were assessed in millimeters by measuring the zone of inhibition using a ruler. MIC and MBC have been considered valuable endpoints of antibacterial activity to serve as a guide for the effective therapy of bacterial infections. In this study, disk impregnated disks at various concentrations have been tested to determine the MIC.

2.8 Data and Statistical analysis

The experimental data were represented as the mean ± standard error of the mean (SEM). Data was examined using PAST software (Version 3.14; Øyvind Hammer, Natural History Museum, University of Oslo, 1999-2016). Chi square was used to calculate the mean observed and expected values; p value ≤ 0.05 was considered as statistically significant.

3. Results

3.1. MDR strains isolated from clinical samples

Total 124 clinical samples were isolated from different specimens. Out of 124 samples, 42 samples were isolated from sputum, 16 from end tracheal tube, 14 from throat swab, 12 from tracheal secretion, 5 from tracheostomal discharge and 1 sample from tracheal tube. Samples isolated from other specimens include wound-14, cerebrospinal fluid (CSF)-9, urine-3, bed sore-3, swab-1, abscess-1 and tissue biopsy-1 (see Table 1).

Table 1. *Acinetobacter* strains recovered from various clinical specimens from the Intensive Care Unit, a tertiary care Centre, Abha, Saudi Arabia between 2013 and 2017

Respiratory tract specimens	Frequency	Percentage	Other specimens	Frequency	Percentage
End tracheal tube	16	12.9	Abscess	1	0.8
Sputum	42	33.9	Bed sore	3	2.4
Throat swab	14	11.3	CSF	9	7.3
Tracheal secretion	12	9.7	Swab	3	2.4
Tracheal tube	1	0.8	Tissue biopsy	1	0.8
Tracheostomal discharge	5	4.0	Urine	3	2.4
Total	90	72.6	Wound	14	11.3
			Total	34	27.4

3.2 Characterization of MDR strains isolated from clinical samples

Clinical isolates were identified and categorized into five different species of *Acinetobacter* as shown in the Table 2. Out of the 124 isolated MDR *Acinetobacter*, strains, 62 (50%) were *Acinetobacter baumannii*; 41 (33%) were *Acinetobacter baumannii* complex; 15 (12%) were *Acinetobacter baumannii-haemolyticus*; 3 (2.4%) were *Acinetobacter haemolyticus,* 3 (2.4%) and 3 (2.4%) were *Acinetobacter lwoffii* (see Table 2). Additionally, *Acinetobacter baumannii* from ATCC19606 was used as a positive control and listed along with the clinical isolates. Thus, total numbers of samples discussed during the study are 125.

Table 2. Identification of the 125 *Acinetobacter* strains and their resistance patterns

Species	Frequency	Percentage	Pattern of resistance
Acinetobacter baumannii (clinical isolates)	62	49.6	MDR
Acinetobacter baumannii (ATCC19606) *	1	0.8	Susceptible
Acinetobacter baumannii complex	41	32.8	MDR
Acinetobacter baumannii-haemolyticus	15	12	MDR
Acinetobacter haemolyticus	3	2.4	MDR
Acinetobacter lwoffii	3	2.4	MDR
Total	125	100	MDR

*ATCC, American type culture collection, P.O. Box 1549. Manassas, VA 20108 USA.

3.3 Effect of various antimicrobial agents on isolated MDR strains

In order to test the antimicrobial sensitivity, Vitek 2 test was used according to the manufacturer. *Acinetobacter baumannii* has shown nearly 100% sensitivity to the following antibiotics: Amoxicillin-potassium clavulanate (Amox / K Clav), Amoxicillin, Ampicillin, Azithromycin, Aztreonam, Cefitriaxone, Ceftiaxone, Cefuroxime, Ertapenem, Fosfomycin, Grepafloxacin, Minocycline, Moxifloxacin, Netilmicin, Nitrofurantoin, Pefloxacin, Penicillin, Rifampin, Tobramycin. relatively, very less significant sensitivity in the range of 95.7-99% was shown against Amikacin, Amp/Subactm, Cefepime, Ceftazidime, Ciprofloxacin, Colistrin Gentamicin, Imipenem, Pip/Tazo, Peperacillin and Trimeth/Sulfa as shown in Table 3.

