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Antibacterial activity of *Syzigium cumini* leaf extracts against multidrug resistant pathogenic bacteria

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ABSTRACT

Staphylococcus aureus (MRSA and MSSA) and *Escherichia coli* strains were isolated from the Clinical specimens such as pus, wound, urine, ear swabs and blood collected from the Health care centers at Lucknow city of Northern India. All strains were tested and detected as multi-drug resistant against penicillin, ampicillin, cefpdoxime, sulphadiazine, nalidixic acid, erythromycin and amoxicillin. Antibacterial activity of *Syzigium cumini* leaf extracts in various solvents was also determined. The efficacy of the extracts was found higher in petroleum ether and ethanolic solvents with the inhibition zone 8-24 mm than all other solvent extracts tested. Extracts of *Syzigium cumini* exhibited prominent activity against both the drug resistant strains of *S. aureus* and *E. coli*. Minimum inhibitory and minimum bactericidal concentrations of *Syzigium cumini* leaf extracts were also determined among the strains. MIC and MBC values varied from 1.56 to 25 mg/ml and 1.56 to 50 mg/ml among the tested strains. The combination of ampicillin with plant extracts exhibited a significant synergistic effects on growth of multidrug resistant strains of both genus tested. Our study thus suggests the use of this medicinal plant in the treatment of various diseases caused by drug resistant species of *S. aureus* and *E. coli.*

INTRODUCTION

The overuse of antibiotics in the treatment of infections has become the global concern for continuous increase in the emergence and spread of multidrug resistant pathogenic strains (Harbottle, 2006). Nowadays most of the antibiotics have become inactive against some pathogenic microbes like methicillin-resistant *S. aureus*, vancomycin-resistant enterococci, multidrug-resistant Gram negative bacteria, etc. An increase in the emergence of multiple drug- resistant bacteria is threatening the world population. Bacterial resistance to antibiotics increases mortality likelihood of hospitalization and length of stay in the hospital. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agent (Gislene, 2000). Resistance to antibiotics is one of the greatest threats to the success of modern medicine. The worldwide emergence of *Escherichia coli, Klebsiella pneumoniae, Haemophilus* and many other ß-lactamase producers has become a major therapeutic problem. Multi-drug resistant strains of *E. coli* are widely distributed in hospitals and are increasingly being isolated from community acquired infections (Khan, 2004; Akram 2007).

Multidrug resistance in clinical bacteria like *Staphylococcus aureus* is responsible for nosocomial infections (Mulligan *et al.*, 1993). The steadily increasing bacterial resistance to existing drugs is a serious problem, and therefore there is a direct need to search for new classes of antibacterial substances, especially from natural sources. Unlike synthetic drugs, antimicrobials of plant origin are not associated with side effects and have a great therapeutic potential to heal many infectious diseases (Chanda *et al.*, 2010; Habbal *et al.*, 2011).

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Medicinal plants like, *Allium sativum, Azadirachta indica, Cordia dichotoma, Holoptelea integrifolia, Ocimum sanctum, Syzigium cumini and Trigonella foenum graecum* etc. have been reported to possess antibacterial properties (Ahmad *et al.,* 1998). Medicinal plants contain antibacterial agents to combat infections and their products are used either internally or externally to heal wounds and other injuries and they are used in relieving pain or cure of common diseases such as diabetes, heart disorders and various cancers (Mohanta *et al.,* 2003). *Syzigium cumini* has been widely used for the treatment of various diseases in traditional and folk medicine (Figure 1). The leaves have antibacterial property and used to strengthen the teeth and gums. The leaves have also been extensively used to treat diabetes, constipation, leucorrhoea, stomachalgia, fever, gastropathy, dermopathy and to inhibit blood discharge in the feces (Gowri and Vasantha, 2010). For this reason, researchers are increasingly turning their attention to herbal products such as *Syzigium cumini*, looking for new leads to develop better drugs against MDR microbe strains (Braga *et al.,* 2005). In the present study; we have selected Indian medicinal plant *Syzigium cumini* to be screened against multi-drug resistant bacteria. The selection of medicinal plants is based on its traditional uses in India. (Chopra *et al.,* 1992; Ahmad *et al.,* 1998; Mehmood *et al.,* 1999). The objective of this study was to determine the antibacterial effect of extracts from the *Syzigium cumini* leaves against multi-drug resistant *Escherichia coli* and *Staphylococcus aureus* strains.

