Efficacy of Aqueous, Ethanolic and Ethyl acetate Extracts of *Curcuma longa* in the Treatment of Multidrug-Resistant *Pseudomonas aeruginosa* Clinical Isolates Associated with Urinary Tract Infections

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Abstract: The present study aimed to investigate the effect of *Curcuma longa* extracts on the drug-resistant *Pseudomonas aeruginosa* clinical isolates. One hundred urine samples were collected from Qena and Nagaa Hammadi General Hospitals in Upper Egypt. Samples were screened for the prevalence of UTI pathogens by biochemical tests and antibiotics sensitivity. The incidence of the isolated urinary tract infection pathogens in the urine sample was 35% in males and 65% in females patients, represented by age under 30 years (10.77 and 14.29%), from 30 to 60 years (81.54 and 68.57%), and more than 60 years (7.69 and 17.14%) for females and males, respectively. The clinical isolates were *Escherichia coli* (36%), *Klebsiella pneumonia* (25%), *Pseudomonas aeruginosa* (14%), *Proteus mirabilis* (12%), *Enterobacter cloacae* (9%), and *Acinetobacter baumanii* (4%). The ethyl acetate and ethanolic extracts of *Curcuma longa* showed varying degrees of antibacterial activities against tested clinical isolates ranging from 7 to 18 mm. Further, major compounds of Hydroquinine (47.55%) , Quinine (35.12%) , o-acetyl-L-serine(6.03%), Copaene (37.39%) , Neophytadiene (6.80%) , Phytol, acetate (6.16%), Phytol (38.51%) , 18- Norabietane (5.35%) were determined among 29 bioactive compounds in the aqueous, ethanolic and ethyl acetate extracts of *Curcuma longa* using gas chromatography-mass spectrometry (GC-MS). These results indicated that the aqueous, ethanolic and ethyl acetate extracts of *Curcuma longa* have promising antibacterial efficacy against multidrug-resistant bacteria and can effectively deal with the Urinary tract infections.

Keywords: *Curcuma longa*, *Pseudomonas aeruginosa*, Turmeric.

Introduction

In the last two decades, the prevalence of multidrug-resistant bacteria has risen considerably. In developing countries, a urinary tract infection (UTI) is one of the most common infections, accounting for more than 35% of hospitalized patients (Ganesh *et al., 2019*). It is more prevalent in sexually active females and rises in people with diabetes, sickle cell disease, or a urinary tract anatomical deformity; other causes of UTI include a swollen prostate gland in men and pregnant women In addition, patients with an indwelling bladder catheter are more susceptible to bacteriuria and urinary tract infections (Mandell *et al.*, 2005). Family Enterobacteriaceae, which includes *Escherichia coli* (which accounts for 77 percent of UTIs), *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Acinetobacter* spp., *Proteus* spp., and *Staphylococcus aureus*, is the most common pathogen found, accounting for the majority of UTIs. (Mansour *et al.*, 2009; Belete *et al.*, 2020). *Pseudomonas aeruginosa* is a multidrug-resistant nosocomial bacterium that has emerged as an emerging opportunistic nosocomial disease (Negi *et al.*, 2014). Treatment of infections caused by Gram-negative pathogens such as *Pseudomonas aeruginosa* is difficult due to the high intrinsic multidrug resistance (Venter *et al.*, 2015). *P. aeruginosa* can form a "biofilm" that protects it from drugs and immune cells, and it is the third most prevalent bacterium associated with urinary tract infections after *E. coli* and *Enterococci* (Cole *et al.*, 2014), and as a result, it has joined the ranks of superbugs, due to its extensive potential for resistance (Breidenstein *et al.*, 2011). New antimicrobial agents,

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on the other hand, have yet to be able to solve this problem. As a result, we must quickly develop new efficient methods to combat the increasing rate of drug-resistant mortality infections (Venter *et al*., 2015). Several investigations on the antimicrobial activity of herbal and plant extracts against drug-resistant pathogens have been carried out to improve the safety of medicinal medications. Alternative sources of natural bioactive chemicals from medicinal plants are being studied to replace the traditional antibiotics and synthesize antimicrobial compounds (Amer *et al*., 2006; Liu *et al*., 2019). More than 80% of people worldwide, mostly in developing countries, employ numerous plant extracts and active compounds as traditional medicine in conventional pharmaceuticals, according to the World Health Organization (Kirbağ *et al*., 2009). Several studies have shown that fragrant herbs including (*Cinnamomum zeylanicum*, *Origanum vulgare*, *Syzygium aromaticum*, *Symus vulgaris* and *Curcuma longa* have antibacterial, antiviral, antifungal, and anticarcinogenic properties (El-Saber Batiha *et al*., 2020).