Subsequently, *Acinetobacter baumannii* have shown resistance towards different antibiotics. Out of 29 antibiotics tested, *Acinetobacter baumannii* was not resistant to six antibiotics namely, Amoxicillin, Ampicillin, Azitromycin, Aztreonam, Cefitriaxone, and Ceftiaxone. However, the strains were resistant to nalidixic acid (50%), mezlocillin (22.2%), cotrimox (18.8%), ticaracillin (12.5%), cefoxitin (11.1%), cephalothin (11.1%), tetracyclin (9.1%), meropenem (7.4%), levofloxacin (6.9%), tigecycline (6.3%), amoxicillin k clavulanate (6.1%) and cefotaxime (5.6%).

Table 3. Sensitivity and resistance rates of *Acinetobacter baumannii* to different antibiotics

S. No	Name of the Antibiotic	Percentage of Sensitive	Name of the Antibiotic	Percentage of Resistance
1	Amikacin	95.9	Amikacin	4.1
2	Amox / K Clav	100	Amox / K Clav	6.1
3	Amoxicillin	100	Amoxicillin	Not resistant
4	Amp / Subactm	95.8	Amp / Subactm	4.2
5	Ampicillin	100	Ampicillin	Not resistant
6	Azithromycin	100	Azithromycin	Not resistant
7	Aztreonam	100	Aztreonam	Not resistant
8	Cefepime	99	Cefepime	1
9	Cefitriaxone	100	Cefitriaxone	Not resistant
10	Ceftazidime	96.3	Cefotaxime	5.6
11	Ceftiaxone	100	Cefoxitin	11.1
12	Cefuroxime	100	Ceftazidime	3.7
13	Ciprofloxacin	97.3	Ceftiaxone	Not resistant
14	Colistin	96.6	Cephalothin	11.1
15	Ertapenem	100	Ciprofloxacin	2.7
16	Fosfomycin	100	Colistin	3.4
17	Gentamicin	99.1	Cotrimox	18.8
18	Grepafloxacin	100	Gentamicin	0.9
19	Imipenem	96.1	Imipenem	3.9
20	Minocycline	100	Levofloxacin	6.9
21	Moxifloxacin	100	Meropenem	7.4
22	Netilmicin	100	Mezlocillin	22.2
23	Nitrofurantoin	100	Nalidixic acid	50.0
24	Pefloxacin	100	Pip / Tazo	2.9
25	Penicillin	100	Piperacillin	2.4
26	Pip / Tazo	97.1	Tetracyclin	9.1
27	Piperacillin	97.6	Ticaracillin	12.5
28	Rifampin	100	Tigecycline	6.3
29	Tobramycin	100	Trimeth / Sulfa	4.3
30	Trimeth / Sulfa	95.7	-	-