Fig. 1: Picture of *Syzigium cumini* leaf.

MATERIALS AND METHODS

Isolation and identification of bacterial strains

Two bacterial species viz., *Escherichia coli* and *Staphylococcus aureus* (MRSA and MSSA) were used in antibacterial assay. These bacterial strains were isolated from the Clinical specimens consisting of pus, wound, urine, ear swabs and blood collected from the Central Pathology Laboratory of Era's Lucknow Medical College and Hospital and Integral Institute of Medical Sciences and Research, Lucknow. The specimens collected were directly streaked onto mannitol salt agar media and EMB agar media specific for each bacterium. The plates were incubated at 37 °C for 24 hours. The bacterial strains were identified on the basis of cultural, morphological and biochemical tests such as catalase, coagulase, gelatin liquefaction, oxidase, Triple Sugar Iron agar (TSI), citrate utilization (Simmon's citrates medium), urease (Christensen's Urea Agar), indole, motility, H_2S production (Sulphide Indole Motility Medium) and sugar fermentation tests. *Staphylococcus aureus* reference strain MTCC 96 and *Escherichia coli* reference strain MTCC 443 were obtained from IMTECH, Chandigarh. All culture media were provided by Himedia Laboratories Pvt. Ltd., India.

Determination of antimicrobial resistance

Pure isolates of identified *Staphylococcus aureus* and *Escherichia coli* were subjected to antimicrobial susceptibility testing using the disc diffusion method as recommended by Kirby Bauer method according to the recommendations of Clinical Laboratory Standard Institute (CLSI, 2010), formerly National Committee for Clinical Laboratory Standards (NCCLS) (2002), using the following antibiotics discs obtained from Hi-Media Laboratories Pvt. Ltd, Mumbai: methicillin (MET) 5 µg, oxacillin (ox)1 µg, penicillin G (PEN) 10 IU, erythromycin (ERYTHRO) 5 µg, nalidixic acid (NA) 30 µg, kanamycin (KAN) 30 µg, nitrofurazone (NR) 100 µg, tetracycline (TET) 10 µg, polymyxin B (PB) 300 µg, ciprofloxacin (CIP) 5 µg, ampicillin (AMP) 10 µg, ofloxacin (OF) 5 µg, sulphadiazine (SZ) 300 µg, amoxicillin (AMX) 10 µg, and cefpodoxime (CPD) 30 µg, chloramphenicol (CH) 30 µg, Gentamycin (GEN) 50 µg, neomycin (NEO) 30 µg and vancomycin (VAN) 30 µg. All isolates were grown in Brain Hearth Infusion broth and incubated at 37°C for 6 h until the turbidity of 0.5 McFarland standards was achieved. The isolates were then swabbed onto Muller Hinton Agar and allowed to dry for 15 min. The antibiotics discs were placed on the centre of the agar plates with the aid of sterile pointed tip forceps and incubated at 37°C for 24 h. The presence of a clear zone around the antibiotic disc is measured with meter rule. *S. aureus* strains were tested for methicillin resistance using the disc diffusion method (Bauer *et al.,* 1966). *S. aureus* isolates are considered to be resistant to methicillin if the inhibition zones are <10 mm while susceptible if the zones of inhibitions were ≥ 10 mm. These isolates were tested and detected as multi-drug resistant against penicillin, ampicillin, cefpdoxime, sulphadiazine, nalidixic acid, erythromycin and amoxicillin. These strains were maintained on agar slants at 4 °C for antimicrobial tests. The microorganisms were incubated overnight at 37 °C in Mueller-Hinton Broth (Oxoid) at pH 7.4.

