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Research Article

Evaluation of antimicrobial properties of some Indian medicinal plants against MDR pathogenic bacteria

Mohammed Imran[1], Mohd. Imran*[1], Shaista Khan[1], Muhammad Arif[2]

[1]Department of Biosciences, Faculty of Science, Integral University, Lucknow-226026, Uttar Pradesh, India
[2]Faculty of Pharmacy, Integral University, Lucknow-226026, Uttar Pradesh, India

ABSTRACT

The antibacterial potential of thirty five extracts from seven plants was investigated against twelve MDR strains of pathogenic bacteria using agar well diffusion method and microbroth dilution assay. *S. aureus* and *E. coli* strains were isolated from the Clinical samples. It was observed that extracts obtained from the Petroleum ether fraction of *Cordia dichotoma, Azadirachta indica*, *Holoptelia integrifolia* and *Syzigium cumini;* Acetone extracts of *Allium sativum* and *Syzigium cumini;* extracts obtained from methanolic fractions of *Allium sativum, Azadirachta indica, Cordia dichotoma* and *Syzigium cumini;* extracts obtained from ethanoloic fractions of *Azadirachta indica*, *Cordia dichotoma*, and *Syzigium cumini* exhibited strong antibacterial effects against almost all pathogenic bacteia tested. The inhibitory effects of *Syzigium cumini* was found to be higher in all solvents with the inhibition zone 8.0±0.20-24.6±0.55 mm in comparison to inhibition zone recorded for other tested plant extracts. Extract obtained from Methanolic fraction of *Syzigium cumini* exhibited a significant antibacterial activity against all the bacteria tested and the MIC was also obtained ranging from 0.78 to 3.12 mg/ml and 1.56 to 6.25 mg/ml against the *S. aureus* and *E. coli* pathogenic strains respectively. Minimum inhibitory concentration of plant extracts were observed ranging from 0.78 to 25 mg/ml and 1.56 to 25 mg/ml against *S. aureus* and *E. coli* respectively. This study suggests that the compounds obtained from these plants can be used as important therapeutic drugs for curing various multidrug resistant bacterial infections.

Keywords: Plant extract; Therapeutic drug; Clinical samples; *S. aureus; E. coli*.

INTRODUCTION

The overuse of antibiotics in curing the diseases has become the cause of concern for an increase in the development and spread of MDR pathogenic bacterial strains (Harbottle et al., 2006). Most of the antibiotics have become fail in the treatment of various infectious microbes like MRSA, vancomycin-resistant enterococci, multidrug-resistant *E. coli* etc. Generally, bacteria are capable to transmit and acquire resistance to antibiotics due to presence of resistant plasmid, which are utilized as curative agent (Gislene et al., 2000). The worldwide emergence of MDR *S. aureus*, *E. coli, Klebsiella pneumoniae, Haemophilus* and many other bacteria are threatening the medical community and identified as a major remedial problem. MDR strains of *S. aureus* and *E. coli* are frequently spread in health care centers and are main cause of nosocomial infections (Khan et al., 2004; Akram et al., 2007). This increase in the antibiotic resistance in bacteria is a public threat, and it is very essential to solve this problem by search for new antibacterial drugs. Unlike allopathic medicines, antibacterial compounds obtained from plant products are not having side effects and have an important curing property to provide body strength and to increase immunity against many diseases (Chanda et al., 2010; Habbal et al., 2011).

There are many Indian plants like*, Allium sativum, Azadirachta indica, Cordia dichotoma, Holoptelea integrifolia, Ocimum sanctum, Syzigium cumini and Trigonella foenum graecum* etc. having antimicrobial properties (Ahmad et al.,1998, Imran et al, 2017). Medicinal plants (Table 2) have antibacterial compound to cure infections and their compound are used in the treatment of pain or cure of many diseases like diabetes, cardiac problems and various carcinomas (Mohanta et al., 2003). This is the main reason; researchers are focussing their attention to plant derived compounds and are hopeful to develop better medicine against MDR pathogenic microbes (Braga et al., 2005). In this study; we have chosen seven Indian medicinal plant *Allium sativum, Azadirachta indica, Cordia dichotoma, Holoptelea integrifolia, Ocimum sanctum, Syzigium cumini and Trigonella foenum graecum* for the evaluation of their antibacterial potential against MDR pathogenic bacteria. These medicinal plants were selected based on their antimicrobial properties against MDR

* Corresponding Author
Email: imranm@iul.ac.in
Contact: +91-8174061004 Fax: +91-522-2890809
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bacteria across the world. (Chopra et al., 1992; Ahmad et al., 1998; Mehmood et al., 1999). The purpose of this study was to find out the antibacterial potential of seven selected Indian medicinal plants (*Allium sativum, Azadirachta indica, Cordia dichotoma, Holoptelia integrifolia, Ocimum sanctum, Syzigium cumini* and *Trigonella foenum-graecum*) against MDR pathogenic strains of *S. aureus* and *E. coli* and to identify the specific plant fraction responsible for the antibacterial activity.