Materials and Methods

Collecting samples, Data, and processing

During the period from January 2018 to January 2019, one hundred urine samples were collected from Nagaa Hammadi general hospital and Qena general hospital, Upper Egypt. The samples were taken from patients between the ages of 6 and 81 years symptomatic with inflammation in the joints, high body temperature, chills, nausea, vomiting, diarrhea, mucus, and a urethra open wound that required treatment urinary catheters and other invasive devices. Each patient who had not received antibiotics in the previous 5 days had clean catch midstream urine specimens were collected into the sterile screw container from each patient who had not received antibiotics within the last 5 days. Every specimen was clearly identified, labeled and transported to the microbiology laboratory on dry ice for additional processing. Guidelines for urine physical condition the pH of the samples, as well as their specific gravity, were determined (Ahmed *et al*., 2021).

Isolation and Identification of UTI Pathogens

Collected urine samples were streaked onto sterile blood agar medium then plates were incubated at 37°C for 24 hrs. The obtained pure colonies were subcultured onto nutrient agar media and identified biochemically by Vitek 2 system.

Antimicrobial susceptibility testing

The 0.5 McFarland bacterial suspensions were diluted to $1.5* 10^7$ CFU/mL in 0.45 % saline. Cards were automatically filled, sealed, and packed into the VITEK 2 system for incubation and reading. The tested antibiotics Ticarcillin (TIC), Ticarcillin/Clavulanic Acid (TIM), Piperacillin (PIP), Piperacillin/Tazobactam (TZP), Ceftazidime (CAZ), Cefepime (FEP), Imepenem (IP), Meropenem (MP), Amikacin (AK), Gentamicin (CN), Tobramycin (TOB), Ciprofloxacin (CIP), and Colistin were used (Gokale & Metgud, 2012; Kirthilaxmi & Benachinmardi, 2014).

Preparation of Plant Extracts

In this study: *Curcuma longa* (turmeric) was collected from commercial sources in Nagaa Hammadi, Qena, Egypt. In order to obtain a homogenous powder, the collected plant materials were washed with sterile water and further dried and then ground before the extraction. We use multiple solvents including aqueous, absolute ethanol, and ethyl acetate to extract the bioactive compounds from medicinal plant *Curcuma longa*. The dried form plant was soaked separately with sterile distilled water, ethanol, and ethyl acetate (100 g in 1 L solvent) for 7 days and extracted by maceration. The obtained extracts were filtered through Buchner funnel with Whatman No.1 filter paper and evaporated by a rotary evaporator (BUCHI R-114, Switzerland) under reduced pressure to dryness at 45°C. The plant crude extracts were stored at 4°C until use. All extracts were re-dissolved in dimethyl sulfoxide (DMSO) except the aqueous extract, which re-dissolved in sterile distilled water at a concentration of 200 mg/mL. Before using bioassay, the reconstituted extract solutions were sterilized by micron syringe filters (0.45μm). (Ahmed *et al*., 2021).

Antibacterial Screening for the Effectiveness of Selected Plants

The antibacterial activity was determined by the standard disc diffusion method as described previously (Yassin *et al*., 2020). Overnight cultures of *P. aeruginosa* clinical isolates (5×10^4 spores/mL) were picked up by sterile swab sticks and streaked on the top of the solid media and allowed to dry completely for 20 min. The plant extract stock concentration (100 mg/mL) was prepared by dissolving the extract in diluted dimethyl sulfoxide (10% DMSO) and sterile filtered through a 0.2 μm pore syringe filter. Sterile Whatman No. 1 filter paper discs of 6 mm diameter were impregnated with each plant crude extract and discs were stored at 4°C before use. Extract-impregnated discs (20 μL) were placed on agar plates and incubated for 24 h at 37°C.

Pure 10% DMSO (20 μL) was used as a negative control, while colistin (10 mg/disc) was used as a positive control. Then, antibacterial activity was determined by measuring the diameter of inhibition

zones in millimeters (mm) against the test bacterial isolates. The experiments were performed in triplicate and the mean values were noted (Yassin *et al.*, 2020).

Determination of the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Overnight prepared cultures of *P. aeruginosa* clinical isolates were adjusted to OD600 of 0.5 McFarland, and 100 μL of each bacterial culture was taken into sterilized 96-well microplate (Salem *et al.*, 2017). Then, 20 μL of the most active extract was added where ten different concentrations were prepared (10^{-1} to 10^{-10}). The 96-well microplate was incubated for 24 h at 37°C. MIC was determined by the addition of 40 μL of (INT) (0.2 mg/mL, Sigma-Aldrich) to the microplate wells and reincubated for 30 min at 37°C; colistin (20%) was used as a positive control. MIC was defined as the lowest concentration at which colour changes and MBC was determined as previously described (Lall *et al.*, 2013; Sirelkhatim *et al.*, 2015).