Collection and authentication of plant materials

Healthy leaves of *Syzigium cumini* were collected from Herbal Garden of Faculty of Pharmacy, Integral University, Lucknow and road side of Kursi road, Lucknow and they were identified and authenticated by Dr. Muhammad Arif, Assistant Professor, Faculty of Pharmacy, Integral University, Lucknow. Voucher specimens were prepared and deposited in the University Herbarium of Pharmacy Department, Integral University, Lucknow, for future reference. The plants were brought to the laboratory and thoroughly washed in running water to remove debris and dust particles and then rinsed using distilled water and finally air dried protected from direct exposure to sun light.

Preparation of plant extract

Syzigium cumini leaves were dried in an oven at temperature below 45 °C for 2-3 days and coarsely powdered. The powdered *Syzigium cumini* leaves were extracted successively with petroleum ether, ethyl acetate, acetone, methanol and ethanol to afford corresponding fractions (Dabur *et al.,* 2004). The filtered extract was left to dryness under reduced pressure on rotary evaporator at 40 °C and stored at 4 °C for further use.

Phytochemical analysis

All the extracts were subjected to phytochemical analysis by using standard procedure for the identification of the various phytoconstituents (Parekh and Chandra, 2007).

Antibacterial Assay

The agar well diffusion method (Perez *et al.,* 1990) as adopted earlier (Ahmed and Beg, 2001) was used. 0.1 ml of diluted inoculums (10^5 CFU/ml) of test organism was spread on Muller- Hinton agar plates. Wells of 6 mm diameter was punched into the agar medium and filled with 50 µl of *Syzigium cumini* leaves extract of 100 mg/ ml concentration and solvent blank (DMSO) separately. The plates were incubated for overnight at 37ºC. The antibacterial activity was evaluated by measuring the zone of inhibition against test organism. The antibiotic disc chloramphenicol (30µg) to which strain is sensitive was used in the test system as positive controls and DMSO, solvent taken in study and ampicillin (10 µg) to which strains were resistant were used as negative control. Each experiment was performed in triplicate.

Determination of minimum inhibitory concentration (MIC) of plant extracts

Minimum inhibitory concentration and minimum bactericidal concentration of plant extracts against drug resistant clinical strains was determined by broth micro dilution method, using specific dye (p-iodonitro tetrazolium violet) as an indicator of growth as described by (Eloff *et al.,* 1998). For each test batch control wells were prepared; the positive control (antibiotic, Mueller-Hinton broth and test organism) and sterility and negative control (Mueller-Hinton broth and DMSO). The plates were incubated at 37 °C for 24 h. The bacterial activity in the test wells was detected by adding 40 µL of 0.2 mg/ml of specific dye (p-iodonitro tetrazolium violet) (Himedia, India) solution dissolved in sterile distilled water to each well. The plates were incubated for further 30 min, and observed visually for any change in color to pink indicating reduction of the dye due to bacterial growth. The lowest concentration (highest dilution) of the plant extract required to inhibit visible growth of the tested microorganism was designated as the MIC.

Data Analysis

All data were measured average value of three replicates and standard error (±). Results were subjected to Microsoft excel 2010. $p < 0.05$ was statistically significant.

RESULTS AND DISCUSSION

The extractive yield of *Syzigium cumini* leaves using different solvents was determined. Maximum extractive yield was recorded in petroleum ether extract followed by ethanol extract. The extraction ability of different solvents from leaves for recovering extractable components followed the order: petroleum ether (5.55%) > ethanol (3.23%) > methanol (2.28%) >acetone (1.98%) > ethyl acetate (1.66%). Alcohol and acetone both are polar solvents but alcohol had more extractive yield than acetone. Non polar solvent petroleum ether had more extractive yield than polar solvent alcohol and acetone while semi polar solvent ethyl acetate had minimum extractive yield. Significant differences of extractive yield among different solvents might be attributed to the varied polarity of the solvents.

The leaf extract of *S. cumini* was evaluated for the presence or absence of diverse phytochemicals. The leaves of *S. cumini* are rich in alkaloids, flavonoids, tannins, saponins, steroids and terpenoids, as shown in Table-1. The leaf methanol extract of *S. cumini* was rich in phenols, saponins, glycosides, flavanoids, alkaloids, steroids, terpenoids, resins and tannins.