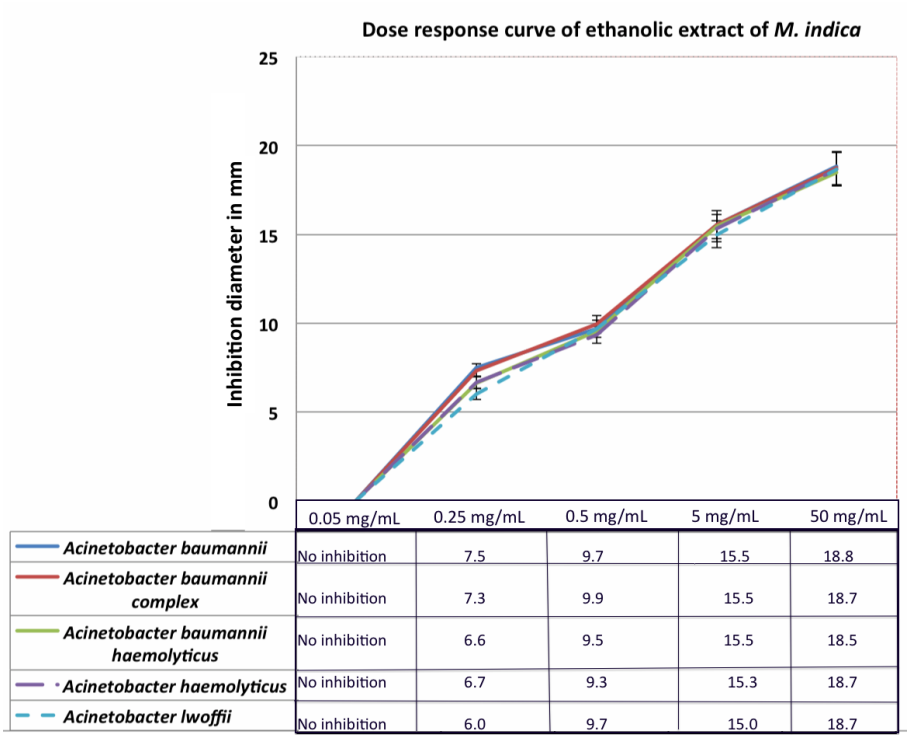
Thus, it was observed that MDR-AB is sensitive to most of the antibiotics ranging from 95.7-100%. Further, out of 29 antibiotics analysed, 18 antibiotics have shown percentage of resistance (<12%), whereas 6 antibiotics have conferred no resistance. However, other 4 antibiotics namely nalidixin acids, mezlocillin, cotrimox, ticaracillin have shown 50%, 22.2%, 18.8% and 12.5% resistance respectively. These observations suggest that MDR-AB is becoming a multi drug resistant and an alternative treatment to inhibit MDR-AB is required.

3.4 Ethanolic extract of *M. indica* possessed antibacterial activity against Acinetobacter

To check the potential antimicrobial activity of the ethanolic extract from *M. indica*, various concentrations (0.05, 0.25, 0.5, 5 and 50 mg/mL) of the extracts were prepared and used against all the 124 different clinical isolates that belong to different *Acinetobacter* species (as shown in Table 2) and reference strain *Acinetobacter baumannii* ATCC19606. Colistin was used as a positive control. A dose response of inhibition of bacterial growth around the impregnated disk was observed in all the five *Acinetobacter* species with no inhibition at 0.05 mg/ml as shown in Fig 1. The average inhibition zones of 62 *Acinetobacter baumannii* isolates; 41 *Acinetobacter baumannii* complex isolates; 15 *Acinetobacter baumannii-haemolyticus* isolates; 3 *Acinetobacter haemolyticus* isolates and 3 *Acinetobacter lwoffii* isolates have been calculated and represented in the below graph (Figure 1). No inhibition was observed at 0.05 mg/mL for all the strains. However, starting from the concentration of 0.25 mg/mL a dose response was observed with an average inhibition zone of 18.8±0.09, 15.5±0.08, 9.7±0.07, 7.5±0.18 mm at the concentrations of 50 mg/mL, 5 mg/mL, 0.5 mg/mL and 0.25 mg/mL respectively for *Acinetobacter baumannii*. A similar pattern was observed for *Acinetobacter baumannii complex with* an average inhibition zone of 18.7±0.16, 16.0±0.14, 10.0±0.05 and 7.0±0.33 at the concentrations of 50 mg/mL, 5 mg/mL, 0.5 mg/mL and 0.25 mg/mL respectively.

In *Acinetobacter baumannii-haemolyticus* ethanolic extracts have shown a comparable result with an average inhibition zone of 18.5±0.35 (5 mg/mL), 15.0±0.27(50 mg/mL), 10.0±0.24(0.5 mg/mL), 7.0±0.55 (0.25 mg/mL) suggesting a dose response. Likewise, in *Acinetobacter haemolyticus* the average inhibition zone was found to be 18.7±0.33 (5 mg/mL), 15.0±0.88(50 mg/mL), 9.0±0.67(0.5 mg/mL), 7.0±1.67(0.25 mg/mL). Finally, for *Acinetobacter lwoffii* the observed average inhibition zones were 18.7±0.88 (5mg/mL), 15.0±0.58(50 mg/mL), 10.0±0.33(0.5 mg/mL), 6.0±1.00(0.25 mg/mL).

