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Extracts of Turmeric (*Curcuma longa* L.) as a Potential Biocontrol of Multidrug-Resistant *Acinetobacter baumannii*

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Abstract: Nosocomial infections with *Acinetobacter baumannii* are increasing every day, making treatment with traditional antibiotics more complicated. This work aimed to investigate the antibacterial activity of *Curcuma longa* extracts against carbapenem-resistant isolates of *A. baumannii*. A total of 90 samples (Sputum, n=40 and Urine, n=50) were collected and biochemically identified via Vitek -2 system. Ten isolates were identified as *A. baumannii* (10/90; 11.1%). (10/90; 100 %) of isolates identified were multi drug resistance (MDR). *Curcuma longa* extracts aqueous and/or ethyl acetate of, exhibited a significant MBC value varying from 43.75 to 50.83 mg ml^{-1} against *A. baumannii* isolates (A1, A2, A3, A5, A6, and A8), respectively. Within isolates A1 and A8, ethanol extracts revealed MBC with significant values (30 to 70 mg ml^{-1}) respectively. The main phytochemical compounds detected among 68 bioactive compounds in the extracts of $^{C. longa}$ over the GC- mass spectra analysis were Turmerone (59.42%), Beta-caryophyllene (16.66 %), Caryophyllene oxide (15.62 %), Isolongifol (12.14 %), Alpha-pinene (12.07 %), Citral (11.68 %), Curdione (11.0 %), B -Sesquiphellandrene (10.47 %), B-myrcene (8.12 %), D-Limonene (8.04 %). Bactericidal activity of *C. longa* against MDR A. baumannii were supported by phytochemical compounds that detected in GC-MS analysis.

Keywords: *Acinetobacter baumannii*, *Curcuma longa*, GC-Mass, Imipenem.

Introduction

Nosocomial infections with *A.baumannii* are a common cause of multiple respiratory, wound and urinary tract infections, and have become one of the most predominant illnesses and representing more than 54% of hospitalized patients (Ayoub & Hammoudi, 2020). Infection with *A.baumannii* is often difficult to treat because of resistance to multiple antibiotics and disinfectants (Williams *et al.*, 2020).They represents second most common gram-negative bacteria after *Pseudomonas aeruginosa*, which identified in clinical specimens (He *et al.*, 2011). In Egypt *A. baumannii* recovered from different location of hospitalized patients (Abdulzahra *et al.*, 2018). *A. baumannii* induced respiratory pneumonia is widespread in clinical environments as their surviving as a commensal on the skin or hair of hospital staff and patients (Vrancianu *et al.*, 2021). Urinary tract infections with *A. baumannii* were increasingly found in immune compromised patients, leading to a high significant morbidity and mortality (Ayenew *et al.*, 2021). *A. baumannii* is generally antibiotic-resistant because of decreased permeability, efflux pumping systems, inactivation of enzymes, and the formation of biofilm (Chakravarty *et al.*, 2020). Thus, they are often β-lactams, aminoglycosides, and quinolones resistant (Motbainor *et al.*, 2020).

β-lactams antibiotics such as cephalosporins, carbapenems, and penicillins represent approximately 60% of the used antibiotics (Magiorakos *et al.*, 2012). *A. baumannii* resistance is mainly due to Extended-Spectrum Beta-Lactamases (ESBLs), which could disrupt various β-lactam antimicrobial agents as penicillins and their derivatives (Ghaima, 2018). *C. longa* commonly known as (Turmeric), is widely used as a spice and colouring agent, and well known for its antibacterial properties (Kumar & Sakhya, 2013). There is also urgently requirement to identify novel pharmacological agent and to understand the function of possible therapies in the treatment of *A. baumannii* infection. In the present study, the prevalence, patient clinical characteristics, antibiogram, of *A. baumannii* isolated from different clinical samples were investigated. Subsequently, the phytochemical investigations of *C. longa* different extracts were carried out using gas chromatography-mass

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spectrometer technique (GC-MS). The antibacterial activity of the extracts or imipenem was tested in vitro against *A. baumannii* isolates.

Materials and Methods

I. Sampling collection, isolation and processing

Ninety specimens; urine (n = 50), sputum (n = 40), and were randomly collected under complete aseptic conditions from different hospitals and clinics in Qena province, Egypt (From January to November 2019). Using sterile container, 5 ml of clean-catch mid-stream urine was collected from patients presumptive for urinary tract infection (UTI). Urine samples were inoculated on MacConkey agar and/or Blood agar (Merck, Germany) at 37ºC for 24 hrs and observed for bacterial growth. Blood agar colonies were counted using colony counter and checked for significant bacteriuria. Culture that grew $\geq 10^5$ CFU/ ml, were measured as a significant bacteriuria.

For heterogeneous colonies, sub-culturing of individual distinct colonies was performed to ensure pure cultures. Sputum samples were taken from patients over a self-induced cough into sterile cups and sent for culture. Samples initially identified using standard laboratory methods including growth on MacConkey agar and/or Blood agar (Merck, Germany) and Gram staining. Plates were incubated at 37ºC and examined for visible bacterial growth after 48 hrs of incubation. The isolated bacteria were further identified using the automated system VITEK-2 (bioMérieux, France).

II. Phenotypic identification of the isolates by ViteK-2 systems

All bacterial isolates were identified through morphological, conventional biochemical tests with Automated Identification (Biomerieux) Vitek-2 System. Forty-one tests, including 18 tests for sugar assimilation, 18 tests for sugar fermentation and 2 decarboxylase tests, and 3 miscellaneous tests (for urease, utilization of malonate, and tryptophane deaminase). With a vacuum device, the cards are inoculated with a 0.5 McFarland suspension of the organism prepared from 18- 20 hours-old blood agar plate (bioMe´rieux) and are then automatically sealed and manually inserted inside the Vitek-2 reader. Inoculators fluorescence is measured every 15 min, and the results of identification are determined after 3 h (Joyanes *et al.*, 2001).