Table 1: Phytochemical anaiysis of *Syzigium cumini* leaf extracts in various solvents.

Phytochemical constituents	Methanol extract	Ethanol extract	Ethyl acetate extract	Acetone extract	Petroleum ether extract
Flavanoids	+	+	-	+	+
Alkaloids	+	+	-	+	+
Glycosoids	+	+	-	+	-
Steroids	+	+	-	+	-
Phenol	+	+	-	+	-
Tannin	+	-	-	+	-
Terpenoids	+	+	-	-	+
Saponins	+	+	-	+	-

In the present study, *Syzigium cumini* leaves were extracted with petroleum ether, ethyl acetate, acetone, methanol and ethanol. Antimicrobial potentiality of the extracts was investigated against six strains of *Staphylococcus aureus* (3 MRSA and 3 MSSA strain) and six strains of *Esherichia coli* and their potential activity were qualitatively and quantitatively assessed by the presence or absence of inhibition zones and MIC values respectively. Results of the antimicrobial activity obtained using the well diffusion assay is summarized in Table 3. The petroleum ether, acetone and ethanol extracts of the investigated *Syzigium cumini* leaves showed antimicrobial activities against all tested bacterial strains. All the plant extracts inhibited almost all the bacterial strains with the zone of inhibition ranging from 8-24 mm. Semi polar solvents ethyl acetate showed minimum inhibitory activity (8-15 mm) and it inhibited 5 clinical strains of *S. aureus* and and 2 clinical strains of *E. coli* and was not effective against SA3 strain and EC10, EC12, EC16, MTCC 443 strains of *S. aureus* and *E. coli* respectively. Maximum inhibitory activity against *S. aureus* was recorded by petroleum ether extract with inhibition zone (22 mm) against MRSA strain (SA17). The highest

antibacterial activity with the zone of inhibition (24 mm) was seen against EC12 isolates of *Escherichia coli* strains by ethanol extract of *Syzigium cumini* leaves. All 5 extracts were compared with 20 standard antibiotics, the results of which are presented in Table 2. The antimicrobial activity of some of the solvent extracts was comparable with that of standard antibiotics. Table 2 illustrates the sensitivity of selected bacterial pathogens towards the standard antibiotics. It was observed that all the pathogens were resistant to the standard antibiotics penicillin, ampicillin, cefpdoxime, sulphadiazine, nalidixic acid, erythromycin and amoxicillin, used in this study. Chloramphenicol was found to be most effective recording its maximum lethal effect against *E. coli* and *S. aureus* strains with 22-28 mm zones of inhibition. *E. coli* and *S. aureus* exhibited maximum sensitivity with 26 mm and 28 mm against chloramphenicol (C) respectively. All strains of *S. aureus and E. coli* were found to be sensitive towards streptomycin (S) recording highest zone of inhibition 22 mm and all *S. aureus* strains were sensitive to vancomycin and less sensitive towards polymyxin B (PB) with highest 15 mm zone of inhibition.

The MIC and MBC values were evaluated against 6 Gram positive bacteria (3 MRSA and 3 MSSA) and 6 Gram negative bacteria (*Escherichia coli*). The values are presented in Table 4a and 4b. For both *Staphylococcus aureus* and *Escherichia coli* strains, MIC and MBC values varied from 1.56 to 25 mg/ml and 1.56 to 50 mg/ml respectively. EC10 and MTCC 443 strains of *Escherichia coli* were the most susceptible gram negative organism to ethanol extract (MIC; 1.56 mg/ml and MBC; 3.12 mg/ml), and MTCC 96 strain of *Staphylococcus aureus* were most susceptible to petroleum ether and acetone extract (MIC; 1.56 mg/ml and MBC; 3.12 mg/ml).

For the standard antibiotics (CH and AMP) MIC and MBC were recorded against *Staphylococcus aureus* strains ranging from 0.0062 mg/ml to 0.05 mg/ml and 0.097 mg/ml to 0.19 mg/ml respectively and for *Escherichia coli* strains, MIC and MBC values were recorded ranging from 0.062 to 0.31 mg/ml and 0.0487 mg/ml to 0.19 mg/ml respectively.

