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RESEARCH ARTICLE

Antimicrobial activity of essential oils of *Curcuma longa* and *Syzygium aromaticum* against multiple drug-resistant pathogenic bacteria

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ABSTRACT

The present study was conducted to investigate the antimicrobial potential of essential oils of *Curcuma longa* and *Syzygium aromaticum* against multidrug-resistant pathogenic bacteria. Four identified bacterial isolates including Methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli, Klebsiella pneumoniae,* and *Acinetobacter baumannii* were selected and their antibiotic sensitivity was checked by disc diffusion assay. *C. longa* and *S. aromaticum* were subjected to steam distillation to obtain their essential oils. The crude essential oils were fractioned by employing column chromatography. Crude essential oils and their fractions were evaluated for their antibacterial activity by agar well diffusion assay and minimum inhibitory concentrations were calculated. All the selected bacterial isolates showed resistance to three or more than three antibiotic groups and were declared as multidrug-resistant (MDRs). Crude essential oils of *C. longa* and *S. aromaticum* exhibited antimicrobial activity against all selected isolates but *S. aromaticum* activity was better than the *C. longa* with a maximum 19.3±1.50 mm zone of inhibition against *A. baumannii* at 1.04 µL/mL MIC. GC/MS analysis revealed the abundance of components including eugenol, eugenyl acetate, β- caryophyllene, and α- Humulene in both crude oil and fractions of *S. aromaticum*. While the main components of *C. longa* essential oil were Ar-tumerone, α–tumerone, β- Tumerone, l-Phellandrene, α-zingibirene, β- sesquiphellandrene, and p- Cymene. This study highlights that plant-based essential oils could be a promising alternative to antibiotics for which pathogens have developed resistance. *C. longa* and *S. aromaticum* carry compounds that have antimicrobial potential against multiple drug-resistant bacteria including MRSA. *E. coli*, *K. pneumoniae* and *A. baumannii*.

Keywords: MDR; essential oil; antimicrobial activity; *Curcuma longa*; *Syzygium aromaticum*.

INTRODUCTION

Many pathogenic gram-positive and gram-negative bacteria such as *Pneumococci, Enterococci, Staphylococci* and *Enterobacteriaceae* have developed resistance against the available antibiotics (Koulenti *et al*., 2019). The effectiveness of many existing antibiotics has been threatened by the emergence of multidrug resistance among these pathogens (Penner *et al*., 2005). But as being opportunistic organisms, pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA), *Klebsiella pneumoniae, Escherichia coli* and *Acinetobacter baumannii* are the pathogens of major concern (Lowy, 1998; Villers *et al*., 1998; Hassan *et al*., 2011). *E. coli* and *K. pneumoniae* are responsible for infections like septicemia, diarrhea, neonatal meningitis, urinary tract infections (UTI), and bacteremia (McDanel *et al*., 2017). MRSA has been accounted as the major cause of nosocomial infections owing to the widespread use of cephalosporin antibiotics (Klevens, 2007). The resistance problem has accelerated in the last two decades as bacteria from clinical and non-clinical setups have developed resistance against conventional antibiotics (Enright *et al*., 2002).

For thousands of years, nature has been facilitating human beings by providing medicinal agents, and a large number of the latest drugs have been isolated from natural sources (Cragg *et al*., 2002). Antimicrobial compounds of plant origin have been proven as potential therapeutics against many infectious diseases (Mukherjee & Wahile, 2006). Essential oils can be used as an alternative to synthetic compounds due to their less harmful side effects (Carson & Hammer, 2011). They have been evaluated for centuries to assess their biological activities against various biological targets (Hussain *et al*., 2013). Essential oils when used in combination with less effective antibiotics show better antimicrobial activity (Pajohi *et al*., 2011). Plant-based essential oils constitute chemical components mainly terpenoids such as monoterpenes, sesquiterpenes, and their oxygenated derivatives (Stephane & Jules, 2020). These compounds exhibit antibiological activities by diffusing and damaging the cell membrane (Stephane & Jules, 2020). These essential oils differ in their chemical composition significantly. Essential oils extracted from clove buds contain eugenol and eucalyptol which have antimicrobial activity (Kumar *et al*., 2010). Eugenol (4-allyl-2-methoxyphenol), the main component of clove and cinnamon oil has antifungal, antiviral

and antibacterial activity against a range of pathogenic bacteria such as *E. coli*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Salmonella enterica*, *S. aureus*, *Lactobacillus sakei*, and *Helicobacter pylori* (Friedman *et al*., 2002; Ali *et al*., 2005; Gill & Holley, 2006).

Curcuma longa commonly known as turmeric is used as a flavoring agent and has antimicrobial properties. *Curcuma longa* extracts consists of alkaloids, tannin, flavonoid, glycoside and carbohydrate that have antimicrobial potential (Niamsa & Sittiwet, 2009). Similarly, *Syzygium aromaticum* essential oil contains eugenol that exhibits antimicrobial activity (Pandey & Singh, 2011). It alters the cell membrane permeability and activates oxidative stress enzymes such as catalase and superoxide dismutase (Ajiboye *et al*., 2016). The objective of the present study was to evaluate the *C. longa* and *S. aromaticum* essential oils for their antibacterial activity against multiple drug-resistant pathogenic bacteria (*A. baumannii, K. pneumoniae, E. coli* and MRSA).