III. 2.3. Antimicrobial susceptibility testing

The antibiograms for all the recovered isolates were determined by disk diffusion method (Bauer *et al.*, 1966). Each bacterial isolates was inoculated in tryptic soy broth and incubated at 37ºC overnight for activation. Bacterial inoculum was adjusted to 0.5 McFarland turbidity, which equal 1.0×10^8 CFU/ml.

The antimicrobial agents used belonged to five different classes of antimicrobials; the aminoglycosides (Amikacin 30 ug/disc, Tobramycin 10 ug/disc and Gentamicin 10 ug/disc), β-lactams (Ticarcillin 100 ug/disc, Ticarcillin/Clavulanic Acid 75/10 ug/disc, Piperacillin 100 ug/disc, Piperacillin/Tazobactam 100/10 ug/disc, Cefotaxime 30 ug/disc, Cefepime 30 ug/disc, Imipenem 10 mg/disc and Meropenam 10mg/disc), Fluoroquinolones (Ciprofloxacin 5ug/disc and Levofloxacin 5 µg), antimetabolites (Trimethoprim/Sulfamethxazole 1.25/23 ug/disc) and Tetracycline (Minocycline 30ug/disc). MIC was carried out in triplicate and average MIC was calculated, as recommended by the (CLSI, 2018). *A. baumannii* isolates with the same phenotype and antibiotic pattern profile were excluded, only different isolates were considered for further experiments.

IV. Plant material and extraction

Curcuma longa (Turmeric) was purchased from local market in Nag hammadi center, Egypt. Three solvents including distilled water, ethanol, and ethyl acetate (Piochem, Egypt), were used as follow. 20 grams of plant powder was soaked in 200 ml of each solvent under stirring conditions (150 rpm), for three consecutive days at room temperature by using bigger bill shaker, USA. The solutions were filtred through Whatman # 1 sterile filter paper and collected in a pre-weighed beaker. The solvent was allowed to evaporate at room temperature tills complete evaporation except for aqueous extract that was concentrated in a water bath until a constant dark sticky residue was obtained. The dried precipitates were dissolved in di-methyl-sulphoxide (DMSO), (10 % v/v) and, then stored in airtight sterilized bottles at - 4ºC.

V. Determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) by INT formazone assay

The determination of MIC and MBC were assayed as described earlier (Salem *et al.*, 2017). The freshly prepared culture of *A. baumannii* isolates was adjusted to OD595 of 0.01. 100 µl of each isolate culture was put into sterilized 96-well plates. Then 20 µl of the original *C. longa* extracts (100 mg ml^{-1}) was added (serial dilutions of 10^{-1} -10^{-10} were used, 8 replicates were made for each dilution into complete raw of the 96-well plate). Imipenem (512 mg ml^{-1}) was tested as a positive

control; un-inoculated media tested as negative control after 24 hrs incubation at 37ºC. MIC was determined by the addition of 40 µl of p-iodonitrotetrazolium violet chloride (INT) (0.2 mg/ml, Sigma-Aldrich) to the plates and re-incubated at 37ºC for 30 min., the lowest concentration which banned color change is the MIC (Eloff, 1998 & Lall *et al.*, 2013). The MBC was determined by transferring 50 ul from each well of an overnight MIC plate (and/or higher) to sterile (TSA) fresh plates. Viable colonies were counted after 24 h at 37ºC. The limit of detection for this assay was 101 CFU/ml.

VI. Gas chromatography–mass spectrometry (GC-MS) analysis

Three different extracts of *C. longa* were subjected to gas chromatography-mass spectrometer technique (GC-MS) (Thermo scientific technologies, Trace 1310-ISQ) with capillary column TG-5 (30m×250µm×0.25µm) system were used. The mass detector used in split mode and helium gas with a flow rate of 1.5 ml/min was used as a carrier. The injector was operated at 230ºC and the oven temperature for the initial setup was 60ºC for 2 min. ramp 10/min. to 300ºC for 8 min. Mass spectra were taken at 70eV, total GC running time was 35 min.

VII. Statistical analysis

The variability degree of results was expressed in form of Means ± Standard Deviation (Mean ±S.D). GraphPad Prism 6.0 (GraphPad Software, San Diego, CA, USA, https://www.graphpad.com) was used to calculate one way analysis of the variance (ANOVA) with multiple comparison tests (Tukey's) to evaluate the effect of the different extract. Differences were considered significant at p values of ≤ 0.05.

Result

Patients and Clinical Characteristics

A total of 90 patients with clinical evidence of nosocomial infection (elevated liver and/ or kidney functions, respiratory diseases, etc.) were enrolled in the study (Table 1). Of them, 51 (56.66) were females and 39 (43.3%) were males. The majority (48.9 % - 29 female; 15 male) of participants were found in the age group between 30- 60 years. Twinty six patients (7 female; 19 male) with a percentage of approximately (28.9 %) of the study participants were above 60 years old. While a percentage of (22.2 % - 18 female; 6 male) were below 30 years old. Table (1) depicts the clinical characteristics of the study participant.