GC-MS Determination of Bioactive Compounds

The selected active solvent extract was dissolved in methanol (100%) and dehydrated with anhydrous sodium sulphate and then filtered through a syringe filter (0.45 μm pore size) before injection. A Trace GC1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) was used for the chromatographic analysis, and the compounds were separated with a direct capillary column TG–5MS (30 m × 0.25 mm × 0.25 μm film thickness). The column temperature was initially 50°C and then increased by 5°C/ min to 230°C with holding 2 min and then increased to 290°C at 10°C/min. ,e injector and MS transfer line temperatures were kept at 250 and 260°C, respectively. Helium was used as a carrier gas at a stable flow rate of 1 mL/ min. The solvent delay was 3 min, and a diluted sample of 1 μL was injected automatically using an autosampler AS1300 coupled with GC in the split mode. EI mass spectra were generated at an ionisation voltage of 70 eV with a mass scan of 40–1000 amu. The ion source temperature was set at 200°C.The extract components were identified by comparison of their retention times and mass spectra with those of WILEY 09 and NIST 11 mass spectral database.

Results

Prevalence of UTI associated bacteria

The incidence of isolated urinary tract infection pathogens in urine samples was recorded from 35 percent males and 65 percent females patients, represented by age under 30 years (10.77 and 14.29 percent), 30 to 60 years (81.54 and 68.57 percent), and more than 60 years (7.69 and 17.14 percent) for females and males, respectively (Table1). All the 100 urine samples that have been properly collected were positive for bacterial growth. The most common predominant organism was *E. coli* (36%), followed by *K. pneumoniae* (25%), *P. aeruginosa* (14%), *Proteus mirabilis* (12%), *Enterobacter cloacae* (9%), and *Acinetobacter baumannii* (4%) (Figure1). The total aerobic bacterial count in those samples ranged from 1.9 X 10^6 to 2.1 X 10^6 CFU/mL, and *P. aeruginosa* isolates (P1 to P14) were biochemically identified and chosen for further study.

Table1. Prevalence of different clinical isolates in urine samples represented in their gender type and age

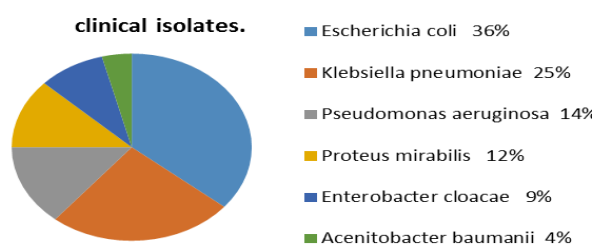| Patient character	Female samples		Male samples	
	65%		35%	
Age (years)				
Under 30 years	7	10.77%	5	14.29%
From 30 to 60 years	53	81.54%	24	68.57%
More than 60 years	5	7.69%	6	17.14%

Figure1. The diversity of *P. aeruginosa* among other bacteria in clinical isolated specimens.

The biochemical tests were also performed, and the results obtained after 10 hurs, and all *P. aeruginosa* isolates were correctly identified (96.8%). Among tested bacteria the VITEK 2 had low discrimination reached up to 6.4%.

Minimum inhibitory concentration (MIC) by Vitek 2 system

The *P. aeruginosa* isolates were validated by VITEK-2 full-automatic bacteria analyzer. Drug resistance, sensitive antibiotics and minimum inhibitory concentration (MIC) were studied. The results showed that all *P. aeruginosa* isolates were resistance to Ticarcillin, Ticarcillin/Clavulanic acid, Piperacillin, Cefepime and Ciprofloxacin, while 90% were resistant to Pipracillin/Tazobactam, Ceftazidime, Gentamicin, Tobramycin and 80% were resistant to Imipenem, Meropenam, Amikacin. Notably, all isolates were sensitive to Colstin (Table 2).

Antibacterial screening for the effectiveness of selected Curcuma longa plant extracts

The antibacterial activity of *Curcuma longa* plant was

investigated against selected MDR *P. aeruginosa* using the disc diffusion method (Table 3). The results revealed that two plant extracts were potentially effective in suppressing *P. aeruginosa* growth with variable potency depending on the concentration of the extract and type of solvent. The ethyl acetate and ethanolic extracts of *C. longa* showed varying degrees of antibacterial activities against tested clinical isolates ranging from (7 – 18 mm). DMSO (10% conc.) was used as negative control, while colistin (10 mg/disc) was used as positive control. Results of antimicrobial activity of *C. longa* showed strong antibacterial activity against selected MDR strains. Hence, experiments were conducted to determine their minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) against *P. aeruginosa* bacterial strains. Moreover, chemical analysis of *C. longa* active extract was performed.