Synergistic activity of petroleum ether extracts of *Syzigium cumini* leaf with standard antibiotics ampicillin and against bacteria is shown in Table 5. The combination of petroleum ether extract and ampicillin exhibited synergistic effect on bacterial growth in terms of their MIC and MBC. It was observed alone and combined MIC ranging from 1.56-6.25 mg/ml and 0.19-0.785 mg/ml against *S. aureus* respectively. MIC ranging from 6.25-25.0 mg/ml and 0.39-3.12 mg/ml was recorded against *Escherichia coli* alone and in combination respectively. Similar trend of synergistic effect was also observed in terms of MBC against both the organisms tested. Combination showed a significant effect against *Staphylococcus aureus* and *Escherichia coli* strains.

The first step towards this study was to find out the antibacterial activity of *Syzigium cumini* leaf extracts against some selected strains of multidrug resistant *Staphylococcus aureus* and *Escherichia coli* strains. The extracts of the *Syzigium cumini* leaf showed significant antimicrobial activity against all tested bacterial strains. Phytochemical studies revealed the presence of various secondary metabolites in the leaf extracts of *S. cumini*. Various phytochemical compounds detected are known to have beneficial importance for human health. The alcohol extract of *S. cumini* was rich in phenols, saponins, glycosides, flavanoids, alkaloids, steroids, terpenoids, resins and tannins. Antibacterial activity of leaf extracts can be attributed due to the presence of these phytochemicals (Cowan, 1999; Padayana *et al.*, 2011). The results of this study support the use of this plant for human diseases and explore the ethnobotanical importance of plant as a potential source of bioactive substances.

The study focuses on antibacterial activity of variety of solvent extracts of *S. cumini.* The present research also observes the sensitivity pattern of selected pathogens towards extracts of *S. cumini* as well as standard antibiotics. In our study, the *S. aureus* and *E. coli* strains were observed multi drug resistant against the common antibiotics used. (Tiwari *et al.*, 2009). *Syzigium cumini* extracts showed significant antibacterial activity against almost all bacterial strains tested. Antimicrobial activity of *S. cumini* was also previously reported by other worker. (Nascimento *et al.*, 2000; Ahmed and Beig, 2001).

In our study the highest zone of inhibition was recorded in petroleum ether leaf extracts of *Syzigium cumini* against *S. aureus* and *E. coli* and it was ranged from 8-22 mm and 12-15 mm respectively which was quite similar with the previous study conducted by Deepak *et al.*, 2014; Yuvraj *et al.*, 2011; Prasad *et al.*, 2013). Our findings are also in agreement with Deepak *et al*, 2014; Elfadil *et al.*, 2015; Pranoti *et al.*, 2014; Satyawati *et al.*, 2014; Yuvraj *et al.*, 2011 with respect to antimicrobial activity of methanolic extract of *Syzigium cumini* leaf extract in which the zone of inhibition was reported ranging from 8-20 mm against *S. aureus* and *E. coli*. Minimum antimicrobial activity was observed in ethyl acetate extract of *Syzigium cumini* leaves ranging from 8-15 mm against both *S. aureus* and *E. coli* strains. Similar observation was also reported by Singh *et al.*, 2016. They also observed the lowest antimicrobial activity in ethyl acetate as compared to other extracts of *Syzigium cumini*. Prasad *et al.*, 2013 recorded the zone of inhibition with 13 mm in acetone extract of *Syzigium cumini* against *S. aureus* which is also similar with our result where zone of inhibition against *S. aureus* was recorded between 8-18 mm. Prasad *et al.*, 2013 reported zone of inhibition in ethanolic extract of *Syzigium cumini* leaves (11mm) against *S. aureus* which is also in agreement of our findings (8-20 mm) against the MRSA and MSSA. For both *Staphylococcus aureus* and *Escherichia coli* strains, MIC and MBC values of *Syzigium cumini* leaf extracts varied from 1.56 to 25 mg/ml and 1.56 to 50 mg/ml respectively. Our MIC and MBC results of *Syzigium cumini* leaf extracts are also similar to the other reports. (Chanudom *et al.*, 2014).