Thus, minimum inhibitory concentration for all the five strains was observed at 0.25 mg/mL. Further, negative control wherein the disk was impregnated in only 50% ethanol has not shown any inhibition. Colistin was used as a positive control with the disk impregnated in 10 g of colistin. The inhibition zone ranging from 13.9 mm-15.0 mm was observed with colistin for the five different strains of *Acinetobacter*. Data for all the five strains of *Acinetobacter,* including positive and negative control is shown in the Figure 2.



	0.05 mg/mL	0.25 mg/mL	0.5 mg/mL	5 mg/mL	50 mg/mL
Acinetobacter baumannii	No inhibition	7.5	9.7	15.5	18.8
Acinetobacter baumannii complex	No inhibition	7.3	9.9	15.5	18.7
Acinetobacter baumannii haemolyticus	No inhibition	6.6	9.5	15.5	18.5
Acinetobacter haemolyticus	No inhibition	6.7	9.3	15.3	18.7
Acinetobacter lwoffii	No inhibition	6.0	9.7	15.0	18.7

Figure 1. Dose response curve illustrating inhibitory activity of *Acinetobacter* strains to different concentrations of ethanolic extract of *M. indica kernels.*

Thus, the ethanolic extracts of *M. indica* have been found to be active against all the five different strains of *Acinetobacter.* Representative images of positive control-ATCC 19606, *Acinetobacter baumannii* (A58, A100, A147 and A150) and *Acinetobacter baumannii complex* (A78) are shown in the Figure 3.

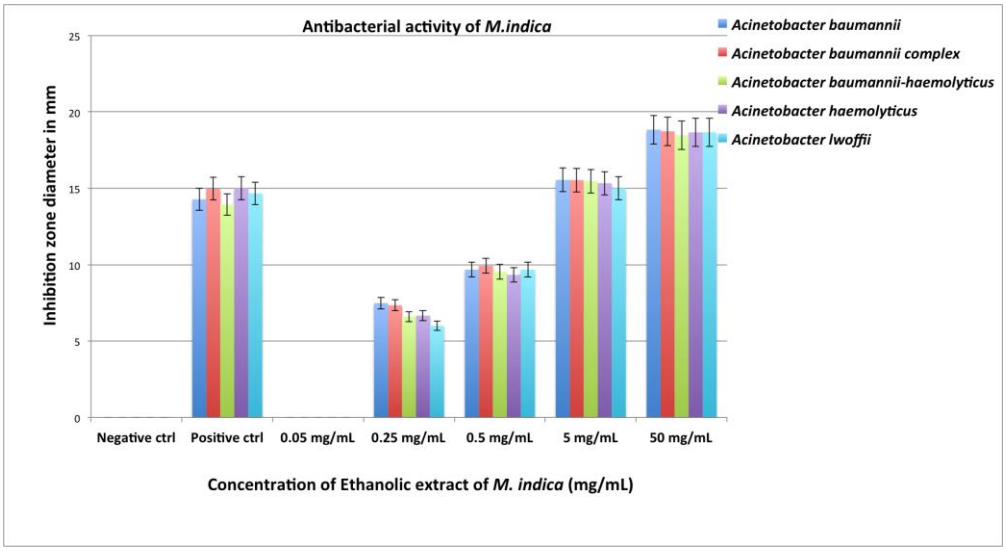


Figure 2. Susceptibility of inhibition of *Acinetobacter* strains to different concentrations of ethanolic extract from *M. indica kernels*