The prevalence of *A. baumannii* isolates among Isolated clinical samples

Among the 90 clinical specimens, the incidence of the isolated bacteria in the clinical samples was illustrated in Table 2 and Figure 1. The most common prevalent organism (from each corresponding clinical samples) was *Klebsiella pneumonia* with a percentage of 45.5% followed by *Escherichia coli* 39.9%, A.baumannii 11.1% and *P. aeruginosa* 4.5% (Figure 1A).The total bacterial count in urine culture showed that 70% (21/30) of female patients had a range of 10^4 to 10^5 CFU/ml compared to 65% (13/20) of male patients. While 5% of female and 2% of male patients had a CFU values ≥ 106 (data not shown). Isolates from urine samples showed that *E. coli* seems to be the predominant with (32/50; 64.0%), followed by *K. pneumonia* (11/50; 22.0%), *P. aeruginosa* (4/50; 8.0%) and *A. baumannii* with (3/50; 6.0%), (Figure 1B). The respiratory tract isolates with *K. pneumonia* showed to be predominant with (30/40; 75.0%), *A. baumannii* (7/40; 17.5%) followed by *E. coli* (3/40; 8.82 %) (Figure 1C).

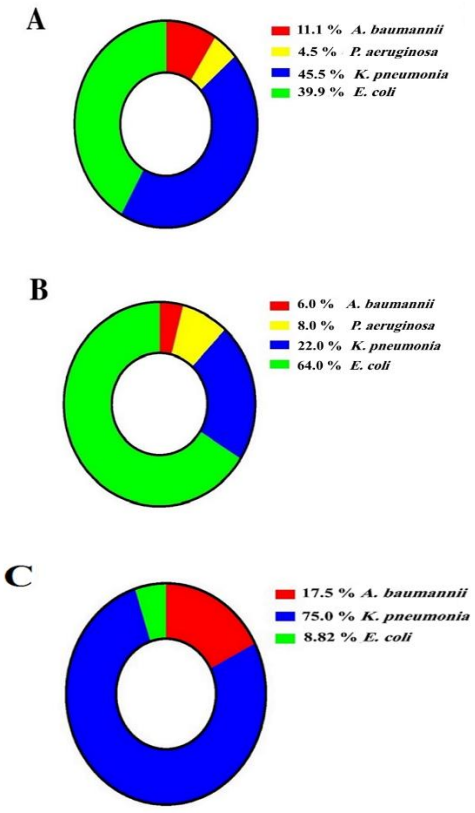

Fig. 1. The prevalence of A. baumannii isolates among Isolated clinical samples. (**A**) the most common prevalent bacterial isolates from each corresponding clinical samples; (**B**) Isolates from urine samples; and (**C**) respiratory tract isolates.

Table 1. Data and patients characteristics for examined clinical samples

Patient character		Male		Female	
		No = 39	43.3%	No = 51	56.66%
Age (year)	> (30)	5	12.82	15	29.41
	between (60 – 30)	15	38.46	29	56.86
	< (60)	19	48.71	7	13.72
Prevalence of underlying disease					
Diabetes mellitus		5	10.63	5	9.8
Diabetic and Hypertension		8	21.3	16	31.37
Respiratory disease		2	6.38	6	11.76
Diabetic and respiratory disease		5	10.63	9	17.64
Diabetic with respiratory disease and hypertension		6	17.02	7	13.74
Patients with elevated liver function test		4	10.63	6	11.76
Patients with elevated kidney function test		7	19.16	2	4.1
Catheter presence		2	10.63	5	9.8

Table 2. The incidence of Acinetobacter baumannii isolates among examined clinical samples

No. of specimen	Type of sample	No. of isolates			
		Acinetobacter baumannii	*Pseudomonas aeruginosa*	*Klebsiella pneumonia*	*Escherichia coli*
Urine	50	3 (6.0 %)	4 (8.0 %)	11 (22.0%)	32 (64.0%)
Respiratory	40	7 (17.5 %)	0 (0.0 %)	30 (75.0%)	3(8.82 %)
Total No. of isolates	**90**	**10 (11.1%)**	**4 (4.5%)**	**41 (45.5%)**	**35 (39.9%)**

Minimum inhibitory concentration MIC (mm)

Ten isolates of *A. baumannii* (11.1%) were recovered from sputum samples (n= 7; 70%), and urine (n = 3; 30%). Antibiotic resistance of *A. baumannii* represented by (Table 3; Figure 2) against 15 antibiotics with different disc potency, was determined that all *A. baumannii* isolates were resistance to Ticarcillin, Ticarcillin/Clavulanic acid, Piperacillin, Piperacillin/Tazobactam, Cefotaxime, Cefepime, Levofloxacin, Ciprofloxacin and Amikacin. While 80 % are resistant to Gentamicin, Tobramycin respectively, 70 % are against Imipenem and Meropenam. Resistance decreased to 60% with Trimethoprim/Sulfamethoxazole and 40 % with Minocycline respectively. For out of 10 *A. baumannii* isolates (codes A4, A7, A9 and A10) were found to share the same phenotype and antibiotic pattern profile. Hence, these isolates were excluded. The rest isolates that showed different phenotype and antibiotic pattern are A1, A2, A3, A5, A6 and A8 were considered for further experiments.

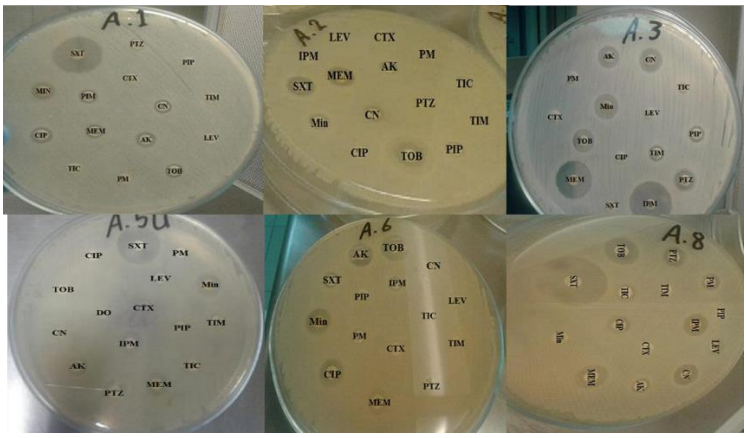

Fig. 2 . Antibiotic susceptibility test of *Acinetobacter baumannii*.