Table 2. MIC (mg/L) of *P.aeruginosa* isolates determined by Vitek 2 system.

Antimicrobial agents (µg)		TIC	TIM	PIP	TZP	CAZ	PM	IPM	MEM	AK	CN	TOB	CIP	CT
P1	MIC (mg/L)	128	128	128	128	64	64	16	16	64	16	16	4	0.5
	Indicate	R	R	R	R	R	R	R	R	R	R	R	R	S
P2	MIC (mg/L)	128	128	128	128	64	64	16	16	64	16	16	4	0.5
	Indicate	R	R	R	R	R	R	R	R	R	R	R	R	S
P3	MIC (mg/L)	128	128	128	128	64	64	16	16	64	16	16	4	0.5
	Indicate	R	R	R	R	R	R	R	R	R	R	R	R	S
P4	MIC (mg/L)	128	128	128	64	64	16	16	64	16	16	16	4	0.5
	Indicate	R	R	R	R	R	R	R	R	R	R	R	R	S
P5	MIC (mg/L)	128	128	128	64	64	64	16	16	64	16	16	4	0.5
	Indicate	R	R	R	R	R	R	R	R	R	R	R	R	S
P6	MIC (mg/L)	128	128	128	64	64	64	16	16	64	16	16	4	0.5
	Indicate	R	R	R	R	R	R	R	R	R	R	R	R	S
P7	MIC (mg/L)	128	128	128	64	64	64	16	16	64	16	16	4	0.5
	Indicate	R	R	R	R	R	R	R	R	R	R	R	R	S
P8	MIC (mg/L)	128	128	128	16	64	16	2	4	8	16	16	4	0.5
	Indicate	R	R	R	I	R	R	I	I	S	R	R	R	S
P9	MIC (mg/L)	128	128	128	64	64	16	16	64	16	16	16	4	0.5
	Indicate	R	R	R	R	R	R	R	R	R	R	R	R	S
P10	MIC (mg/L)	128	128	128	64	64	16	16	64	16	16	16	4	0.5
	Indicate	R	R	R	R	R	R	R	R	R	R	R	R	S
P11	MIC (mg/L)	128	128	128	64	64	16	16	64	16	16	16	4	0.5
	Indicate	R	R	R	R	R	R	R	R	R	R	R	R	S
P12	MIC (mg/L)	128	128	128	128	8	64	2	1	16	4	1	4	0.5
	Indicate	R	R	R	R	I	R	S	S	S	S	S	R	S
P13	MIC (mg/L)	128	128	128	128	64	64	16	16	64	16	16	4	0.5
	Indicate	R	R	R	R	R	R	R	R	R	R	R	R	S
P14	MIC (mg/L)	128	128	128	128	64	64	16	16	64	16	16	4	0.5
	Indicate	R	R	R	R	R	R	R	R	R	R	R	R	S

TIC=Ticarcillin, TIM= Ticarcillin/Clavulanic acid, PIP=Piperacillin = PIP, TZP= Pipracillin/Tazobactam, CAZ= Ceftazidime, PM= Cefepime, IPM= Imipenem, MEM=Meropenam, AK= Amikacin, CN= Gentamicin, TOB= Tobramycin, CIP= Ciprofloxacin, CT = Colistin. **S** = Sensitive, **I** = Intermediate, **R** = Resistance, *P.aeruginosa* clinical isolates (**P1-P14**).

Minimum Inhibitory Concentration (MIC) of the most active tested plants

The MIC was the lowest concentration that inhibited bacterial growth and was determined using the micro-dilution method with the help of (INT) reduction assay. The MIC values of tested plant extracts were ranged from 10 to 100 mg/mL, where the MIC of Colistin was ranged from 1 to 2 mg/mL against all the tested isolates. The MIC of aqueous, ethanolic and ethyl acetate extracts were (10 to 20, 30 to 50 and 28.75 to 71.25 mg/mL, respectively), and the aqueous extract from C. longa, showed the minimum MIC activity against bacterial isolate P13 with a MIC value of 10 mg/mL among tested isolates, while P4 showed the highest MIC value at 20 mg/mL (Table 4).

An overview of the automated microtiter 96-plates results, including tested strains and its activity against various extracts are illustrated in (Figure2).