MATERIALS AND METHODS

Sample collection and processing

Forty (n=40) clinical samples were collected from the Tertiary Care Hospital in Lahore and processed for isolation of multi-drug resistant bacteria.

Isolation and identification of bacteria

Culturing and identification of isolates were performed following the standard guidelines for microbiological examination (Collee *et al*., 1996; Winn, 2006). Processed samples were plated on Nutrient agar plates and incubated aerobically at 37°C for 24-48 h. After growth, based on colony characteristics representative colonies were selected and transferred to selective and differential media by sub-culturing to obtain pure colonies. Organisms were further identified biochemically by following standard methods (Collee *et al*., 1996).

Antimicrobial susceptibility testing

Initially bacterial strains were screened for their antibiotic sensitivity and the zone of inhibition was measured. On the basis of the zone of inhibition against different antibiotic classes, bacterial strains were declared as MDR, XDR and PDR (Magiorakos *et al*., 2012). Antibiotic sensitivity was ascertained by using the Disc diffusion technique as per protocols of the national committee for clinical laboratory standers (NCCLS). Bacterial cultures suspension was set to 0.5McF standards containing 1×10^5 CFU/mL by suspending bacterial colonies in 4-5 mL of Muller-Hinton (MH) broth.

All selected isolates were tested against antibiotics representative of all important groups which included standards i.e., Linezolid and Vancomycin for MRSA while Imipenem and Meropenem for gram-negative bacteria. Others included were Penicillin (10 mcg), Cefotaxime (30 mcg), Cefotaxime (30 mcg), Trimethoprim-Sulphamethoxazole (1.25 mcg/23.75mcg), Tetracycline (30 mcg), Methicillin, Fusidic acid (10 mcg), Chloramphenicol (25 mcg), Piperacillin/Tzobactum (110 mcg), Linezolid (30 mcg), Ampicillin (10 mcg).

Zones of inhibition (ZOI) of each antimicrobial agent were reported as "Resistant", "moderate/Intermediate sensitive", and "Sensitive" by using the interpretative chart. The result interpretation was performed according to CLSI standards 2012. The isolates subjected to antibiotic resistance were further classified into multi-drug resistant (MDR) organisms on the base of their resistance pattern against the antibiotics. The organisms, that showed resistance against three or more than three groups of antibiotics were declared as multidrug-resistant

Extraction of essential oils

Dried rhizome of the *Curcuma longa* and fruit buds of the *Syzygium aromaticum*, each of 10 Kg were distilled by steam distillation to obtain their essential oils and percentage yield (volume/weight) was calculated.

Fractionation of essential oils on the basis of solvent polarity

Crude essential oils of the plants was fractionated by performing Column chromatography. A column of 50 × 3.7 cm was made by Silica gel (60 mesh) slurry in n-Hexane at 40-60°C and left undisturbed for 12 h for complete saturation with standard solvent. Different solvents and their combination on the basis of their polarity (from lower to high) were used to fractionate essential oils. The fraction was obtained by using the solvents n-Hexane (100%), chloroform, and n-Hexane (1:1) Chloroform (100%), Chloroform, and Ethyl-acetate (1:1), Ethyl-acetate+Methanol (1:1) and Ethyl acetate (100%) in separate containers and were concentrated by rotary evaporator at 30°C to 50°C (according to nature of solvent).

Evaluation of the antibacterial activity of essential oils

Crude essential oils and their fractions were evaluated for their antibacterial activity by agar well diffusion assay and minimum inhibitory concentrations were calculated. Wells were punched (6-8 mm) and 50 µL of each essential oil and effective fraction was poured. Plates were incubated at 37°C for 24 h and ZOI was measured. Essential oils and their fraction with maximum ZOI were selected for further evaluation.

Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) of essential oils and their effective fractions were evaluated against the selected resistant strains by broth microdilution method. Two antibiotics including Imipenem and Meropenem were used as references for *E. coli, A. baumannii,* and *K. pneumoniae* while Linezolid and Vancomycin were used as reference drugs against MRSA. In brief, 50 µL of essential oil and antibiotic diluted in Mueller Hinton (MH) broth were inoculated with 100 µL inoculums of the bacterial isolates. Standard turbidity of 1 McFarland was adjusted by suspending colonies from MH agar plates into 5 mL of 0.85% of normal saline solution and diluted as 1:1000 in MH broth. OD values were recorded at zero time and after incubation of 24 h. The minimum concentration at which inhibition of growth was observed was considered as the MIC value of essential oils (Lalitha, 2004).