*__TIC__, Ticarcillin: **TIM**, Ticarcillin/Clavulanic Acid: **PIP**, Piperacillin: **PTZ**, Piperacillin/ Tazobactam: **CTX**, Cefotaxime: **PM**, Cefepime: **IPM**, Imipenem: **MEM**, Meropenam: **AK**, Amikacin: **CN**, Gentamicin: **TOB**, Tobramycin: **LEV**, Levofloxacin: **CIP**, Ciprofloxacin: **Min**, Minocycline: **CT**, Colistin **SXT**, Trimethoprim/Sulfamethxazole. **A1**: **A8** are the *A.baumannii* isolates code. **A1** and **A2** for urine isolates, sample no: **A3**, **A5**, **A6** and **A8** for respiratory isolates.

Table 3. Minimum inhibitory concentration MIC (mm).

Antibiotics used*	Isolates No.*									
	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10
TIC	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)
TIM	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)
PIP	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)
PTZ	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)
CTX	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)
PM	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)	0(R)
IPM	0 (R)	0 R)	26(S)	23(S)	0(R)	0(R)	0(R)	0(R)	0(R)	19(S)
MEM	0(R)	12(R)	15(I)	0(R)	15(I)	0(R)	0(R)	16 (I)	0(R)	0(R)
AK	9(R)	0(R)	0(R)	0(R)	0(R)	19(S)	0(R)	0(R)	0(R)	0(R)
CK	0(R)	0(R)	14(I)	0(R)	0(R)	0(R)	0(R)	14(I)	0(R)	0(R)
TOB	0(R)	0(R)	0 (R)	0(R)	0(R)	16(S)	0(R)	16(S)	0(R)	0(R)
LEV	0(R)	0(R)	0 (R)	0(R)	0(R)	16(S)	0(R)	16(S)	0(R)	0(R)
CIP	0(R)	0(R)	0 (R)	0(R)	0(R)	0 (R)	0(R)	0 (R)	0(R)	0(R)
Min	0(R)	0(R)	21(S)	23(S)	12(I)	22(S)	0(R)	0(R)	14(I)	23(S)
SXT	23(S)	0(R)	0(R)	0(R)	26(S)	0 (R)	0(R)	27(S)	0(R)	19(S)

Gas Chromatography-Mass Spectrometry (GC/MS) Analysis

The main bioactive components obtained by GC-MS analysis of *C. longa* aqueous extract, revealed the presence of 26 compounds, were identified and accounted for 95.78 % of the total area. The mean compounds identified was Turmerone (55.42%). While intermediate compound was B-myrcene (8.12%), and D-Limonene (8.04%). The minor compounds identified in *C. longa* aqueous extract were p-cymene (4.14%), and other compounds were presented in (Table 4; Figure 3). Ethanolic extract were identified to possesses 18 signal peaks for 18 compounds present and accounted for 99.81% of the total. Turmerone (59.42%), alpha pinene (12.07%) and Citral (11.68%), were identified as a major compound. Ethylacetate extract, illustrated the presence of 24 compounds, were accounted 97.58 % of the total area obtained by analysis of *Curcuma longa* extract. Caryophyllene (16.66%), Caryophyllene oxide (15.62%), (-)-isolongifolol (12.14%), Curdione (11.0%), B-Sesquiphellandrene (10.47%), and identified as a major compound.

Table 4. Bioactive components identified by GC-MS of *Curcuma longa* extract *

Aqueous extract					
No	RT* (min)	Compound Name	M. formula*	M. wt*	Area (%)
1	5.33	β-pinene	$C_{10}H_{16}$	136	1.55
2	7.28	B-myrcene	$C_{10}H_{16}$	136	8.12
3	9.08	D-Limonene	$C_{10}H_{16}$	136	8.04
4	13.74	eucalyptol	$C_{10}H_{18}O$	154	1.89
5	14.42	Linalyl propionate	$C_{13}H_{22}O_2$	210	0.74
6	16.08	Cubebenes	$C_{15}H_{24}$	204	0.20
7	16.67	Decanoic acid, methyl ester	$C_{11}H_{22}O_2$	185	0.18
8	17.12	beta – basbolene	$C_{15}H_{24}$	204	0.66
9	17.79	Beta - elemene	$C_{15}H_{24}$	204	1.01
10	18.54	Phytol ketone	$C_{18}H_{36}O$	268	0.10
11	21.10	beta -sesquiphellandrene	$C_{15}H_{24}$	204	6.80
12	21.19	Beta -farnesene	$C_{15}H_{22}$	202	0.68
13	22.03	Curcumene	$C_{15}H_{24}$	204	1.42
14	22.23	Calarene epoxide	$C_{15}H_{24}O$	220	0.16
15	23.75	Farnesol (E), methyl ether	$C_{16}H_{28}O$	236	0.47
16	24.32	Globulol	$C_{15}H_{26}O$	222	0.14
17	24.81	Geranyl-2-methylbutyrate	$C_{15}H_{26}O_2$	238	1.76
18	26.20	Cubenol	$C_{15}H_{26}O$	222	0.15
19	28.78	Caryophyllene oxide	$C_{15}H_{24}O$	220	2.01
20	30.15	trans-Valerenyl acetate	$C_{17}H_{26}O_2$	262	0.17
21	30.41	Corymbolone	$C_{15}H_{24}O_2$	236	0.94
22	34.84	Turmerone	$C_{15}H_{22}O$	218	55.42
23	36.35	Eupatoriochromene	$C_{13}H_{14}O_3$	218	0.76
24	37.13	Phytol	$C_{20}H_{40}O$	296	0.20
25	42.93	Anthraquinone	$C_{14}H_8O_2$	208	1.6
26	53.48	Heptacosane	$C_{27}H_{56}$	380	0.65
Total 95.78					