The zone of inhibition of chloramphenicol against *S. aureus* was reported 26 mm, 21 mm, by Deepak *et al.*, 2014; Yuvraj *et al.*, 2011 respectively which is in agreement with our result in which zone of inhibition against Chloramphenicol was 26-30 mm.

Table2: Antimicrobial activity of antibiotics against *Staphylococcus aureus* and *Escherichia coli* strains.

S. No.	Antibiotics	Zone of inhibition(mm)											
		Staphylococcus aureus strains						*Escherichia coli* strains					
		SA3	SA4	SA10	SA17	SA19	M96	EC10	EC11	EC12	EC16	EC17	MTCC443
1	PEN (10 IU)	20	-	28	12	10	28	-	-	-	-	-	-
2	CIP (5 µg)	20	-	14	12	12	12	16	12	18	14	-	14
3	KAN (30 µg)	14	24	18	10	16	16	20	14	14	10	10	11
4	AMP (10 µg)	-	-	-	-	-	-	-	-	-	-	-	-
5	ST (10 µg)	16	18	16	12	14	12	18	20	20	22	22	16
6	NR (100 µg)	15	18	16	14	12	14	22	18	22	20	20	20
7	NEO (30 µg)	ND	ND	ND	ND	ND	ND	14	16	20	12	20	14
8	CPD (30 µg)	10	-	-	-	-	-	-	10	15	-	-	12
9	OF (5µg)	16	12	10	-	12	-	-	12	12	10	10	11
10	TET (10 µg)	14	18	16	12	10	18	20	12	8	12	15	14
11	SZ (300 µg)	-	-	-	-	-	8	-	-	-	-	-	-
12	NA (30 µg)	-	-	8	8	-	-	-	-	-	-	-	-
13	CH (30 µg)	26	22	24	24	28	24	22	26	25	22	22	24
14	PB (300 µg)	12	-	-	12	-	15	12	12	-	15	10	10
15	ERY (5 µg)	10	12	10	8	10	-	-	10	-	-	8	12
16	AMX (10 µg)	12	10	12	10	12	16	-	-	-	-	12	10
17	GEN (50 µg)	ND	ND	ND	ND	ND	ND	18	20	20	22	22	24
18	VAN (30 µg)	15	17	13	16	16	18	ND	ND	ND	ND	ND	ND
19	MET(5 µg)	10	-	10	-	-	12	ND	ND	ND	ND	ND	ND
20	oxacillin (1 µg)	15	-	16	-	-	16	ND	ND	ND	ND	ND	ND

AM - Amikacin; AP - Ampicillin; CH - Chloramphenicol;; ERY- Erytromycin, GEN - Gentamicin; KAN - Kanamycin; MET - Methicillin; NA - Nalidixic Acid, NR - Nitrofurazone, PEN -Penicillin, SZ - Sulphadiazine, TET - Tetracycline; VAN - Vancomycin , CIP- ciprofloxacin, NEO- neomycin, AMX- amoxicillin, PB- Polymyxin B, NA- Nalidixic acid, OF- Ofloxacin, CPD- cefpodoxime, ST-Streptomycin.

Table 3: Antimicrobial activity of *Syzigium cumini* extracts in various solvents against clinical strains of *Staphylococcus aureus* and *Escherichia coli*.