Identification of bioactive compounds by (GC-MS)

The GC-MS analysis profile of the aqueous extract of *C. longa* showed that only 7 compounds were detected and by comparing those with entries in the NIST database, the nearest compound resembling those peaks were identified. The highly quantity detected compounds were, hydroquinine (47.55%), quinine (35.12%), o-acetyl-L-serine (6.03%). While the GC-MS analysis profile of the ethanolic extract of C. longa and its constituents illustrated that a total of 12 various compounds were detected and by comparing those with entries in the NIST database, the nearest compound

resembling those peaks were identified, as well as their molecular weight, retention time, and other characteristics. It was observed that the ethanolic extract contained majority copaene (37.39%), neophytadiene (6.80%), Phytol acetate (6.16%), 18-norabietane (5.35%), tetra adecanioc acidmethyl ester (4.51%). All the obtained compounds are known, and the peak area of each compound is directly proportional to its quantity in the extract. It was observed that the ethyl acetate extract of *C. longa* contained various types of chemical compounds including mainly: phytol (38.51%), 7-hydroxy-6-methyl-oct-3-enoic acid (26.54%). All data were presented in Table 5 and illustrated in Figure 3.

Table 3. Antibacterial screening for the effectiveness of *Curcuma longa* plant extracts (100 mg/mL) against the six clinical isolates of *P. aeruginosa*.

Clinical isolates	Tested plant "*Curcuma longa*"		
	Aq. extract	EOH. extract	E.A. extract
P4	18±0.2	9±0.1	8±0.1
Positive Control*	20±0.21		
P8	13±0.6	10±0.1	8±0.2
Positive Control	15±0.29		
P9	12±0.2	10±0.1	7±0.1
Positive Control	14±0.8		
P12	11±0.1	8±0.2	7±0.1
Positive Control	12±0.6		
P13	10±0.1	8±0.1	8±0.2
Positive Control	13±0.36		
P14	11±0.1	9±0.1	8±0.1
Positive Control	13±0.1		

*Colistin (10 mg/disc); P4–P14: selected strains; — = no activity; inhibition zones including the diameter of the paper disc (6 mm). Data are means of three replicates ($n= 3$) ± standard error. It was confirmed that 10% DMSO had no inhibitory effect on any isolate. Aq = Aqeous extract, EOH= Ethanolic extract, E.A= Ethyl acytate extract. According to the vitek-2 compact system test.three out of 14 P.aeruginosa clinical isolates codes P1, P2, P3, P5, P6, P7, P10, P11 were found to share the same phenotypic and antibiotic pattern profile, so these isolates were skipped.

Table 4. The MICs of different plant extracts against *P. aeruginosa* tested strains by disc diffusion method measured by (mm).

Tested strains No.	The MICs of Aqueous extract of *C. longa*						
	R1	R2	R3	Mean	SD	Mean+SD	Mean-SD
P4	16.9	19.1	18.0	18	0.898146	18.89815	17.10185
P8	14.8	15.2	9.0	13	2.833137	15.83314	10.16686
P9	14.1	13.2	8.7	12	2.362202	14.3622	9.637798
P12	13.3	11.7	8.0	11	2.21961	13.21961	8.78039
P13	9.6	8.1	12.3	10	1.737815	11.73781	8.262185
P14	11.9	13.2	7.9	11	2.255364	13.25536	8.744636
Tested strains No.	The MICs of Ethanolic extract of *C. longa*						
	R1	R2	R3	Mean	SD	Mean+SD	Mean-SD
P4	8.6	10.3	8.1	9	0.94163	9.94163	8.05837
P8	12.1	11.2	6.7	10	2.362202	12.3622	7.637798
P9	9.2	12.1	8.7	10	1.498888	11.49889	8.501112
P12	9.5	7.0	7.5	8	1.080123	9.080123	6.919877
P13	10	8.1	5.9	8	1.675311	9.675311	6.324689
P14	8.3	7.6	11.1	9	1.512173	10.51217	7.487827
Tested strains No.	The MICs of Ethyl acetate extract of *C. longa*						
	R1	R2	R3	Mean	SD	Mean+SD	Mean-SD
P4	9.3	8.0	6.7	8	1.061446	9.061446	6.938554
P8	9.7	7.0	7.3	8	1.208305	9.208305	6.791695
P9	5.9	6.2	8.9	7	1.349074	8.349074	5.650926
P12	6.5	9.2	5.3	7	1.630951	8.630951	5.369049
P13	9.2	6.3	8.5	8	1.235584	9.235584	6.764416
P14	5.8	10.1	8.1	8	1.756891	9.756891	6.243109