Table 4 (Continued). Bioactive components identified by GC-MS of *Curcuma longa* extract *

Ethanolic extract					
NO	**RT* (min)**	**Compound Name**	**M. formula***	**M. wt***	**Area (%)**
1	17.79	Limonene	$C_{10}H_{16}$	136	0.26
2	18.04	alpha phellandrene	$C_{10}H_{16}$	136	0.44
3	19.54	alpha-pinene	$C_{10}H_{16}$	136	12.07
4	20.14	Eucalyptol	$C_{10}H_{16}$	136	3.44
5	21.01	Citral	$C_{10}H_{16}O$	152	11.68
6	26.07	Terpinen-4-ol	$C_{10}H_{18}O$	154	2.02
7	26.63	Ascaridole	$C_{10}H_{16}O_2$	168	0.57
8	31.21	7-epi-cis-sesquisabinene hydrate	$C_{15}H_{26}O$	222	0.13
9	32.54	Caryophyllene	$C_{15}H_{24}$	204	0.17
10	33.74	cis-Farnesol	$C_{15}H_{26}O$	222	2.45
11	34.36	Butylated Hydroxytoluene	$C_{15}H_{24}O$	220	3.30
12	34.83	α-cedrene	$C_{15}H_{24}$	204	0.18
13	35.09	γ-curcumene	$C_{15}H_{24}$	204	0.09
14	36.82	7-epi-cis-sesquisabinene hydrate	$C_{15}H_{26}O$	222	0.97
15	37.47	Caryophyllene oxide	$C_{15}H_{24}O$	220	0.64
16	37.74	β-curcumene	$C_{15}H_{24}$	204	0.38
17	41.78	germacrone	$C_{15}H_{22}O$	218	1.60
18	42.33	Tumerone	$C_{15}H_{22}O$	218	59.42
Total 99.81					
Ethyl acetate extract					
NO	**RT* (min)**	**Compound Name**	**M. formula***	**M. wt***	**Area (%)**
1	8.48	1- dimethyl(isopropyl)silyloxy-2-phenylethane	$C_{13}H_{22}OSi$	222	0.13
2	15.67	1,8-cineole	$C_{10}H_{18}O$	154	1.44
3	25.59	alfa.-Copaene	$C_{15}H_{24}$	204	1.81
4	25.79	Zingiberene	$C_{15}H_{24}$	204	1.57
5	25.95	Corymbolone	$C_{15}H_{24}O_2$	236	0.40
6	26.73	Beta-Caryophyllene	$C_{15}H_{24}$	204	16.66
7	26.88	Ylangene	$C_{15}H_{24}$	204	0.77
8	27.11	Alloaromadendrene	$C_{15}H_{24}$	204	0.44
9	27.44	B-Curcumene	$C_{15}H_{24}$	204	2.36
10	27.68	B -Sesquiphellandrene	$C_{15}H_{24}$	204	10.47
11	28.08	agarospirol	$C_{15}H_{26}O$	222	1.67
12	28.26	B-bisabolene	$C_{15}H_{24}$	204	0.81
13	28.69	Copaene	$C_{15}H_{24}$	204	5.13
14	28.91	*Cis*-Z-a-bisabolene epioxide	$C_{15}H_{24}O$	220	3.40
15	29.20	B-CADINENE	$C_{15}H_{24}$	204	1.14
16	29.57	Cubedol	$C_{15}H_{26}O$	222	0.24
17	30.61	(-)-isolongifolol	$C_{15}H_{26}O$	222	12.14
18	30.96	Curdione	$C_{15}H_{24}O_2$	236	11.0
19	31.27	(-)-Spathulenol	$C_{15}H_{24}O$	220	6.18
20	32.55	Elemene	$C_{15}H_{24}$	204	1.62
21	33.20	cis-11-Eicosenoic acid	$C_{20}H_{38}O_2$	310	0.70
22	33.37	Caryophyllene oxide	$C_{15}H_{24}O$	220	15.62
23	35.82	6-epi-shyobunone	$C_{15}H_{26}O$	222	0.46
24	38.19	Oleic Acid	$C_{18}H_{34}O_2$	282	1.42
Total 97.58					

*RT: Retention time per minute; active compound detected by GC/MS; area (%) percentage of compound; *M. Formula: Molecular formula; *M. wt: molecular weight of the compound (g/mol).

In vitro assay for the antibacterial activity of Curcuma longa extract

Antibacterial activity of *C. longa* different extracts and Imipenem against *A. baumannii* isolates was analyzed by minimal inhibitory concentrations (MIC) by determining the bacterial viability using a colorimetric INT-formazan assay. As a result, we determined the minimal bactericidal concentrations (MBC) which confirmed the killing of *A. baumannii* isolates over time (24 hrs). The individual use of *C. longa* aqueous and/or ethyl acetate extracts against *A. baumannii* isolates (A1, A2, A3, A5, A6, and A8) exhibited MBC values

varying from 40 to 100 mg mL^{-1}, respectively in (Table 5 and figure 4). Within isolates A1 and A8, ethanol extracts revealed MBC values (40 to 90 mg ml^{-1}) respectively when compared to imipenem figure 4A, B and C. It is worth mentioning that Imipenem showed a great bactericidal efficacy against all isolates with a concentration of 0. 2 mg mL^{-1} are adequate to kill all the tested *A. baumannii* isolates.