Ext	Zone of inhibition (mm) (Mean ± SD)						
	Staphylococcus aureus						p-value
	SA3	SA*4	SA10	SA*17	SA*19	MTCC96	<0.001*
PE	20.4±0.95	8.1±0.75	20.0±1.32	22.1±0.6	21.6±1.10	16.3±0.40	<0.001*
EA	0	8.1±0.70	10.3±0.88	15.3±0.6	14.0±0.68	10.0±0.72	<0.001*
AC	8.4±0.75	10.4±0.8	15.5±0.70	18.3±0.7	16.1±0.40	18.1±0.30	<0.001*
ME	8.1±0.30	0	20.4±0.85	20.6±0.9	17.6±0.75	20.4±0.88	<0.001*
ET	10.4±0.70	8.1±0.35	20.0±0.51	10.2±0.4	12.2±0.4	10.2±0.41	<0.001*
DM	0	0	0	0	0	0	0
CH	28±1.52	27±1.50	28.3±1.05	27.6±0.5	28.3±0.66	27.4±0.60	>0.05
Ap	0	0	0	0	0	0	0

Zone of inhibition (mm) (Mean ± SD)						
Escherichia coli						p-value
EC10	EC11	EC12	EC16	EC17	M443	<0.001*
12.5±0.7	14.3±0.6	15.5±0.9	15.8±0.6	14.4±0.6	15.4±0.9	<0.001*
0	8.4±0.7	0	0	8.2±0.15	0	<0.001*
16.1±0.3	17.6±0.9	15.3±0.4	20.4±0.9	22.2±0.5	18.1±0.4	<0.001*
20.4±0.7	19.1±1.9	20.5±0.9	15.4±0.8	15.6±0.9	18.4±0.8	<0.001*
22.4±0.8	20.2±0.4	24.4±0.7	10.5±0.8	10.2±0.4	20.4±0.9	<0.001*
0	0	0	0	0	0	0
28.4±0.6	27.9±0.4	27.4±1.0	27.8±0.4	28.5±0.7	28.1±0.5	>0.05
0	0	0	0	0	0	0

Data represented as Mean±SD. SD: Standard Deviation, *p<0.05, considered statistically significant. Ext= *Syzigium cumini* leaf extracts, PE= Petroleum ether, EA= ethyl acetate, AC = acetone, ME =methanol, ET = ethanol, DM = DMSO, CH = chloramphenicol, AP=ampicillin, SA= methicillin sensitive *S. aureus*, SA*= Methicillin resistant *S. aureus*, M443= MTCC reference strain of *E. coli*, DM= DMSO, EC= *Escherichia coli*.

Table 4a: MIC and MBC values (mg/ml) of *Syzigium cumini* leaves extract against *Staphylococcus aureus* strains compared with Chloramohenicol and Ampicillin.

***S. aureus* strains**	**SA3**		**SA*4**		**SA10**		**SA*17**		**SA*19**		**MTCC 96**	
Solvent extracts	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Petroleum ether	3.125	6.25	1.56	3.12	1.56	3.12	6.25	6.25	1.562	3.12	1.56	3.12
Ethyl acetate	12.5	25.0	25.0	25.0	6.25	12.5	25.0	25.0	12.5	25.0	6.25	12.5
Acetone	3.125	6.25	6.25	12.5	3.12	6.25	6.25	12.5	12.50	12.5	1.56	3.12
Methanol	3.125	12.5	6.25	12.5	6.25	12.5	12.5	12.5	3.125	6.25	6.25	12.5
Ethanol	6.25	12.50	12.5	25.0	3.12	6.25	12.5	25.0	6.25	6.25	3.125	6.25
Chloramphenicol	0.006	0.012	0.01	0.02	0.01	0.02	0.012	0.025	0.006	0.012	0.012	0.05
Ampicillin	0.097	0.097	0.09	0.19	0.09	0.19	0.19	0.19	0.097	0.19	0.19	0.19

MIC and MBC values (mg/ml) of *Syzigium cumini* leaves extract against *Staphylococcus aureus* strains compared with chloramohenicol and ampicillin.

Table 4b: MIC and MBC values (mg/ml) of *Syzigium cumini* leaves extract against *Escherichia coli* strains compared with Chloramohenicol and Ampicillin.