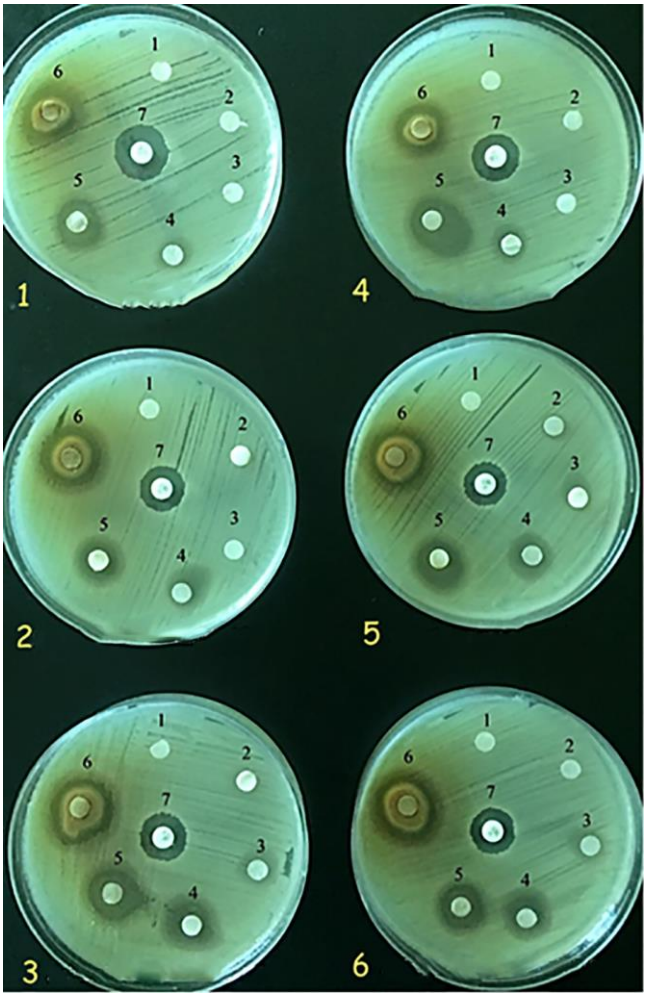


Figure 3. Susceptibility of clinical isolates of *Acinetobacter baumannii* and *Acinetobacter baumannii* complex to different concentration of ethanolic extract of *M. indica kernels.* Plate No-1 corresponds to *Acinetobacter baumannii ATTCC 19606,* Plate No-2 *Acinetobacter baumannii* complex and the Plates No-3-6 corresponds to *Acinetobacter baumannii.* Inside the plate abbreviations: 1-negative control (ethanol impregnated disc); 2-0.005 mg/mL ethanolic *M. indica* extract; 3-0.05 mg/mL; 4-0.5 mg/mL; 5-5 mg/mL; 6-50 mg/mL and 7, colistin disc (10µg; Bio-Rad).

To conclude, the results of the disk impregnation method have shown that *Acinetobacter* MDR-AB isolates could be inhibited by ethanolic extract of *M. indica at concentrations ranging from 50-0.5 mg/mL* with an average inhibition zones of 18.74±0.09, 15.51±0.08 and 9.74±0.06 mm, respectively, indicating a dose dependant decrease in the rate of antagonisms (R^2= 0.947). Inhibitory effect exhibited by the extract of *M. indica* were comparable to that of colistin (14.98±0.72mm) that was used as a positive control; However, colistin showed minor increase over *M. indica* at the concentration 0.5 mg/ mL (p= 0.5384). No inhibition was noticed at the concentration 0.05-mg/ mL as well as with the negative control (ethanol impregnated discs).

4 Discussion

MDR *Acinetobacter* species have transpired as an important threat giving rise to nosocomial infections in the south region, Abha, Saudi Arabia. There are minimal ways to survive the emerging MDR super bugs, which is a major

concern among researchers and clinicians about these MDR strains. Although modern medicine is highly efficient, herbal medicines have maintained recognition not only based on their effectiveness but also due to cultural and historic features for more than 5000 years (25). The herbal medicinal plants undoubtedly contain antimicrobial properties and it has been shown that nearly 50% of modern drugs use plant products (26) and many natural products could lead to new drug discoveries (27). In the current study, we explore the unmet need and insufficiently explored areas to highlight the potential of a plant product from mango tree *M. indica* as an effective antimicrobial agent towards MDR *Acinetobacter* species and predict their therapeutic value.