Identification of components of essential oils by GC/MS analysis

The active crude plant's essential oils and their effective fractions were evaluated for their components through GCMS analysis. The ascertained components were compared with reference V.2.0 GCMS library. Essential oils were analyzed on an Agilent technologies GCMS triple quad. A capillary-Colum, Optima- 5 (30m×250µm×0.25 µm) was used while helium gas was used as a carrier at a flow rate of 3.3245 ml/min. the temperature was maintained at 250°C. MS quad was 150°C while MS sours were adjusted at 230°C. Mass spectra were observed at 70 eV by using, /z 30 to 500 Mass range (Fasola *et al*., 2011).

Statistical analysis

Result data were analyzed through a statistical package for social sciences (SPSS), one-way ANOVA, and post hos Duncan model were used.

RESULTS

Identification of bacterial isolates

Bacterial isolates were identified as *Escherichia coli,* methicillin-resistant *Staphylococcus aureus, Acinetobacter baumannii, and Klebsiella pneumoniae* on the basis of their colonial characteristics, microscopic morphology, and biochemical tests (Table 1). Ten isolates of each bacterium were selected for further studies.

Table 1. Cultural, microscopical and biochemical characteristics of selected bacterial isolates

Characteristics	*Staphylococcus aureus*	*Klebsiella pneumoniae*	*Escherichia coli*	*Acinetobacter baumannii*
Colony morphology	Round, Convex, Yellowish colony	Round, Convex, Creamy pink	Round, Raised, Beige colony	Round, Convex, Pink colony
Gram staining	+ve cocci	-ve rod	-ve rod	-ve coccobacilli
Growth medium with color	Mannitol salt agar (yellow colonies with color change)	MacConkey Agar (pink colonies)	MacConkey Agar (dark pink colonies)	MacConkey Agar (without salt) pinkish
Catalase test	+ve	+ve	+ve	+ve
Oxidase test	-ve	-ve	-ve	-ve
Coagulase test	+ve	-ve	-ve	-ve
Indole test	-ve	+ve	-ve	-ve
Methyl red test	+ve	-ve	+ve	-ve
Voges proskauer test	+ve	+ve	-ve	+ve
Citrate utilization test	+ve	+ve	-ve	+ve
Mannitol fermentation	+ve	+ve	+ve	+ve

Antimicrobial resistance profile

All the selected bacterial isolates exhibited resistance to more than three antibiotic classes and were declared as multidrug-resistant MDRs (Figure 1). MRSA showed 100% resistance to penicillin and tetracyclines followed by trimethoprim/sulphamethoxazole and chloramphenicol (90%) and piperacillin/tazobactum (80%). While, glycopeptides (vancomycin) and oxazolidines (linezolid) inhibited their growth. In the case of *E. coli, the* highest resistance 100% was observed against extended-spectrum cephalosporins and penicillins followed by Antipseudonomal penicillins+βlactamase inhibitors (80%), aminoglycoside, cephalosporins and aminoglycosides (70%). However, the isolates were completely susceptible to antipseudomonal carbapenems (imipenem).