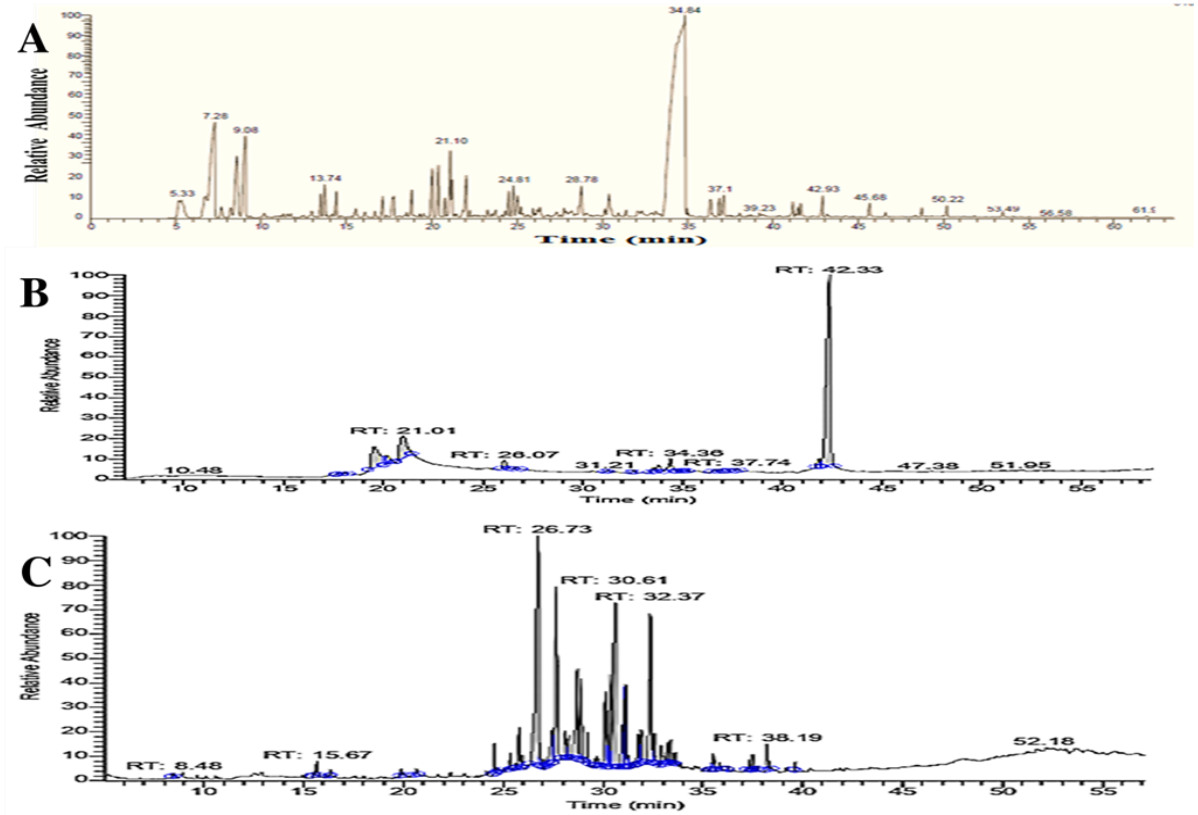

Fig. 3. Chromatogram obtained from GC/MS of *Curcuma longa*. (A): for aqueous extract, (B): for ethanolic extract and (C): for ethyl acetate extract.

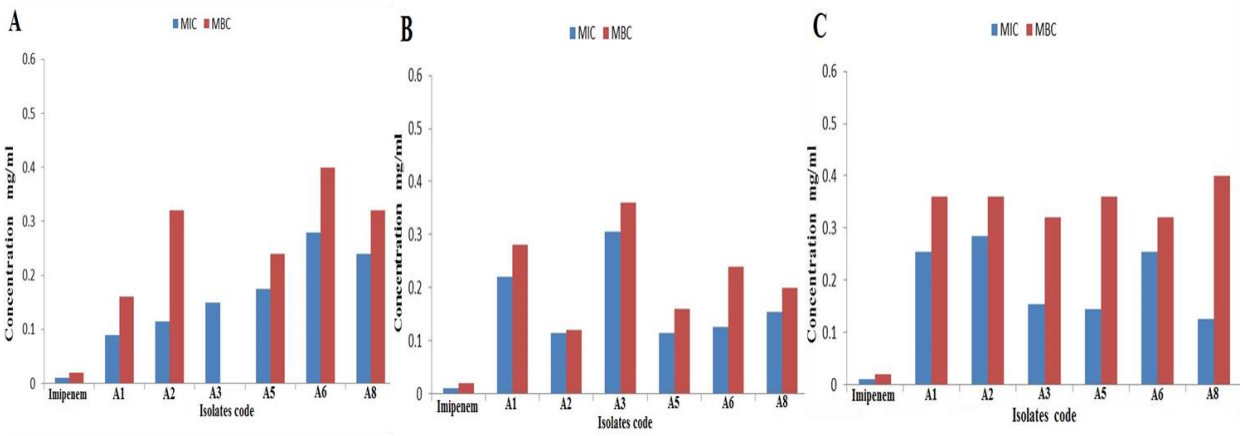

Fig. 4. MIC and MBC of Imipenem and *Curcuma longa* against *A. baumannii* isolates. MIC (blue) and MBC (red). *Curcuma longa* different extracts (100 mg ml^{-1}) (A): aqueous extract; (B): ethanolic extract; and (C): ethyl acetate extract. (Imi): Imipenem (0.512 mg ml^{-1}). A1: A8 are the *A. baumannii* isolates code. A1and A2 isolates from urine sample no: A3, A5, A6 and A8 isolates for respiratory sample.

Table 5. MIC and MBC of *Curcuma longa* extracts*.