MATERIALS AND METHODS

Isolation and identification of pathogenic bacteria

Two pathogenic strains of *E. coli* and *S. aureus* (MRSA and MSSA) were used in the determination of antibacterial activity. These clinical strains of bacteria were isolated from the 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 samples collected were transported to Microbiology research laboratory of Integral University and streaked on the mannitol salt agar media and EMB agar media which is specific for the isolation of *S. aureus* and *E. coli*. The plates were kept in incubator at 37°C for 24 hours. The bacterial strains were confirmed by their cultural characteristics, morphological and biochemical properties. Such as catalase, coagulase, gelatin liquefaction, oxidase, Triple Sugar Iron agar (TSI), citrate consumption (Simmon's citrates medium), urease (Christensen's Urea Agar), indole, motility, H_2S production (Sulphide Indole Motility Medium) and sugar fermentation tests. Reference strain MTCC 96 of *S. aureus* and MTCC 443 of *E. coli* were taken from IMTECH, Chandigarh. Culture media were supplied by Himedia Laboratories, Mumbai, India.

Antibiotic susceptibility determination

All the isolates of *S. aureus* and *E. coli* bacteria were tested for their susceptibilty to antimicrobial agents by means of disc diffusion method (Bauer et. al., 1996) as per recommendation of CLSI, 2010. Concentrations of antibiotics were used in µg/disc. Following Antibiotics discs were purchased from Hi-Media Laboratories, Mumbai: methicillin (5µg), oxacillin (1µg), penicillin G (10 IU), erythromycin (5µg), nalidixic acid (30µg), kanamycin (30µg), nitrofurazone (100µg), tetracycline (10µg), polymyxin B (300µg), ciprofloxacin (5µg), ampicillin (10µg), ofloxacin (5µg), sulphadiazine (300µg), amoxicillin (10µg), and cefpodoxime (30µg), chloramphenicol (30µg), Gentamycin (50µg), neomycin (30µg) and vancomycin (30µg). The above antibiotics were aseptically placed on seeded nutrient agar plates. There after all the plates were incubated at 37°C for 24 hrs. The diameters of zones of inhibition surrounding the discs were accurately measured in mm and their relative susceptibility pattern was determined. *S. aureus* isolates were confirmed to be resistant to methicillin if the inhibition zones are <10 mm while susceptible if the zones of inhibitions were ≥ 10 mm.

The isolated *S. aureus* and *E. coli* bacteria were maintained on nutrient agar slants at 4°C for further use.

Collection and authentication of plant sample

Azadirachta indica, Cordia dichotoma, Holoptelia integrifolia, Ocimum sanctum, and Syzigium cumini plant leaves were collected from Integral University, Lucknow campus and road side of Kursi road, Lucknow. *Trigonella foenum- graecum* seeds and buds of *Allium sativum* were collected from local market. The plant materials were identified and authenticated by Dr. Arshad Hussain, Associate Professor and Head Department of Pharmacognosy and Phytochemistry of Integral University, Lucknow (Voucher specimen Number: IU/PHAR/HRB/14/09-18). The plants materials were thoroughly washed with tap water for the removal of debris and dust materials and finally washed with distilled water thereafter dried under shade. The dried plant materials were homogenized to coarse powder.

Preparation of plant extract

Plant parts were dried in an oven at temperature 40-45^{0}C for 2-3 days and coarsely powdered. The powdered plant materials were extracted with petroleum ether, ethyl acetate, acetone, methanol and ethanol as described previously (Imran et al., 2017). The extracts were filtered through Whatman filter paper No. 2 and left to dryness under reduced pressure on rotatory evaporator at 40°c. Then, 100 mg of the dried plant extracts were dissolved in 1 ml sterile distilled water and stored at 4°c for further use.

Phytochemical analysis

Phytochemical investigation of the all extracts was done as described previously for the identification of the various phytoconstituents (Sachan et al., 2011).

Antibacterial test

Antibacterial tests of the selected plant extracts was done by agar well diffusion method (Perez et al., 1990) as previously adopted by Ahmed and Beg (2001). 100 µl of diluted inoculums (10^5 CFU/ml) of tested cultures were swabbed on the top of nutrient agar plates. 6 mm diameter wells were cut into the agar medium and these wells were filled with 50 µl of plant extracts of 100 mg/ ml concentration and solvent blank (DMSO) separately. These plates were incubated at 37°C for 24 hrs. The antibacterial activity was determined by measuring the zone of inhibition against tested bacteria. The antibiotic disc chloramphenicol (30µg) was included in the experiment as positive controls and control without plant extracts, DMSO, solvent included in experiment and ampicillin (10µg) to which strains were resistant were used as negative control. Each experiment was run in triplicate.