Acinetobacter species are widely distributed in the human body. Under normal physiological conditions, *Acinetobacter* species are commonly found in the normal flora of the body. It can also be easily isolated in a healthy individual's body from the hand, throat, nose, trachea, ear, conjunctiva and humid inhabiting areas (28). In hospitalized patients, the main areas of the human body populated by these *Acinetobacter* species are skin, oropharynx, and digestive tract (29). Similarly, in our study, 124 isolated clinical samples have shown that the primary sample source that inhabited *Acinetobacter* species were from oropharynx region. Among them, 42% (sputum), 16% (end tracheal tube), 14% (throat swab), 12% (tracheal secretion), 5% (tracheostomal discharge) and 1% (tracheal tube) were identified. Other sources included 14% (wound), 9% (CSF), 3% (urine), 3% (bed sore), 1% (swab), 1% (abscess) and 1% (tissue biopsy). In contrast, *Acinetobacter* species isolated from Cantonal Hospital Travnik, Bosnia and Herzegovina, have shown that most of the *Acinetobacter* infections correspond to abscess and wound infections (30). However, the authors have elucidated that increased isolation was performed on pus samples followed by endotracheal aspirates. Conclusively, in comparison with our study *Acinetobacter* species prevalence occurred more in the respiratory tract (32%) followed by wound (19.5%), blood (16%) and urine (9%) (31).

Acinetobacter baumannii is now a common and considered a serious pathogen included in the acronym "ESKAPE," standing for *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* species (32). As discussed, the genus *Acinetobacter* consists of 51 species and *Acinetobacter calcoaceticus–Acinetobacter baumannii* complex encompasses six species: *Acinetobacter calcoaceticus* (genomic species 1), *Acinetobacter baumannii* (genomic species 2), *Acinetobacter pittii* (known previously as genomic species 3) and *Acinetobacter nosocomialis* (previously named as genomic species 13 TU), *Acinetobacter seifertii* and *Acinetobacter dijkshoorniae* (closely related to *Acinetobacter pittii*). Out of all the species only five are known to cause infections in humans that include *Acinetobacter baumannii, Acinetobacter pittii, Acinetobacter nosocomialis, Acinetobacter seifertti and Acinetobacter dijkshoorniae.*

Acinetobacter calcoaceticus does not cause disease and recognized as a nonpathogenic environmental organism isolated from the soil (33). In the present study, the clinical isolates were categorized as per the classification and it was observed that 50% were *Acinetobacter baumannii;* 33% were *Acinetobacter baumannii* complex; 12% were *Acinetobacter baumannii-haemolyticus;* 2.4% each were *Acinetobacter haemolyticus* and *Acinetobacter lwoffii.* Thus, *Acinetobacter*

baumannii was found to be the primary pathogen among others similar to the study done by Rebic et al., (30) where the authors have shown that *Acinetobacter baumannii* was responsible for 84.3% *Acinetobacter* infections out of 125 isolates (30).

Further, it is well known that *Acinetobacter baumannii* is susceptible to many antimicrobial antibiotics that belong to different classes such as penicillins and cephalosporins, fluroquinolones, aminoglycosides, carbapenems, polymyxins and tigecycline. However, *Acinetobacter baumannii* have evolved resistance to antibiotics that are categorized as MDR-resistant to penicillins, cephalosporins, fluroquinolones, and aminoglycosides; XDR-resistant to carbapenems in addition to resistance against MDR class of antibiotics and finally, PDR-resistant to polymyxins and tigecycline. A study has shown that 69% of isolates collected from south region, Abha, Saudi Arabia were resistant to test antibiotics suggesting that further sensitivity assessment is required (21). In this study, we have shown that although *Acinetobacter baumannii* is sensitive to most of the antibiotics, the bacteria have shown resistance to nalidixic (quinolone antibacterial agent), beta-lactum antibiotics: mezlocillin and ticaracillin and cotrimox (sulfonamide). Resistance towards nalidixic acid is due to the decreased accumulation in the *Acinetobacter* cells when efflux pump was inhibited (34). Additionally, a study from Snami et al., has shown that modulation of cellular permeability, outer membrane protein A (OmpA) led to decreased MIC with nalidixic as it is linked to efflux pump (35). Although in our study, cotrimox has exhibited 18.8% resistant in *Acinetobacter baumannii,* cotrimox in conjunction with colistin was able to show sensitivity in Galleria mellonella model when administered against carbapenem-resistant *Acinetobacter baumannii* (CRAB) (36). Nevertheless, the emergence of MDR cannot be undervalued and alternative approaches are indispensable to fight against these super bugs.