Tested strains No.	*MIC and MBC of *Curcuma longa* aqueous extract (100 mg ml^{-1})												
	R1	**R2**	**R3**	**R4**	**R5**	**R6**	**R7**	**R8**	**MIC**	**SD**	**MIC+SD**	**MIC-SD**	**MBC**
A1	20	20	20	20	20	20	30	30	22.5	4.6291	27.1291	17.8709	40
A2	30	20	30	30	30	30	30	30	28.75	3.535534	32.28553	25.21447	80
A3	30	30	30	40	40	40	40	50	37.5	7.071068	44.57107	30.42893	100
A5	40	40	40	40	40	50	50	50	43.75	5.175492	48.92549	38.57451	60
A6	70	70	70	70	70	70	70	70	70	0	70	70	100
A8	60	60	60	60	60	60	60	60	60	0	60	60	80
Tested strains No.	MIC and MBC of *Curcuma longa* ethanolic extract (100 mg ml^{-1})												
	R1	**R2**	**R3**	**R4**	**R5**	**R6**	**R7**	**R8**	**MIC**	**SD**	**MIC-SD**	**MIC+SD**	**MBC**
A1	50	50	50	60	60	60	60	50	55	5.345225	49.65478	60.34522	70
A2	20	30	30	30	30	30	30	30	28.75	3.535534	25.21447	32.28553	30
A3	70	70	70	80	80	80	80	80	76.25	5.175492	71.07451	81.42549	90
A5	20	30	30	30	30	30	30	30	28.75	3.535534	25.21447	32.28553	40
A6	30	30	30	30	40	30	30	30	31.25	3.535534	27.71447	34.78553	60
A8	30	40	40	40	40	40	40	40	38.75	3.535534	35.21447	42.28553	50
Tested strains No	MIC and MBC of *Curcuma longa* ethylacetate extract (100 mg ml^{-1})												
	R1	**R2**	**R3**	**R4**	**R5**	**R6**	**R7**	**R8**	**MIC**	**SD**	**MIC-SD**	**MIC+SD**	**MBC**
A1	60	60	60	60	60	70	70	70	63.75	5.175492	58.57451	68.92549	90
A2	60	70	70	70	70	70	80	80	71.25	6.408699	64.8413	77.6587	90
A3	30	40	40	40	40	40	40	40	38.75	3.535534	35.21447	42.28553	80
A5	30	30	30	40	40	40	40	40	36.25	5.175492	31.07451	41.42549	90
A6	60	60	60	60	60	70	70	70	63.75	5.175492	58.57451	68.92549	80
A8	20	30	30	30	30	30	40	40	31.25	6.408699	24.8413	37.6587	100
Tested strains No	MIC and MBCof Imipenem (512 mg ml^{-1})												
	R1	**R2**	**R3**	**R4**	**R5**	**R6**	**R7**	**R8**	**MIC**	**SD**	**MIC-SD**	**MIC+SD**	**MBC**
A1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.035	0.147	0.077	0.2
A2	0.1	0.1	0.11	0.1	0.1	0.1	0.1	0.1	0.1	0.003	0.104	0.097	0.4
A3	0.1	0.1	0.1	0.1	0.1	0.11	0.1	0.1	0.1	0.003	0.104	0.097	0.2
A5	0.2	0.2	0.2	0.2	0.2	0.2	0.22	0.2	0.2	0.007	0.209	0.195	0.2
A6	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.11	0.1	0.003	0.104	0.097	0.4
A8	0.1	0.1	0.1	0.1	0.1	0.1	0.11	0.1	0.1	0.003	0.104	0.097	0.2

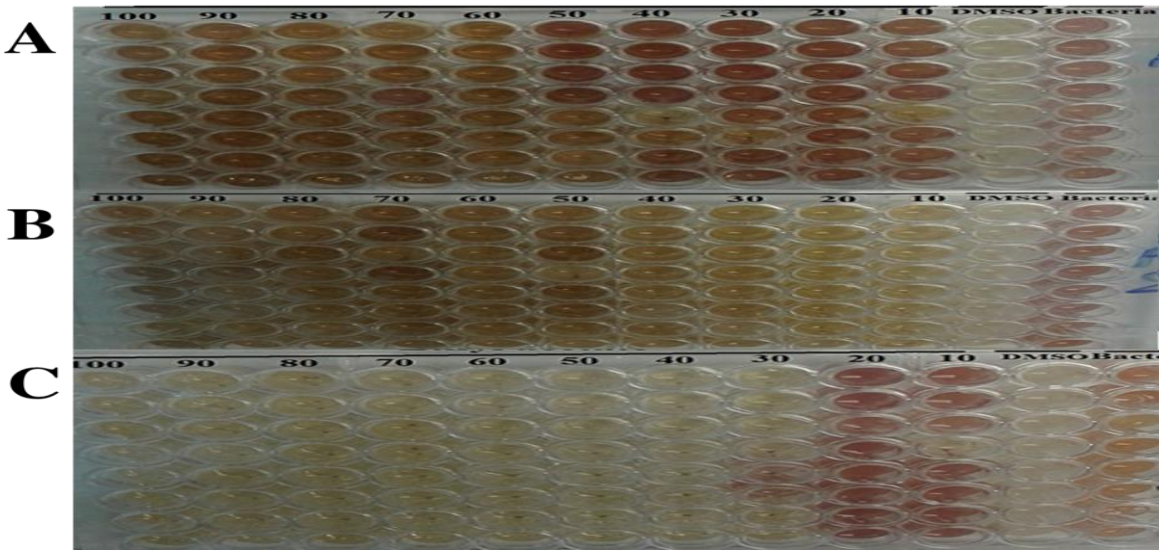

Fig. 5. MIC of *Curcuma longa* extract against *A. baumannii* isolates.(A):for aqueous extract; (B):for ethanolic extract; (C): for ethyl acetate extract. A1: A8 are the *A. baumannii* isolates code. A1and A2 isolates from urine sample no: A3, A5, A6 and A8 isolates for respiratory sample.