Determination of minimum inhibitory concentration (MIC) of different plant extracts

Minimum inhibitory concentration of plant extracts against antibiotic resistant bacterial strains was determined by micro dilution method, dye (p- iodonitro tetrazolium violet) was used as an indicator of growth as previously used by Eloff et al. (1998). Positive control (antibiotic, bacterial inoculum and Mueller-Hinton broth) and sterility and negative control (Mueller-Hinton broth, DMSO) was included in experiment. The test plates were kept in incubator at 37 °C for 18 h. The bacterial activity in the test wells was visualized 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 test plates were incubated again for 30 min, and if color of solution changed to pink it was the sign of reduction of the dye due to bacterial growth. The minimum concentration of dilutions of the extracts that inhibited visible growth of the tested microorganism was considered as the MIC of plant extracts.

RESULTS AND DISCUSSION

Table 1 illustrates the sensitivity of selected bacterial pathogens towards the standard antibiotics. All the tested strains of bacteria were found to be resistant against standard antibiotics penicillin, ampicillin, cefpdoxime, sulphadiazine, nalidixic acid and amoxicillin tested. Chloramphenicol was found to be most effective against *E. coli* and *S. aureus* strains with 22-28 mm zones of inhibition. All *S. aureus* strains were susceptible to vancomycin.

In the present study, all the selected plant materials were extracted with petroleum ether, ethyl acetate, acetone, methanol and ethanol. Antibacterial activity of the extracts was checked against six strains of each *S. aureus* and *E. coli* and the efficacy of plant extracts were qualitatively and quantitatively assessed by the presence or absence of zone of inhibition and MIC values respectively.

Table 3 and 4 show antibacterial activities of plants extracts against the stains tested. Petroleum ether extracts of *Azadirachta indica*, *Cordia dichotoma*, *Holoptelia integrifolia* and *Syzigium cumini;* acetone extracts of *Allium sativum* and *Syzigium cumini;* methanolic extracts of *Allium sativum, Azadirachta indica, Cordia dichotoma* and *Syzigium cumini;* ethanoloic extracts of *Azadirachta indica*, *Cordia dichotoma* and *Syzigium cumini* showed significant antibacterial activity against almost all bacterial strains tested. The potency of *Syzigium cumini* was found to be higher in all solvents with the inhibition zone 8.0±0.20 to 24.6±0.55 mm than all other plant extracts tested.

Petroleum ether extracts of *Azadirachta indica*, *Cordia dichotoma*, *Holoptelia integrifolia* and *Syzigium cumini* showed zone of inhibition ranging from 8.4±0.20 to 24.6±0.55 mm and 10.5±0.70 to 22.5±1.05 mm against *S. aureus* and *E. coli* strains respectively. Methanolic extracts of *Allium sativum, Azadirachta indica, Cordia dichotoma* and *Syzigium cumini* also showed good antibacterial activity with the inhibition zone ranging from 8.13±0.41 to 23.46±0.55 mm and 8.6±1.06 to 20.53±0.6 mm respectively. Ethyl acetate, acetone and ethanol extracts of selected plants also showed inhibition zone ranging from 8.13±0.25 to 22.33±0.80 mm and 8.40±0.72 to 22.40±0.65 mm against *S. aureus* and *E. coli* strains respectively.

The MIC values of plant extracts were also recorded against each tested bacterial strains (Table. 5). All selected plant extracts showed their MIC ranging from 0.78 to 25 mg/ml and 1.56 to 25 mg/ml against *S. aureus* and *E. coli* strains respectively. The highest MIC values were observed with ethyl acetate fractions of all plant extracts while the petroleum ether and methanolic fractions showed a significantly lower MIC values against the strains tested. Methanolic extracts of *Syzigium cumini* showed a highest antibacterial activity against each bacterial strains tested and the MIC value was recorded ranging from 0.78 to 3.12 mg/ml and 1.56 to 6.25 mg/ml against the *S. aureus* and *E. coli* strains respectively. *Trigonella foenum graecum* extracts showed no activity including ethanol extracts of *Allium sativum*, acetone extracts of *Cordia dichotoma* and *Holoptelia integrifolia,* ethyl acetate extract of *Syzigium cumini* against MDR *S. aureus* and *E. coli* strains within the tested concentrations.

For the standard antibiotics (Chloramphenicol) MIC value was recorded against *S. aureus* and *E. coli* strains in the range 0.006 mg/ml to 0.01 mg/ml and 0.03 mg/ml to 0.12 mg/ml respectively.

Various phytochemical compounds are reported having beneficial effects on human health. The extracts of the tested plants were found rich in phenols, saponins, glycosides, flavanoids, alkaloids, steroids, terpenoids, resins and tannins. Antibacterial activity of the plant extracts can be credited due to the presence of these phytochemicals (Cowan, 1999; Padayana et al., 2011).

Our study focused on antibacterial activity of various solvent extracts of some selected plant extracts obtained from different solvents against MDR *S. aureus* and *E. coli.*

Petroleum ether extracts of *Azadirachta indica*, *Cordia dichotoma*, *Holoptelia integrifolia* and *Syzigium cumini;* Acetone extracts of *Allium sativum* and *Syzigium cumini;* methanolic extracts of *Allium sativum, Azadirachta indica, Cordia dichotoma* and *Syzigium cumini;* ethanoloic extracts of *Azadirachta indica*, *Cordia dichotoma*, and *Syzigium cumini* exhibited considerable antibacterial activity against each bacterial strains tested.