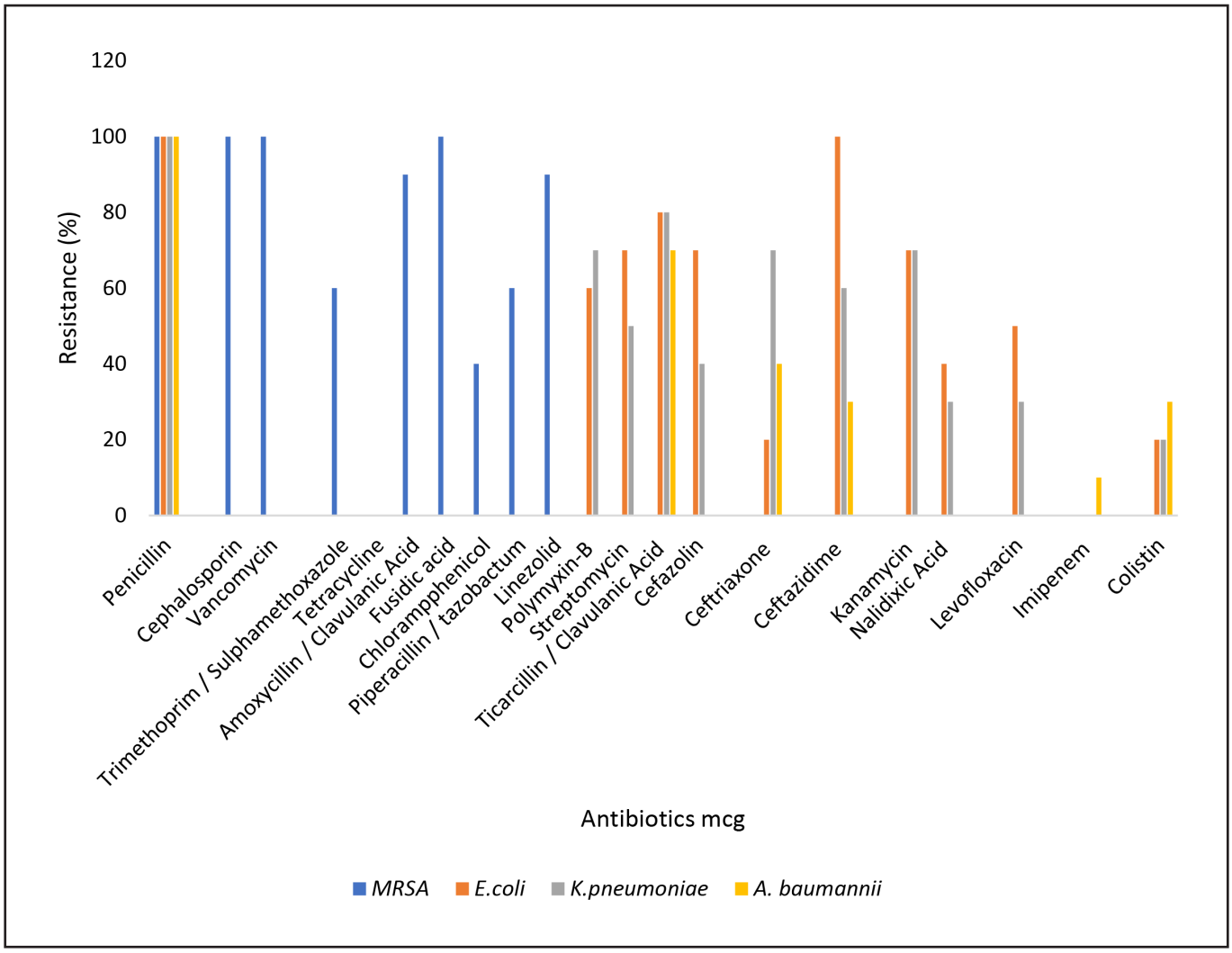

Figure 1. Antibiotic resistance pattern of selected bacterial isolates against antibiotics selected as per CLSI guidelines.

All the isolates of *K. pneumoniae* and *A. baumannii* showed maximum (100%) resistance to penicillin. *K. pneumoniae* showed moderate resistance to antipseudomonal penicillin's+βlactamase inhibitors (50%), and aminoglycosides (60%). Reduced resistance was observed against polymyxins and colistin (30%) Similar pattern was observed for *A. baumannii*, while some of the isolates were recorded XDR.

Antimicrobial screening of essential oils and their fraction

Crude essential oils of the selected plants i.e., *C. longa* and *S. aromaticum* were recovered by hydrodistillation and the condensate was collected. Maximum 1.8% and 1.2% yield was obtained from *C. longa* and *S. aromaticum,* respectively. Fractionation was performed by Colum chromatography. Six fractions (F-1 to F-6) were obtained from the fractionation of crude essential oils of both selected plants.

Crude essential oils and their fractions obtained by column chromatography were preliminarily screened for their antibacterial potential against selected bacterial isolates by using well diffusion method. Not a single fraction of *C. longa* exhibits antibacterial activity, but its crude essential oil inhibited bacterial growth (Table 2). On the other hand, in the case of *S. aromaticum,* all the fractions as well as crude essential oil exhibits antibacterial activity against all selected strains of the multi-drug resistant bacteria. Fraction F-1 (100% N-Hexane + 50% Chloroform) showed maximum activity only against MRSA (11 mm ZOI) and *K. pneumoniae* (16 mm ZOI) while F-5 (50% Ethyl acetate + 50% Methanol) showed the good results against all the bacterial isolates as compared to other fractions but the zone of inhibition observed was less than crude essential oil. Crude essential oil exhibited antimicrobial activity against all selected isolates, maximum against *E. coli* with 20 mm ZOI (Table 3). Those fractions which showed prompt antibacterial effects were further evaluated in detail for their antibacterial activity by comparing their zone of inhibition with standard selected antibiotics.

Syzygium aromaticum showed good antibacterial potential against MRSA (18.7±1.59 mm ZOI), as compared to their fractions which are close to the zone of inhibition exhibited by vancomycin (Table 4). Fraction F-1 activity was greater than F-5 but both fractions showed very less antibacterial activity as compared to standard drugs. Essential oil of the *C. longa* showed very less antibacterial activity against MRSA as compared to *S. aromaticum* and standard drugs (Table 5). Similarly, *S. aromaticum* showed better antibacterial activity against *K. pneumoniae* with ZOI 16.8±1.48 mm which is almost half the ZOI exhibited by meropenem (31.4±2.21 mm) as compared to *C. longa* (Table 5). However, the individual fractions of *S. aromaticum* were less effective against *K. pneumoniae* than crude essential oil as well as the standard drugs. The antibacterial activity of fraction F-1 was greater than F-5 but both fractions showed very less antibacterial activity as compared to standard drugs (Table 5).

Against *E. coli* and *A. baumannii*, *S. aromaticum* exhibited antibacterial activity with ZOI 19.3±1.50 mm and 15.4±3.23 mm, respectively which is near to imipenem and meropenem. The antibacterial activity of fraction F-1 was greater than F-5 but both fractions showed very less antibacterial activity as compared to standard drugs.