Discussion

Antibiotic-resistance risks in particular of nosocomial infections with *A. baumannii*, *P. aeroginosa* are increasing and treatment of bacterial infection especially multi-drug resistant become the major of concern (Ibrahim *et al*., 2021). The current study showed the proportion of nosocomial infections due to the gram-negative bacilli *A. baumannii* isolated from clinical samples as urine (6.0%), and sputum (17.5%) among the patients (Figure 1). A total 90 patient were included, categorized to male 43.12%, female 56.88% patients.

Demographic data, risk factors for *A. baumannii* (Table 1). *A. baumannii* are a common cause of urinary tract infections (UTI) that upset the kidney, leading to pyelonephritis, as well as the bladder, resulting in cystitis (Thomson & Armitage, 2010). UTI symptoms included elevated kidney function and high levels of *A. baumannii* in the patients' sputum (70%). This result is conceivable since UTI symptoms are not a reliable indication of illness, the presence of bacteria must be confirmed by urine culture in order to diagnose UTI (Elamary *et al*., 2020). The urine culture in our results indicated the presence of bacteriuria in 70% of female patients and 65% of male patients (Table 1). The percentage of *A. baumannii* in our study agreed with (Al-Agamy *et al*., 2014).The incidence of *A. baumannii* isolates among examined clinical samples was (10/110; 9.09%), *K. pneumonia* was the predominant with (49/110; 44.5%), *E. coli* (46/110; 41.8%) and *P. aeruginosa* (5/110; 4.5%) (Fouad *et al*., 2013; Sarangi *et al*., 2017). The highest prevalence reported for *A. baumannii* isolates (Table 3; Figure 1) were recovered from sputum samples (7/40; 17.5%), followed by urine (2/50; 4.0%) and pus (1/20; 5.0%) (Abdulzahra *et al*., 2018).The antibiotic susceptibility test for *A. baumannii* isolates indicated that all isolates (n = 10; 100%) were multi-drug resistant to Ticarcillin, Ticarcillin/Clavulanic Acid, Piperacillin, Piperacillin/Tazobactam, Cefotaxime, Cefepime, Ciprofloxacin, and Amikacin, while 70–80% are extensively drug-resistant. Resistance decreased to 40% with Minocycline and all isolates were sensitive to Colistin (Figure 2). Our findings are in covenant with earlier results concerning the MDR isolates (Younis *et al*., 2019). Bactericidal activity of *C. longa* A very promising the best result were obtained with ethanolic extract with average MBC (43.12 mg ml-1), aqueous and ethyl acetate extract of *C. longa* (43.75 – 50.83 mg ml-1) (Table 5) (Ahmed *et al*., 2021).

(GC-MS) of *C. longa* extract (Table 4; Figure 3A, B and D) revealed the presence of 68 compounds, turmerone (55.42%), B-myrcene (7.45%), P-cymene (4.14%), D-limonene (3.9%), β-sesquiphellandrene (2.19%). Aromatic turmerone and turmerone ($C_{15}H_{20}O$ and $C_{15}H_{22}O$), were identified as two major sesquiterpenes ketonic compound responsible for the aroma of turmeric by (Nair, 2019).

Antibacterial activity of *C. longa*, aromatic turmerone and turmerone were reported together (Negi *et al*., 1999; Lee *et al*., 2011). The minor compounds identified in our results p-cymene (4.14%), 1,8-cineole (1.05%), β-pinene (0.84%) and cis-sabinol (0.27%) agreed with (Jayaprakasha *et al*., 2005). Antibacterial activity of P-cymene, 1, 8-cineole, β-pinene and cis-sabinol against E.coli isolated from UTI patients were reported by (Lagha *et al*., 2019). Moghaddam *et al.* (2018) were reported antimicrobial activity of β-ocimene, other compound present in (Table 4) of Ferulago angulata essential oil. The major compounds identified by GC-MS of *C. longa* ethyl acetate extract were caryophyllene, caryophyllene oxide, (-)-isolongifolol, curdione, B-sesquiphellandrene (Chowdhury *et al*. 2008). Rania *et al*. (2002) and Naz *et al.* (2010) reported Caryophyllene, susquiphellandrene as a major constituent of *C. longa* rhizome. Mehra & kumar. (2019) were reported antibacterial activity of compounds (-)-Spathulenol, Copaene, Cis-Z-a-bisabolene epioxide, β-curcumene, alfa-copaene agarospirol, zingiberene, 1,8-cineole and oleic acid in methanol extract of *C. longa*. Moo *et al*. (2020) revealed antibacterial activity β-caryophyllene against *Bacillus cereus* due to changing in membrane permeability and integrity leading to membrane damage and intracellular content leakage, which eventually caused cell death. Santos *et al.* (2019) regarded the mechanism of bactericidal of limonene, caryophyllene oxide, citral, trans caryophyllene, and 1, 8-cineole to the direct damage caused to the integrity of the cell membrane by the lipophilic components of the essential oil. Woo *et al.* (2020) revealed the inhibitory effects of β-caryophyllene on *Helicobacter pylori* infection because of interruption of bacterial replication via down regulation genes by β-caryophyllene.

Conclusion

Nosocomial infection with *A. baumannii* has increased year by year in human medicine and it is also considered as a major cause of mortality worldwide, so there is a need to decrease the prevalence of nosocomial infections while improving the healthcare quality and patient safety. Although ethanol or/aqueous extract of *C. longa* was promising for the in vitro study against *A. baumannii*. GC/MS of C. longa reveal many active compounds that may act as antibacterial agent against MDR *A. baumannii*.

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