Table 1: Multidrug resistance in pathogenic strains of *Staphylococcus aureus* and *Escherichia coli*

S.no	Antibiotics Conc^{n} (µg/ml)	Zone of inhibition (mm)											
		Staphylococcus aureus strains						*Escherichia coli* strains					
		SA1	SA2	SA3	SA4	SA5	MTCC96	EC1	EC2	EC3	EC4	EC5	MTCC443
1	PEN (10 IU)	-	28	20	-	-	28	-	-	-	-	-	-
2	CIP (5 µg)	18	-	20	20	12	12	12	22	-	8	8	14
3	KAN (30 µg)	-	14	14	24	20	16	19	-	13	16	18	11
4	AMP (10 µg)	-	-	-	-	-	-	-	-	-	-	-	-
5	ST (10 µg)	14	15	16	18	18	14	16	15	13	16	24	16
6	NR (100 µg)	16	12	15	18	14	14	16	15	16	15	15	20
7	NEO (30 µg)	ND	ND	ND	ND	ND	ND	14	16	20	12	20	14
8	CPD (30 µg)	8	12	10	-	10	-	14	-	-	10	11	12
9	OF (5µg)	14	12	16	12	-	-	10	16	8	8	10	11
10	TET (10 µg)	16	10	14	18	12	18	11	9	-	16	-	14
11	SZ (300 µg)	-	-	-	-	-	8	-	13	-	-	-	-
12	NA (30 µg)	10	10	8	-	-	-	-	-	-	-	-	-
13	CH (30 µg)	24	22	26	28	26	24	22	26	24	24	26	24
14	PB (300 µg)	15	15	12	-	12	15	13	12	15	13	15	12
15	ERY (5 µg)	12	10	10	12	14	-	18	18	8	8	-	12
16	AMX (10 µg)	14	10	12	10	08	16	-	-	-	-	10	12
17	GEN (50 µg)	ND	ND	ND	ND	ND	ND	20	19	16	16	26	22
18	VAN (30 µg)	16	15	14	17	13	18	ND	ND	ND	ND	ND	ND
19	MET(5 µg)	-	-	-	10	12	12	ND	ND	ND	ND	ND	ND
20	oxacillin (1 µg)	-	9	8	15	10	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, (-) means no zone of inhibition, ND means not detected.

Antibacterial activities of various plants such as *Azadirachta indica*, *Cordia dichotoma*, *Holoptelia integrifolia* and *Syzigium cumini; Allium sativum, Syzigium cumini;* methanolic *Allium sativum, Azadirachta indica, Cordia dichotoma* and *Syzigium cumini;* ethanoloic, *Azadirachta indica*, *Cordia dichotoma*, and *Syzigium-cumini* these selected plants were also previously reported by other workers (Gull et al., 2012; Sethi et al*.,* 2012; Bharathi et al., 2014; Laxmi et al., 2015; Ahmed and Beig, 2001).

In our study all the selected plant materials were extracted with petroleum ether, ethyl acetate, acetone, methanol and ethanol. The potency of *Syzigium cumini* was found to be higher in all solvents with the inhibition zone 8.0±0.20 to 24.6±0.55 mm than all other plant extracts tested.

Petroleum ether extracts of *Azadirachta indica*, *Cordia dichotoma*, *Holoptelia integrifolia* and *Syzigium cumini* showed zone of inhibition ranging from 8.4±0.20 to 24.6±0.55 mm and 10.5±0.70 to 22.5±1.05 mm, while methanolic extracts of *Allium sativum, Azadirachta indica, Cordia dichotoma* and *Syzigium cumini also* exhibited its range from 8.13±0.41 to 23.46±0.55 mm and 8.6±1.06 to 20.53±0.6 mm against MDR *S. aureus* and *E. coli* strains respectively.

Ethyl acetate, acetone and ethanol extracts of selected plants also showed a zone of inhibition ranging from 8.13±0.25 to 22.33±0.80 mm and 8.40±0.72 to 22.40±0.65 mm against *S. aureus* and *E. coli* strains respectively (Imran et al., 2012).

Our results also showed similarity with the various previous reports (Imran et al*.,* 2010; Roula et al., 2010; Yuvraj et al., 2011; Gull et al., 2012; Sethi et al., 2012; Nandedkar et al., 2013; Prasad et al., 2013; Bharathi et al., 2014; Deepak et al., 2014; Laxmi et al., 2015; Tantry et al., 2015; and Mawahib et al., 2015).