Evaluation of minimum inhibitory concentration of essential oils and their effective fractions

Minimum inhibitory concentrations (MIC) of essential oils and their fractions were evaluated by the micro broth dilution method. Essential oils of *C. longa, S. aromaticum* and their effective fraction were tested against selected bacteria. Vancomycin and linezolid were taken as standards for *S. aureus,* and meropenem and imipenem for *K. pneumoniae, E. coli,* and *A. baumannii*. *S. aromaticum* showed maximum antibacterial activity against *A. baumannii* at 1.04 µL/mL MIC followed by MRSA, *K. pneumonia,* and *E. coli*. MIC values evaluated for *S. aromaticum* in case of MRSA were very close to vancomycin (Table 6).

Table 2. Preliminary antibacterial screening of essential oil of *C. longa* and its fractions

Solvent Used for Fractionation	Fraction of *C. longa*	Zone of Inhibition (mm)			
		MRSA	*K. pneumoniae*	*A. baumannii*	*E. coli*
100% N Hexane	F-1	0*	0*	0	0
50% Chloroform + 50% N Hexane	F-2	0*	0*	0*	0
100% Chloroform	F-3	0	0	0	0
50% Chloroform + 50% Ethyl acetate	F-4	0*	0*	0	0*
50%Ethyl acetate + 50% Methanol	F-5	0	0	0	0
100% Ethyl Acetate	F-6	0	0	0	0
Crude Essential Oil		**4**	**10**	**6**	**6**

0*: indicating that very nominal antibacterial activity observed not comparable with crude essential oil.

Table 3. Preliminary antibacterial screening of essential oils of *S. aromaticum* and its fractions

Solvent Used for Fractionation	Fraction of *S. aromaticum*	Zone of Inhibition (mm)			
		MRSA	*K. pneumoniae*	*A. baumannii*	*E. coli*
100% N Hexane	F-1	11	16	0	0
50% Chloroform + 50% N Hexane	F-2	0**	0	0**	0
100% Chloroform	F-3	0	0	0**	0**
50% Chloroform + 50% Ethyl acetate	F-4	0*	0*	0	0
50%Ethyl acetate + 50% Methanol	F-5	9	8	7	8
100% Ethyl Acetate	F-6	0	0	0	0
Crude Essential Oil		**18**	**17**	**15**	**20**

0* and 0**: indicating that very nominal antibacterial activity observed not comparable with crude essential oil.

Table 4. Antibacterial activity of crude essential oil of *C. longa* and *S. aromaticum* compared to reference drugs against selected multi-drug resistant bacterial isolates

Bacterial isolates	Mean Zone of Inhibition (ZOI)					
	C. longa	*S. aromaticum*	Vancomycin	Linezolid	Imipenem	Meropenem
MRSA	3.8±2.21	18.7±1.59	22.3±3.24	32±3.34		
K. pneumoniae	9.7±1.26	16.8±1.48			28.7±1.76	31.4±2.21
A. baumannii	8.9±3.03	15.4±3.23			23.8±2.95	24.8±2.37
E. coli	5.5±4.3	19.3±1.50			27.9±1.92	31.4±1.81

The sum means values differ significantly at p≤0.05 from each other in one set, under one-way ANOVA.

Table 5. Antibacterial activity of fractions of *S. aromaticum* compared to reference drugs against selected multi-drug resistant bacterial isolates

Bacterial isolates	Mean Zone of Inhibition (ZOI)						
	S. aromaticum	Vancomycin	Linezolid	Imipenem	Meropenem	F-1 (100% N-Hexane)	F-5 (50% Ethyl acetate + 50% Methanol)
MRSA	18.7±1.48	22.3±3.39	32±2.38			12±1.78	9.3±1.97
K. pneumoniae	16.8±1.62			28.7±1.80	31.4±2.55	10.5±2.20	7.3±3.11
A. baumannii	15.4±3.29			23.8±2.94	24.8±2.46	10.5±2.10	7.5±2.76
E. coli	19.3±1.47			27.9±2.10	31.4±2.01	11±4.29	7.9±2.12

The sum means values differ significantly at p≤0.05 from each other in one set, under one way ANOVA.

Table 6. MIC (µl/ml) values of *Syzygium aromaticum* and *C. longa* essential oil and F-1 and F-5 fractions against selected bacterial strains

Plants	Oils and fractions	MIC (µL/ml) of essential oil *S. aromaticum and C. longa*			
		S. aureus (MRSA)	*K. pneumoniae*	*E. coli*	*A.baumannii*
S. aromaticum	Crude oil	1.69±0.76	2.06±0.08	3.12±1.17	1.04±0.42
	F-1	12.5±6.25	14.58±7.79	16.67±5.89	17.69±7.58
	F-5	31.3±13.97	13.5±8.39	25.0±12.50	26.0±12.75
C. longa	Crude oil	37.5±13.69	47.92±30.17	41.67±12.19	31.25±15.30
Meropenem			0.78	0.78	0.39
Imipenem			0.78	0.78	0.39
Vancomycin		1.56			
Linezolid		0.78			

The sum means values differ significantly at p≤0.05 from each other in one set, under one way ANOVA.