R1= first replicate, R2= second replicate, R3=third replicate, SD = standard deviation. P4-P14= tested strains.
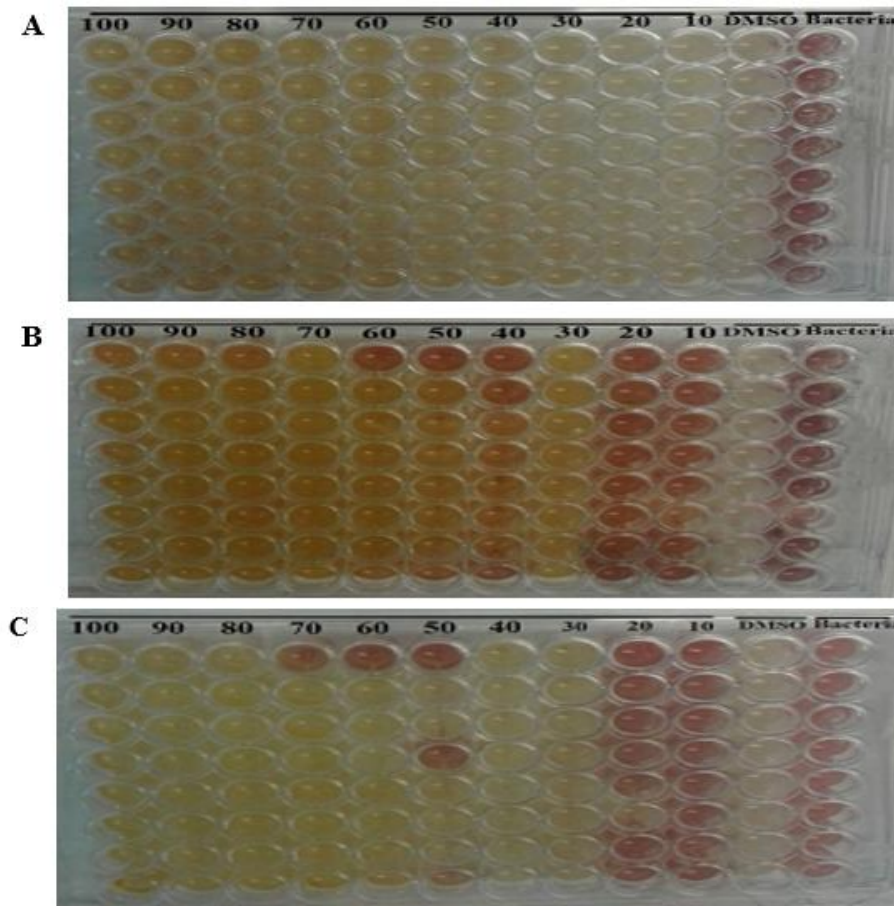
Figure 2. Effect of Aqueous , Ethanolic, and Ethyl acetate extract of *C. longa* against target *P.aeruginosa* isolates determined by 96-Microtiter plate. (A); Aqueous extract:(B); Ethanolic extract: (C); Ethyl acetate extract.

Discussion

Uncontrolled use of antimicrobial drugs to treat infections has resulted in the emergence of resistance among various microbial strains (Méndez-Vilas, 2013). Drug resistance of *P. aeruginosa* from various clinical specimens in Egypt is one of the main active pathogens, and these organisms associated with UTIs have received little attention (Wassef *et al*., 2015). From January 2018 to January 2019, one hundred urine samples were collected from patients suffering from urinary tract infection (UTI). The VITEK2 system identified the isolates as *E. coli*, *K. pneumonia*, *P. aeruginosa*, *P. mirabilis*, *E. cloacae*, and *A. baumanii* (Woo *et al*., 2000). According to the results of our urinary sample analysis, approximately 71.5 % (n = 10/14) of *P. aeruginosa* isolates were antibiotic resistant. A similar Egyptian study found that 45 percent of *P. aeruginosa* isolates were resistant to ceftazidime antibiotics (Al-Agamy *et al*., 2011). Our findings reported that all isolates were sensitive to Colistin, while 90% were resistant to Pipracillin/Tazo-bactam, Ceftazidime, Gentamicin, Tobramycin and 80% were resistant to Imipenem, Meropenam, Amikacin, 100 % of isolates were resistance to Ticarcillin, Ticarcillin/Clavulanic acid, Piperacillin, Cefepime and Ciprofloxacin.