The emerging resistance of MDR's occurs due to the excessive usage of antimicrobial agents per patient per square area (37). There is an urgent need to explore the possibility of inexpensive and potent alternatives to enforce the infection control in order to reduce the spread of resistant *Acinetobacter* species. One of the potential candidates is the extract of plant product from the mango kernel (*Mangifera indica* L). In this study, *Acinetobacter* species clinical isolates were challenged with the ethanolic extracts of mango kernel to assess its antimicrobial activity. Five different concentrations were administered in a disc diffusion method against five different strains: *Acinetobacter baumannii*, *Acinetobacter baumannii* complex, *Acinetobacter baumannii-haemolyticus*, *Acinetobacter haemolyticus* and *Acinetobacter lwoffii*. A dose dependent kinetic was observed suggesting that the high phenolic content of pentagalloylglucopyranose (PGG) (61.28%) and smaller amounts of methyl gallate (MG) (0.68%) and gallic acid (GA) (0.44%) (38) of mango kernel ethanolic extract was able to inhibit *Acinetobacter* species. The average MIC observed was 18.74±0.09, 15.51±0.08 and 9.74±0.06 mm for 50 mg/ mL, 5 mg/mL and 0.5 mg/mL appropriately. No significant difference was observed in between the strains. Likewise, it has been shown that mango kernel ethanolic extracts were efficient when used against the strains of *Staphylococcus aureus* (39-41).

Thus, our study has identified *Acinetobacter* species in ICU clinical samples and has provided the means to combat these emerging resistance strains with inexpensive plant product.

Nevertheless, it is necessary to perform such studies periodically to identify the prevalence and sensitivity, which will help the clinicians manage *Acinetobacter* infections in an effective and safe manner.

5 Conclusion

A notable activity has been shown with the ethanolic extracts of mango kernel in inhibiting MDR-AB, which is comparable to that of Colistin. Although Colistin along with many other antibiotics is effective, the cost of treatment for the evolving MDR-AB resistant strains is proving to be a constraint to healthcare. Hence, it becomes extremely beneficial to uncover the potential of plant-based products. The present investigation has shown a promising result that mango kernel as low as 0.25 mg/mL is able to inhibit MDR-AB. Further, the study of the antimicrobial properties of the mango kernel can be extended on other MDR *Acinetobacter* strains and also on animal models to assess the toxicity and the mechanism of action.

Informed consent

The study design was informed to all the individuals participated in the study and their due consent was obtained.

Data Availability

The authors will furnish any additional information in regard to be data incorporated in the research article on request.

Conflict of interest

The authors have no conflicts of interest to declare related to this study.

Author's contribution

M.E.H. and A.D. conceived and designed the research project. M.E.H., A.D., A.A.B, M.R.P.J. and A.A.A. were responsible for the methodology. A.A.B, M.R.P.J., A.A.H., A.K., M.A.K., A.M.A., and H.C.C. collected the data for the study. M.E.H., A.D., H.C.C. and A.M. performed the data analysis. M.E.H., A.D. and A.A.A. provided the resources. M.E.H. and A.D. supervised and performed the funding acquisition for the study. M.R.P.J. carried out the project administration. M.E.H., A.D., A.A.A. and M.R.P.J. wrote the original draft. H.C.C., A.D. and M.E.H. reviewed and edited the article. Ali Al Bshabshe and Martin R.P. Joseph equally contributed to the work.

Acknowledgements

The authors wish to thank all the members of the Microbiology Departments at both Aseer Central Hospital and the College of Medicine, King Khalid University, Abha for their generous support in the accomplishment of the research project. The research was funded by a grant to Anantharam Devaraj from the Deanship of Scientific Research, King Khalid University, Abha, Saudi Arabia (Project number: G.R.P 179-39).

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