MIC value of the effective fraction (F-1) of the *S. aromaticum* was evaluated against the selected strains of the bacteria. It was observed that the MIC value was higher than the standard drugs as well as the crude essential oils. Against MRSA, fraction F-1 showed MIC ranging from 6.25 to 2 µL/mL which is quite high as compared to 1.56 µL/mL and 0.78 µL/mL in the case of vancomycin and linezolid, respectively. Similarly, MIC values ranging from 6.25 to 25 µL/mL were observed against *K. pneumoniae, A. baumannii,* and *E. coli* which are higher than imipenem and meropenem (Table 6).

Curcuma longa showed very less antibacterial potential against the selected strains of MRSA*, K. pneumonia*, *E. coli,* and *A. baumannii*. The MIC values ranged from 25 µL/mL-50 µL/mL for *S. aureus*, 12.5µL/mL-100 µL/mL for *K. pneumoniae*, 25 µL/mL-50 µL/mL for *E. coli* and 12.5µL/mL to 50 µL/mL for *A. baumannii* which are quite higher than the standard drugs. Furthermore, at the preliminary stage, not a single fraction of the *C. longa* essential oil showed antimicrobial activity (Table 6).

GC/MS analysis of essential oils and their fractions

By the GC-MS analysis of *S. aromaticum* almost 35 compounds were detected (Table 7). The major constituent of *S. aromaticum* essential oil was eugenol (73.09%), eugenyl acetate (15.78%) β- caryophyllene (14.45%), α- Humulene (2.5%). The amount of these components were also detected in F-1 and F-2 fractions of *S. aromaticum.* Eugenol was abundant in both fractions i.e., 50.09% and 35.25% in F-1 and F-2, respectively. Other constituents in very low concentration were also detected such as chavicol (0.31%), α- copaene (0.70%), valencene (0.17%), α–selinene (0.13%), β-Cadinene (0.12%) and caryophyllene oxide (1.09%).

Table 7. Chemical composition of *S. aromaticum* essential oil as determined by GC-MS analysis and comparison with the collected fractions

Sr. No	Names of Components	Retention Time/ RT (minutes)	Relative Peak Area %		
			Essential Oil *Syzygium aromaticum*	F-1 Fraction (100% n-Hexane)	F-2 Fraction (50% Ethyl acetate + 50% methanol)
1	5-Hexan-2 one	9.10	–	+	0.51
2	2-Heptanol acetate	.9.26	0.013.		+
3	2- Nonanone	.10.52	+	–	+
4	(E)- 4,8-Dimethyl-1,3,7-nonatriene	11.03	0.01	–	–
5	Benzyl Acetate	12.03	0.05	–	+
6	Acetic acid, phenylmethyl ester	12.33	–	–	++
7	Methyl Salicylate	13.05	0.06	+	+
8	Bicyclobutylidiene	14.02	+	–	–
9	Chavicol	14.56	0.31	+	+
10	α- Cubebene	16.68	0.02		
11	Eugenol	16.97	73.09	50.09	35.25
12	α- Copaene	17.28	0.7	+	0.32
13	Unknown	17.45	–	–	–
14	β- Elemene	17.61	0.03	–	+
15	β- Caryophyllene	18.21	14.45	10.3	5.09
16	Valencene	18.27	0.20	+	+
17	α- Guaiene	18.29	0.06	0.02	+
18	α- Gurjunene	18.32	0.05	–	–
19	β- Ylangene	18.69		–	–
20	α- Humulene	18.92	2.5	++	1.2
21	Alloaromadendrene	19.12	0.07	+	-
22	δ- Cadinene	19.24	0.05	–	+
23	(E)-5-Acetyl-2,2-dimethyl-1-1-(3'-methyl-1',3'-butadien-1'-yl)bicyclopentan	19.27	0.35	–	–
24	α- Muurolene	19.33	0.13	–	–
25	α- Amorphene	19.38	0.09	–	–
26	γ-Muurolene	19.47	0.16	–	–
27	β- Selinene	19.56	0.06	+	–
28	α- Selinene	19.73	0.13	–	–
29	β- Cadinene	19.83	0.12	+	-
30	Unknown	19.95	0.03	–	–
31	γ- Cadinene	20.10	0.02	–	–
32	Eugenyl Acetate	20.44	15.78	7.05	5.23
33	Carophyllene oxide	21.13	1.09	0.62	0.45
34	Benzyl Benzoate	21.97	0.04	0.055	+
35	Hexadecanoic Acid	22.5	–	0.5	+

For *C. longa* 30 compounds were detected (Table 8). Ar-Tumerone was the most abundant (36.92%) component followed by α-tumerone (34.76%) and β-tumerone (25.89%). Other major components include I-phellandrene (7.9%), α-zingibirene (5.7%), β-sesquiphellandrene (4.3%), and p-cymene (4.96%). In addition, other traces were also found but in very small quantities.