Bioactive compounds derived from medicinal plants have been investigated for decades and have proven to be one of the most effective sources of drug treatments for bacterial infections (Gray *et al*., 2020). Many studies around the world have shown that medicinal plants and their extracts have multi-antimicrobial properties (Al-Juraifani, 2011). The current study showed that *C. longa* ethyl acetate and ethanolic extracts demonstrated varying degrees of antibacterial activity against tested clinical isolates ranging from 7 to 18 mm. *C. longa* and its sequential extracts (aqueous, ethanol, and ethyl acetate) were tested for antibacterial activity against MDR *P. aeruginosa* bacterial strains that were resistant to various antibiotics. The ethanolic extract of *C. longa* was found to be very effective against *P. aeruginosa* isolates, with a maximum inhibition zone of about 10 mm. Ahmed *et al*., (2021) found that the ethanolic extract of *S. aromaticum* has bactericidal activity against *P. aeruginosa* and bacteriostatic activity against *P. aeruginosa* clinical isolates. Other studies conducted concurrently looked into the highly effective methanolic extract of *S. aromaticum* against MDR *P. aeruginosa* demonstrated that the large variation in MIC values indicates a selective activity of the extract, which is consistent with the data obtained (Karou *et al*., 2005; Sujatha *et al*., 2011; Rath *et al*., 2014).

Table 5. Bioactive chemical components of *Curcuma longa* extracts.

	No.	Compounds	RT	Molecular Formula	Molecular weight	Area %
Aqueous extract of *C. longa.*	1	Octadecanoic acid	12.44	$C_{18}H_{36}O_2$	284	1.46
	2	o-acetyl-L-serine	15.39	$C_5H_9NO_4$	147	6.03
	3	2-(2-Furyl)-2-methoxyethanol	16.19	$C_7H_{10}O_3$	142	1.34
	4	Quinine	17.90	$C_{20}H_{24}N_2O_2$	342	35.12
	5	Hydroquinine	20.99	$C_{20}H_{26}N_2O_2$	326	47.55
	6	Mannose	24.66	$C_6H_{12}O_6$	180	1.533
	7	2-D,2-Pentadecyl-1,3-Dioxe Pane	28.52	$C_{20}H_{39}O_2$	313	0.66
Ethanolic extract of *C. longa.*	1	3-Octadecene, (E)-	11.05	$C_{18}H_{36}$	252	1.53
	2	Tetradecanoic acid, methyl ester	16.28	$C_{15}H_{30}O_2$	242	4.51
	3	Neophytadiene	21.15	$C_{20}H_{38}$	278	6.80
	4	Copaene	23.03	$C_{15}H_{24}$	204	37.39
	5	Phytol, acetate	26.84	$C_{22}H_{42}O_2$	338	6.16
	6	Isobornyl acetate	36.88	$C_{12}H_{20}O_2$	196	2.01
	7	Heptacosane	43.20	$C_{27}H_{56}$	380	0.99
	8	Celidoniol, deoxy-	51.31	$C_{29}H_{60}$	408	3.40
	9	Erucic acid	51.91	$C_{22}H_{42}O_2$	338	0.61
	10	18-Norabietane	53.56	$C_{19}H_{34}$	262	5.35
	11	Propanoic acid,2-(3-acetoxy-4,4,14-trimethylandro st-8-en-17-yl)-	57.47	$C_{27}H_{42}O_4$	430	0.93
	12	1-Docosene	62.00	$C_{22}H_{44}$	308	3.86
Ethyl acetate extract of *C. longa.*	1	17-Octadecynoic acid	5.58	$C_{18}H_{32}O_2$	280	1.55
	2	1,11-undecanediol	12.34	$C_{11}H_{24}O_2$	188	0.97
	3	Iridomyrmecin	15.12	$C_{10}H_{16}O_2$	168	3.23
	4	Ethyl oleate	16.43	$C_{20}H_{38}O_2$	310	0.84
	5	7-hydroxy-6-methyl-oct-3-enoic acid	17.67	$C_9H_{16}O_3$	178	26.54
	6	Linoleic acid ethyl ester	18.38	$C_{20}H_{36}O_2$	308	2.37
	7	Phytol	20.36	$C_{20}H_{40}O$	296	38.51
	8	8,11,14-Eicosatrienoic acid,	21.09	$C_{20}H_{34}O_2$	260	2.55
	9	Ethyl 9-hexadecenoate	23.31	$C_{18}H_{34}O_2$	282	0.57
	10	12-Heptadecyn-1-ol	24.93	$C_{17}H_{32}O$	252	0.44
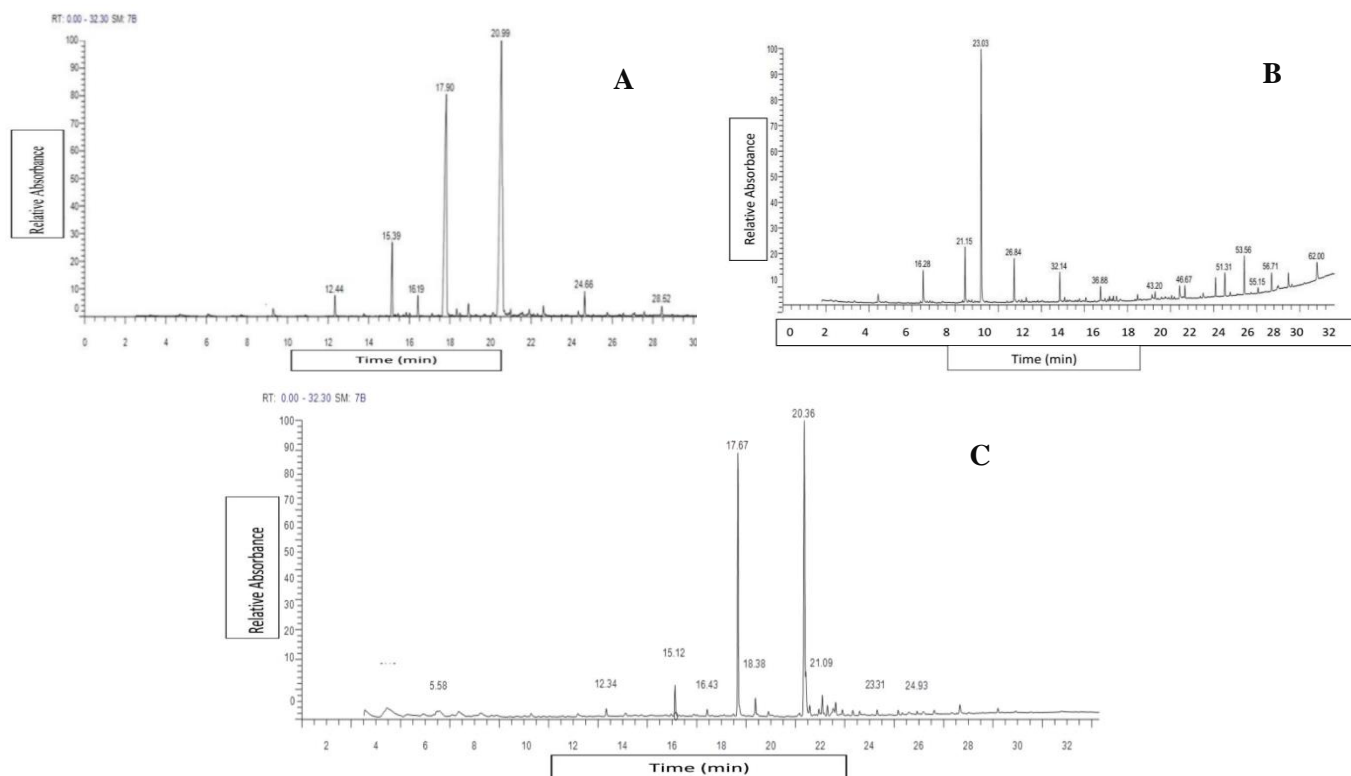
Figure 3. GC-MS chromatogram of extracts of *C. longa.* (A); aqueous extract: (B); ethanolic extract: (C); ethyl acetate extract.