Imran et al., (2010) and Bharathi et al., (2014) evaluated the antibacterial activity of *Azadirachta indica* leaf extracts against *S. aureus* where they recorded the zone of inhibition 10 - 25 mm respectively this is closure to our results where zone of inhibition produced by *Azadirachta indica* against *S. aureus* was 8.23±0.35 to 21.9±0.43 mm. (Table. 4)

Sethi et al., (2012) evaluated the antibacterial activity of ethanolic and methanolic extracts of *Azadirachta indica* leaf against *E. coli* and reported a zone of inhibition with 21 mm and 18 mm respectively. Nandedkar et al., (2013) evaluated antibacterial potentiality of the extracts of *Cordia dichotoma* against *S. aureus* and *E. coli* where methanol extract showed 10.2 and 7.2 mm zone of inhibition against *S. aureus* and *E. coli* respectively. Sethi et al., (2012); Bharathi et al., (2014) and Tantry et al., (2015) also reported the antimicrobial

Table 2: Selected Indian medicinal plant species and their reported properties

Plant Name (Family)	Plant Part used	Ayurvedic/Ethnomedicinal uses (Chopra et al., 1992)	Known phytoconstituents of part used
Allium sativum (Liliaceae)	Buds	Used in relieving flatulence, coughs, fevers. Juice used in the treatment of skin diseases, ear aches, indigestion, severe pain in abdomen, treatment for many respiratory diseases and also it makes the immune system strong. (Borek. 2001).	Allicin 0.06-0.1%, Diethyl catechol, protocatechuic acid, allistin 1 and 2, ajoene, allyl propyl sulphide (Harborne and Baxter, 1995; Ratnakar and Murthy, 1995; Nagenawa et al., 1996).
Azadirachta indica (Maliaceae)	Leaves	Anthelmintic, antifungal, antidiabetic, antibacterial, antiviral, prevention of pregnancy, sedative, anti acne. The extract from leaves, fruits and root are used to control leprosy, intestinal helminthiasis and respiratory disorders in children. Neem oil possesses a broad spectrum of antibacterial action against Gram negative and Gram positive bacteria.	Azadirachtin, nimbin salannin, Octacosane (Helmy et al., 2007), Tetratriacontane (Nand et al., 2012), Octadecanoic acid, methyl ester (Senthil Kumar et al., 2012), Hexadecanoic acid, Heptacosane methyl ester (Akpuaka et al 2013).
Cordia dichotoma (Boraginaceae)	Leaves	Used for the treatment of ulcers and headaches. Decoction used for relieving sore throat and as a liver tonic, posses anti inflammatory activity, diuretic and a laxative property. (Ficarra et al., 1995)	It Posses pyrrolizidine, alkaloids, coumarins, flavonoids, saponins, terpenes and sterols (Alarcon, D. L et al., 1994).
Holoptelia integrifolia (Ulmaceae)	Leaves	Paste of leaf, bark stem and seeds is topically used for wound-healing, treatment of leucoderma, cutaneous infections, scabies and ring worm (Mahmud et al., 2010, Benjamin et al., 2009, Sharma et al., 2010). Bark and leaves are antihelminthic, and are used for the treatment of leprosy, skin diseases, diabetes, intestinal disorder, rheumatoid arthritis (Prajapati et al., 2010).	1, 4-naphthalenedione is obtained from leaves of *Holoptelea integrifolia* and is reported to have antibacterial activity against *Staphylocopccus aureus* (Vinod et al., 2010).
Ocimum sanactum (Labiaceae)	Leaves	Used in the treatment of gastric disorders, bronchitis, ear ache. It posses antiseptic, diaphoretic, hepatoprotective, antiasthmatic activity, antibacterial activity, anticancer activity, anticonvulsant activity, antiemetic activity, antithyroidic activity, antianxiety activity, antidepressant activity.	It posses 71.3% euginol, 3.7% carvacrol, 20.4% methyl Euginol, 1.7% cayophyllene, Orientin and Vicenin, (Chopra et al 1992). Aesculectin, Aesculin, Apgenin, Caffiec acid, Chlorgenic acid, Circineol, Gallic acid, Galuteolin, Isorientin, Isovitexin (Mondal et al., 2009).
Syzigium cumini (Myrtaceae)	Leaves	Used as diuretic, stomachic, astringent, antidiabetic, antioxidant property, useful in relieving flatulence, Dysentery. In the Unani system of medicine it is used in the preparation of liver tonic, enriches blood, strengthens teeth and gums and forms good lotion for removing ringworm infection of the head. Antiallergic activity, antibacterial activity, antifungal activity (Naural Product Research, 2010). The essential oil obtained is responsible for antibacterial activity of *Syzigium cumini* leaves.	Terpene, 1- Limonene and dipentene, sesquiterpenes of cadalane type and sesquiterpene of azulene type. Acylated flavanol, glycosides (Timbola et al., 2002), quecetin, myricitin (Gupta et al., 1974), esterase, gallyl corboxylase (Mortan, 1987).
Trigonella foenum graecum (Fabaceae)	Seeds	Seeds are used in the treatment of diabetes and excess of cholesterol in blood stream (Basch, et al., 2003), anti pyretic, antiemetic, tonic, anthelmentic, appetizer, astringent, It is also used for curing leprosy, bronchitis, piles; useful in several heart disease.	Lysine, and L-tryptophan rich proteins, mucilaginous fiber, saponins, coumarin, fenugreekine, nicotinic acid, sapogenins, phytic acid, scopoletin and trigonelline [Billaud, 2001, Ribes et al., 1986].