DISCUSSION

Development of resistance by pathogenic bacteria against commonly used antibiotics is a very alarming condition. The emergence of multiple drug resistance is one of the major reasons for treatment failure leading to high mortality and morbidity rate in hospitals as well as in the community (Ventola, 2015). Gram-positive bacteria, particularly, methicillin-resistant *Staphylococcus aureus* are an extreme risk to public health (Penner *et al*., 2005). As an opportunistic organism, with intrinsic virulence and the ability to survive in different environmental conditions *Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia,* and *Acinetobacter baumannii* are the pathogens of great concern (McDanel *et al*., 2017). Their pathology spectrum can vary from mild to moderate skin infections to severe life-threatening sepsis syndrome. To find out

Table 8. Chemical composition of *C. longa* essential oil as determined by GC-MS analysis and comparison with the collected fractions

Sr. No	Names of Components Relative Peak Area %	Retention Time/ RT (minutes)	Relative Peak Area %
			Essential Oil (*Curcuma longa*)
1	α- Thujene	7.48	0.67
2	Vinyl propionate	7.89	1.8
3	α- Pinene	7.71	0.6
4	α- Myrcene	9.50	0.81
5	I-Phellandrene	9.93	7.9
6	p- Cymene	10.44	4.96
7	1,8-Cineole	10.73	2.5
8	Limonene	10.79	0.6
9	α- Terpineolene	12.64	0.76
10	Camphor	15.02	0.09
11	α- Terpineol	16.95	0.4
12	Trans-Caryophyllene	22.47	1.95
13	α- Caryophyllene	23.31	0.3
14	α- Curcumene	23.82	4.3
15	α- Zingibirene	24.28	5.7
16	α- Bisabolene	24.57	0.7
17	α- Sesquiphellandrene	24.96	4.3
18	2-Phenyl-1-1D1-Aziridine	25.88	1.29
19	α- Caryophyllene	26.15	0.8
20	α- Atlantone	26.28	0.7
21	1,3,5-Cycloheptatriene	26.41	1.3
22	Pyrazine	26.46	0.6
23	α- Bisabolene	26.89	0.5
24	γ- Curcumene	28.21	2.4
25	α- Cadinol	30.62	1.2
26	β- Tumerone	34.91	25.89
27	Ar- Tumerone	35.13	36.92
28	α – Tumerone	35.95	34.76
29	(6R, 7R)Bisabolone	37.52	2.1
30	α- Atlantone	40.22	1.24

new treatment options against multiple drug resistant bacteria is a high-value concern for the pharmaceuticals industry. Plant extracts and essential oils have been shown to exert tremendous biological and antimicrobial activities *in vitro* and *in vivo*, which justify research on traditional medicinal plants and focus on the characterization of their antimicrobial and safety profile.

The current study focused to ascertain the resistant pattern of the most infamous pathogenic bacteria including *K. pneumonia*e, *A. baumannii*, and *E. coli* as well as evaluating the antibacterial potential of indigenous plant extracts against them. All of the selected isolates showed resistance to the majority of antibiotics and declared as multidrug-resistant bacteria. These results are in accordance with the previously reported studies (Niranjan & Malini, 2014; Subedi *et al*., 2018; Karameşe & Özgür, 2020).

Medicinal plants serve as a significant source of natural organic compounds that are widely used as a therapeutic agent. Essential oils are naturally volatile, aromatic, concentrated hydrophobic liquids produced by plants and extracted by specific methods of distillation. It has been reported that these essential oils have excellent antimicrobial, antioxidant, insecticidal, and preservative properties. A number of plants are used to provide a substitute solution, against antibiotic resistance problems (Ventola, 2015). Many essential oils exhibit their antimicrobial effect by interrupting the normal functioning and synthesis of key elements required for the normal growth of microbes. They may produce a bactericidal effect by targeting the structure and function of the cell wall, cell membrane, DNA enzymes, protein synthesis, cytoplasmic changes, and pH disturbance (Faujdar *et al*., 2020). In the present study, *S. aromaticum* and *C. longa* were evaluated for their antimicrobial activity against the selected bacterial isolates. *S. aromaticum* showed good activity against MRSA, however, *C. longa,* was less effective as compared to *S. aromaticum.* It could be due to the absence of compounds essential for antimicrobial potential. These results are in agreement with a study reported by Gonnalves *et al*. (2019). Essential oils of cinnamon and thyme have been reported to be effective against MRSA at the lowest concentration of 25 µL/mL (Gonnalves *et al*., 2019). In another study, a combination of citricidal and geranium oil has been reported as the greatest anti-bacterial agent against MRSA (Uzair *et al*., 2017).