***E. coli* strains**	**EC10**		**EC11**		**EC12**		**EC16**		**EC17**		**MTCC 443**	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Pet. ether	12.5	25.0	6.25	25.0	25.0	25.0	6.25	12.5	12.5	25.0	6.25	25.0
Ethyl acetate	25.0	50.0	12.5	25.0	25.0	50.0	12.5	12.5	6.25	12.5	12.5	25.0
Acetone	6.25	12.5	6.25	12.5	3.12	12.5	3.12	12.5	6.25	25.0	6.25	12.5
Methanol	3.12	6.25	1.56	3.12	3.12	6.25	6.25	12.5	1.56	3.12	3.12	6.25
Ethanol	1.56	3.12	3.12	12.5	3.12	6.25	1.56	3.12	3.12	6.25	1.56	3.12
CH	0.06	0.12	0.12	0.12	0.03	0.06	0.15	0.31	0.03	0.06	0.06	0.12
Ampicillin	0.04	0.09	0.09	0.19	0.09	0.19	0.09	0.19	0.04	0.19	0.04	0.09

MIC and MBC values (mg/ml) of *Syzigium cumini* leaves extract against *Escherichia coli* strains compared with chloramohenicol and ampicillin.

Table 5: Synergistic effect of *Syzigium cumini* leaves petroleum ether extracts (SC) with Ampicillin (AP).

Bacteria	**Strain**	**Extract and antibiotics (alone)**				**Extract+Antibiotics (combination)**	
		MIC		**MBC**		**MIC**	**MBC**
		SC	**AP**	**SC**	**AP**		
S.aureus	SA3	3.12	0.097	6.25	0.19	0.785	1.56
	SA*4	1.56	0.097	3.12	0.19	0.195	0.39
	SA10	1.56	0.097	3.12	0.19	0.195	0.78
	SA*17	6.25	0.195	12.5	0.39	0.785	1.56
	SA*19	1.56	0.097	3.12	0.19	0.195	0.78
	MT96	1.56	0.195	3.12	0.39	0.39	0.39
E. coli	EC10	12.5	0.048	25.0	0.096	1.56	3.12
	EC11	6.25	0.097	25.0	0.19	0.39	3.12
	EC12	25.0	0.097	50.0	0.19	3.12	6.25
	EC16	6.25	0.097	12.5	0.19	0.39	0.78
	EC17	12.5	0.048	25.0	0.096	3.12	3.12
	M443	6.25	0.048	25.0	0.096	0.78	3.12

MT 96= MTCC 96, M 443= MTCC 443, all values are expressed in mg/ml. SA= *S. aureus*, EC= *E. coli*, *= Methicillin resistant *S. aureus.*

MIC of ampicillin was recorded against *S. aureus* was 0.097-0.19 mg/ml which is in agreement with Bonyadi *et al*. (MIC of ampicillin against *S. aureus* was 0.048 mg/ml). The zone of inhibition and minimum bactericidal concentration (MBC) are two different attributes and there is no linear relationship between zone of inhibition and MBC.

Combined therapy has been justified to decrease the bacterial resistance and produce a desirable significant synergistic effect (Esimone *et al.,* 2006). Antibiotic synergism with bioactive plant extracts is useful in treating infectious diseases.

In our study, synergistic effect was verified resulting from the combination of ampicillin with petroleum ether extracts of *Syzigium cumini*. Our results are in agreement with the other studies which reported synergistic effects with significant reduction in the MICs of the antibiotics in combination with different crude plant extracts against *drug resistant bacteria* (Esimone *et al.,* 2006; Braga *et al*., 2005; Sibanda and Okok, 2007). It was observed a significant increase in activity of ampicillin when combined with tested plant extract (Table 5). Combinations of two or more compounds are generally superior to the use of a single compound, especially for the treatment of infections caused by drug resistant bacteria.

CONCLUSION

Our study certifies that the extract of *Syzigium cumini* has a significant potential antimicrobial activity against the multidrug resistant bacteria and also serves an important data regarding the valuable research in treating infectious diseases. It also reveals that the petroleum ether may be an effective solvent in future for antimicrobial studies of *Syzigium cumini*. The research data are also comparable with the common antibiotics used against *S. aureus* and *Escherichia coli*.

A considerable synergism was also obtained between the *Syzigium cumini* and antibiotics used against *S. aureus* and *Escherichia coli*, so *Syzigium cumini* may be an effective alternative of antibiotics in the treatment of infectious diseases.

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