Our GC/MS analysis indicated that the main compounds of *C. longa* ethanolic extract were Copaene (37.39%), While the *C. longa* ethyl acetate extract main compounds were Phytol (38.51%), 7-Hhydroxy-6-methyl-oct-3-enoic acid (26.54%). On the other hand, the aqueous extracts of *C. longa* main compounds were Hydroquinine (47.55 %) and Quinine (35.12 %) (Mansouri *et al.*, 2021; Rahman *et al.*, 2021). Other intermediate compounds are also reported to have antibacterial activity (Moo *et al.*, 2020). Measuring the response of a specific microbial pathogen to a specific antibiotic in clinical isolates is crucial. Recent evidence suggests that medicinal plants have been used against resistant microbial pathogens of clinical isolates, and that when compared to single compounds, plant extracts have more profound pharmacological activity due to their multiple mechanisms of action or synergistic interaction between the different compounds in a mixture (Livermore *et al.*, 2003).

Conclusion

Nosocomial infections with *P. aeruginosa* are increasing every day, making treatment with traditional antibiotics more complicated, so there is a need to decrease the prevalence of nosocomial infections while improving the healthcare quality and patient safety. Biomolecules generated by medicinal plants, especially *Curcuma longa*, is important, and it is important to explore novel therapeutic alternative solutions for the treatment of *P. aeruginosa* associated UTIs.

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