Against *K. pneumoniae, S. aromaticum* showed better antibacterial activity which indicates that *S. aromaticum* has comparatively good potential as the values were very close to the MIC for the meropenem. Similarly, in the case of *E. coli, C. longa* was less effective than *S. aromaticum* with MIC 41.6 µL/mL. Antibacterial activity of thyme and peppermint essential oil alone and in combination with ciprofloxacin against *K. pneumoniae* has been reported (Edwards-Jones *et al*., 2004). Similarly, for *A. baumannii S. aromaticum* showed better activity at the lowest MIC that was closer to the reference drugs. This indicates that essential oils have antibacterial activity against the MDR pathogenic bacteria but a high dose is required to meet the standards cut point. In literature, essential oils obtained from Cinnamon (Mohamed *et al*., 2018), *Corriandrum sativum* (Intorasoot *et al*., 2017), *Listea cubeba* (Alves *et al*., 2016) and Oregano (Hao *et al*., 2021) have been reported effective against MDR *A. baumannii.* The studies suggest that these essential oils are effective both alone and in synergy with antibiotics.

In this study, essential oils with swift antibacterial effects were fractionated by using the column chromatography method. The fraction of essential oils was separated by using different solvents and combinations with different polarities. Seven fractions were eluted for *S. aromaticum* and *C. long*. All seven fractions of *C. longa* were evaluated for antibacterial activity by well diffusion method to measure the zone of inhibition, but antibacterial efficacy was not observed for any fraction. This indicated that crude oil is more effective than that of individual fractions. In case of *S. aromaticum* two fractions which were obtained by using the 100% N-hexane and 50% ethyl acetate+50% methanol (combination) as mobile phase, showed anti-bacterial activities. Both Fractions exhibit less antibacterial efficacy as compared to the crude oils which demonstrated that the antibacterial effect is the collective phenomenon of essential oils and by fractionation different individual constituents of the crude oils have either no or very less antibacterial activity.

By GC/MS analysis of the *S. aromaticum,* 35 chemical components were detected. eugenol was identified as a major constituent followed by eugenyl acetate, β-caryophyllene, and α-humulene. These major components were also detected in the effective fractions (F-1 and F-2) of *S aromaticum* but the concentration was quite less than the crude essential oils. Many trace components detected in crude essential oils were absent in fractions. This could be the main reason for the less antibacterial activity of fractions as compared to crude oil. Different studies reported eugenol as the most abundant component (ranging from 55% to 75%) which is comparable with our study. Similarly, eugenyl acetate and β-caryophyllene reported in this study are comparable with different studies available in the literature (Bhuiyan *et al*., 2010; Amelia *et al*., 2017; Lu *et al*., 2018). Eugenol is a phenolic aromatic compound that has a pleasant odor and taste, belonging to a group of phenylpropanoids. A free hydroxyl group present in it is responsible for antimicrobial activity. Studies have reported different mechanisms of antimicrobial action. One of the major actions is the disruption of cytoplasmic membrane permeability

(Sulistyoningrum *et al*., 2017). Abdullah *et al*. (2015) evaluated the antimicrobial activity of clove essential oil against four multi-drug resistant (MDR) bacteria (*A. baumanni*, *P. aeruginosa*, *S. aureus*, and *E. faecalis*) isolated from different clinical samples. Maximum activity was observed against *A. baumanni* at a concentration of 10% (v/v). These results are similar to those reported in the present study.

By GC/MS analysis of *C. longa,* thirty compounds were identified such as Ar-tumerone, α-tumerone, β-tumerone, I-phellandrene, α-zingibirene, β-sesquiphellandrene, p-cymene. Some trace components were also detected. Different studies available in the literature reported different numbers of constituents in different ranges. The variation of the concentration could be based on the method used for distillation, the part used, and the nature of the plant (dried or fresh) (Awasthi & Dixit, 2009; Dosoky *et al*., 2019; Singh *et al*., 2011).

The emergence of antibiotic resistance is inevitable, while the development of new drugs is a time taking process (Koulenti *et al*., 2019). In light of this, recent medicinal plants have received great attention owing to their low toxicity, pharmacological effectiveness, and economical value (Pajohi *et al*., 2011). Studies have been focused on plant-derived phytochemicals and their mode of action of antimicrobial activity. It has been reported that essential oils obtained from plants have antioxidant and antimicrobial properties. These plant essential oils carry a wide range of complex and diverse compounds. They have antibacterial, antifungal, and antiviral activities and have been screened globally for the treatment of infectious diseases. Therefore, essential oils and their fractions are a powerful alternative to combat resistance (Lu *et al*., 2018).

CONCLUSION

In conclusion, essential oils of *S. aromaticum* and *C. longa* have the potential to inhibit the growth of multi-drug resistant pathogens. The crude oils of these plants were more effective than their fractions. However, in case of *A. baumannii*, *S. aromaticum* surpassed the *C. longa* antimicrobial activity at 1.04 µL/mL MIC. The essential oils of these plants carried various compounds that are responsible for antimicrobial activities. In this study, crude oil and their fractions of S. aromaticum carried an abundance of eugenol, eugenyl acetate, β- caryophyllene, and α- Humulene, while in *C. longa* Ar-tumerone, α–tumerone, β- Tumerone, I-Phellandrene, α-zingibirene, β- sesquiphellandrene, and p- Cymene were found. This study shows that plant essential oils have the potential to inhibit various pathogens and could be a promising alternative for antibiotics to tackle resistance problems.

Conflict of interest statement

There is no conflict of interest

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