Table 3: Antibacterial activity of selected plant extracts against multi drug resistant pathogenic strains of Escherichia coli (Zone of inhibition (mm) (mean ± SD)

Plants	Fractions	EC1	EC2	EC3	EC4	EC5	MTCC 443	p-value
A*. sativum	PE	ND	ND	ND	ND	ND	ND	-
	EA	12.4±0.91	-	10.5±0.7	-	12.5±0.65	-	<0.001
	AC	-	10.37±0.60	8.47±0.85	-	-	-	<0.001
	ME	-	-	8.6±1.06	-	-	-	<0.001
	ET	-	-	-	-	-	-	-
A. indica	PE	16.5±0.75	16.47±0.61	15.6±0.91	16.33±0.87	10.5±0.70	14.37±0.80	<0.001
	EA	-	-	-	-	8.47±0.81	10.47±0.70	<0.001
	AC	13.4±0.65	14.5±0.70	14±0.35	12.6±0.80	10.57±0.97	12.43±0.97	<0.001
	ME	15.43±0.80	12.40±0.65	15.53±0.90	8.37±1.10	-	8.40±0.72	<0.001
	ET	8.43±0.86	10.40±0.85	8.40±0.70	8.53±0.80	-	8.40±0.72	<0.001
C. dichoto-ma	PE	16.37±1.05	16.43±0.85	15.40±0.77	18.46±0.80	10.5±0.70	18.47±0.70	<0.001
	EA	8.63±0.75	-	-	-	-	8.4±0.91	<0.001
	AC	-	-	-	-	-	-	-
	ME	16.5±0.65	10.47±0.70	8.46±0.83	8.67±0.90	10.36±0.73	10.43±0.60	<0.001
	ET	12.40±0.75	10.40±0.65	10.47±0.85	8.47±0.81	10.33±0.61	8.60±0.85	<0.001
Holoptelia integrifolia	PE	16.4±0.60	18.30±0.75	18.1±0.45	20.46±0.90	15.53±0.70	14.5±0.55	<0.001
	EA	-	-	-	-	8.37±0.66	-	<0.001
	AC	-	-	-	-	-	-	-
	ME	16.33±0.75	10.57±0.86	-	-	8.53±0.7	8.5±0.78	<0.001
	ET	-	12±0.20	10.40±1.20	-	-	-	<0.001
O. sanctum	PE	18.06±0.30	16.06±0.20	14.63±0.85	20.3±0.85	18.46±0.90	18.43±0.85	<0.001
	EA	-	8.53±0.45	-	-	8.33±0.66	-	<0.001
	AC	-	12.46±0.70	-	10.46±0.65	-	10.37±0.66	<0.001
	ME	10.46±0.70	10.7±0.66	-	-	8.47±0.70	8.57±0.55	<0.001
	ET	8.5±0.79	-	10.73±0.30	-	8.33±1.33	8.56±0.75	<0.001
S. cumini	PE	12.4±1.10	14.3±1.15	15.37±0.81	20.6±0.79	22.5±1.05	18.57±0.78	<0.001
	EA	-	-	-	-	-	-	-
	AC	16.47±0.70	17.97±0.35	15.63±0.88	20.6±0.72	22.4±0.65	18.47±0.80	<0.001
	ME	20.53±0.61	18.5±0.76	20.5±0.5	15.6±0.80	16.5±0.81	18±0.60	<0.001
	ET	22.36±1.26	20.76±0.55	22.17±0.60	10.6±0.72	10.63±0.77	20.43±0.77	<0.001
Trigonella Foenum Graecum	PE	-	-	-	-	-	-	-
	EA	-	-	-	-	-	-	-
	AC	-	-	-	-	-	-	-
	ME	-	-	-	-	-	-	-
	ET	-	-	-	-	-	-	-
DMSO		-	-	-	-	-	-	-
Ampicillin(10 µg)		-	-	-	-	-	-	-
CH (30 µg)		26±1.53	28±1.05	28±0.60	27±0.50	26±0.67	26±1.0	<0.001

PE = petroleum ether, EA = ethyl actate, AC = acetone, ME = methanol, ET = ethanol, CH = chloramphenicol, DMSO = Dimethyl sulphoxide, *A* = *Azadirachta,* ***A**** = *Allium*, Data represented as Mean±SD. SD: Standard Deviation, *p<0.05, considered statistically significant, Diameter of inhibition zone = The mean of three replicates of recorded zones of inhibition, ND = not detected, (-) = Absent/No activity.

potential of *Ocimum sanctum* leaf extracts with the zone of inhibition ranging from 13-21mm against *S. aureus* and *E. coli* respectively which is similar to our findings 8.0±0.40 to 20.4±0.91 mm against the same organisms.

Petroleum ether leaf extracts of *Syzigium cumini* showed effective activity against *S. aureus* and *E. coli* with a zone of inhibition ranging from 8.0±0.20 to 24.6±0.55 mm which was quite similar with the previous studies (Deepak et al., 2014; Yuvraj et al., 2011and Prasad et al., 2013). Our findings of methanolic extract of *Syzigium cumini* leaf against MDR *S. aureus* and *E. coli* were also found to be similar with Deepak et al., (2014); Elfadil et al., (2015); Pranoti et al., (2014); Satyawathi et al., (2014) and Yuvraj et al., (2011).

Similar study of antibacterial activity of crude extracts, methanolic and petroleum ether fractions of *Trigonella foenum graecum* was also recorded by Roula et al., (2010) and Mawahib et al., (2015). Minimum inhibitory concentration of plant extracts were found in the

Table 4: Antibacterial activity of selected plant extracts against multi drug resistant pathogenic strains of *Staphylococcus aureus* (Zone of inhibition (mm) (mean ± SD)

Plants	Fractions	SA1	SA2	SA3	SA4	SA5	MTCC 96	p-value
A. sativum*	PE	ND	ND	ND	ND	ND	ND	-
	EA	18.13±0.50	17.62±0.81	15.4±0.96	15.13±0.30	16.46±0.70	14.73±0.51	<0.001
	AC	10.43±0.77	20.26±0.41	8.66±0.60	17.43±0.61	12.16±0.35	10.36±0.97	<0.001
	ME	15.23±0.40	20.4±0.72	15.23±0.47	15.16±0.30	10.53±0.70	16±0.36	<0.001
	ET	15.76±0.55	20.46±0.75	12.76±0.66	12.1±0.30	10.1±0.55	17.7±0.46	<0.001
A. indica	PE	16±0.20	8.4±0.20	20.16±0.40	10.43±0.35	16.3±0.45	20.1±0.40	<0.001
	EA	18.23±0.35	16.93±0.35	-	-	16.06±0.35	-	<0.001
	AC	10.2±0.40	8.23±0.35	-	12.2±0.52	14.2±0.46	-	<0.001
	ME	10.3±0.46	15.13±0.38	21.9±0.43	10.16±0.58	12.1±0.30	-	<0.001
	ET	-	16.13±0.25	20.43±0.87	10.66±0.57	15.9±0.55	11.2±0.40	<0.001
C. dichotoma	PE	12.16±0.40	17.93±0.30	20.26±0.75	20.86±0.30	18.1±0.30	16.23±0.40	<0.001
	EA	-	-	12.36±0.66	-	-	-	<0.001
	AC	-	-	-	-	-	-	-
	ME	21.96±0.38	20.03±0.40	17.83±0.75	21.63±0.65	8.33±0.35	12.36±0.15	<0.001
	ET	22.16±0.35	15.06±0.30	12.16±0.45	19.6±0.85	10.76±0.47	14.63±0.65	<0.001
H. integrifolia	PE	-	-	20.23±0.55	16.76±0.76	10.8±0.61	11.5±0.75	<0.001
	EA	12.1±0.36	8.03±0.25	-	-	-	-	<0.001
	AC	-	8.1±0.36	-	-	-	-	<0.001
	ME	15.43±0.65	-	14.06±0.32	17.16±0.40	11.93±0.30	15.66±0.75	<0.001
	ET	16.43±0.75	12.06±0.45	15.13±0.41	15.3±0.95	12.43±0.70	18.56±0.85	<0.001
O. sanctum	PE	-	20.36±0.90	-	-	-	8.0±0.40	<0.001
	EA	-	10.46±0.70	8.3±0.98	-	12.26±0.87	10.46±0.70	<0.001
	AC	-	-	8.3±0.62	-	-	-	<0.001
	ME	8.46±0.65	16.3±0.75	18.4±0.80	10.43±0.77	-	-	<0.001
	ET	10.3±0.81	20.4±0.91	-	-	10.5±0.75	10.5±0.81	<0.001
S. cumini	PE	20.46±0.76	10.5±0.75	20.6±0.7	24.6±0.55	22.23±0.70	20.33±0.61	<0.001
	EA	-	8.06±0.20	10.36±0.66	15.43±0.65	8.3±0.75	12.3±0.95	<0.001
	AC	8.13±0.25	18.43±0.70	20.26±0.96	20.36±1.05	23.33±0.80	20.5±0.95	<0.001
	ME	8.13±0.41	18.53±0.80	20.5±0.98	20.43±0.85	23.46±0.55	20.56±0.95	<0.001
	ET	10.53±0.75	8.56±0.86	20.46±0.76	10.46±0.90	22±0.40	22.36±1.26	<0.001
Trigonella Foenum Graecum	PE	-	-	-	-	-	-	-
	EA	-	-	-	-	-	-	-
	AC	-	-	-	-	-	-	-
	ME	-	-	-	-	-	-	-
	ET	-	-	-	-	-	-	-
DMSO		-	-	-	-	-	-	-
Ampicillin (10 µg)		-	-	-	-	-	-	-
CH (30 µg)		26±1.5	28±1.05	28±0.60	27±0.50	26±0.66	26±70	<0.001

A= Allium*, A = *Azadirachta*, C = *Cordia*, O = *Ocimum*, S = *Syzigium*, T. f = *Trigonella foenum*

PE = Petroleum ether, EA = Ethyl acetate, AC = Acetone, ME = Methanol, ET = Ethanol, CH = Chloramphenicol.

(-) = No Activity/Absent, ND = Not determined.

range 0.78-25 mg/ml and 1.56-25 mg/ml against *S. aureus* and *E. coli* respectively. Methanolic extracts of *Syzigium cumini* exhibited minimum MIC ranging from 0.78 to 3.12 mg/ml and 1.56 to 6.25 mg/ml against the *S. aureus* and *E. coli* strains, respectively. The other extracts of *Allium sativum, Azadirachta indica, Cordia dichotoma, Holoptelia integrifolia* and *Ocimum sanctum* in various solvents showed significant antibacterial activity in a range of 1.25 to 25 mg/ml. our findings are similar to the other reports (Prasannabalaji et al., 2012, Bishnu et al., 2011, Solomacos et al., 2008, Kavishankar et al, 2011, Bimlesh et al., 2011, Mahesh et al., 2015, Dahia and Purkayastha 2012).

Prasannabalazi et al., (2012) reported 0.156 mg/ml and 0.625 mg/ml MIC of *Ocimum sanctum* leaves extracts in methanol extract against *S. aureus* and *E. coli* respectively. Solomacos et al., (2008) reported MIC ranging from 30-400 mg/ml of *S. cumini* leaves in ethanol extracts against *S. aureus*.

Kavishanker et al., (2011) reported 50 mg/ml MIC of *Azadirachta indica* and *S. cumini* leaves methanolic

Table 5: MIC of plant extracts against the selected MDR strains of *Staphylococcus aureus* and *Escherichia coli*

Plant		MIC (mg/ml)											
		Staphylococcus aureus strains						*Escherichia coli* strains					
		S1	S2	S3	S4	S5	MTCC 96	E1	E2	E3	E4	E5	MTCC 443
Allium sativum	P. ether	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	E. acetate	3.12	3.12	6.25	12.5	3.12	6.25	6.25	-	12.5	-	6.25	-
	Acetone	12.5	1.56	12.5	3.12	6.25	12.5	25.0	12.5	25.0	25.0	15.0	12.5
	Methanol	6.25	3.12	3.12	6.25	12.5	3.12	25.0	-	25.0	-	-	-
	Ethanol	6.25	3.12	12.5	12.5	12.5	1.56	25.0	-	-	-	-	-
Azadirachta indica	P. ether	6.25	12.5	3.12	25.0	3.12	3.12	3.12	3.12	1.56	3.12	12.5	3.12
	E. acetate	1.56	6.25	25.0	25.0	6.25	25.0	25.0	25.0	6.25	25.0	25.0	12.5
	Acetone	-	25.0	12.5	6.25	12.5	-	12.5	6.25	25.0	6.25	12.5	6.25
	Methanol	12.5	6.25	1.56	12.5	6.25	-	6.25	6.25	3.12	12.5	-	25.0
	Ethanol	25.0	3.12	3.12	25.0	3.12	12.5	25.0	12.5	12.5	25.0	-	25.0
Cordia dichotoma	P. ether	3.12	3.12	3.12	3.12	6.25	3.12	1.56	3.12	3.12	3.12	12.5	1.56
	E. acetate	12.5	25.0	6.25	12.5	25.0	-	12.5	25.0	12.5	25.0	12.5	25.0
	Acetone	25.0	12.5	25.0	25.0	-	-	25.0	6.25	12.5	25.0	25.0	25.0
	Methanol	12.5	1.56	3.12	1.56	25.0	6.25	1.56	12.5	3.12	-	25.0	12.5
	Ethanol	25.0	6.25	12.5	3.12	12.5	6.25	6.25	6.25	3.12	-	25.0	12.5
Holoptelia integrifolia	P. ether	25.0	25.0	1.56	3.12	12.5	12.5	1.56	1.56	1.56	1.56	3.12	3.12
	E. acetate	6.25	25.0	12.5	25.0	-	-	25.0	25.0	12.5	-	25.0	25.0
	Acetone	25.0	12.5	-	12.5	-	-	25.0	25.0	12.5	-	12.5	25.0
	Methanol	12.5	6.25	6.25	3.12	12.5	3.12	6.25	12.5	6.25	-	12.5	12.5
	Ethanol	6.25	12.5	6.25	3.12	6.25	1.56	25.0	6.25	3.12	-	25.0	25.0
Ocimum sanctum	P. ether	25.0	1.56	25.0	25.0	25.0	25.0	1.56	3.12	3.12	1.56	1.56	1.56
	E. acetate	25.0	6.25	12.5	12.5	12.5	12.5	25.0	25.0	6.25	25.0	12.5	25.0
	Acetone	12.5	6.25	12.5	25.0	-	-	6.25	6.25	1.56	6.25	25.0	6.25
	Methanol	6.25	3.12	3.12	12.5	25.0	-	6.25	12.5	12.5	-	25.0	12.5
	Ethanol	12.5	1.56	25.0	25.0	12.0	12.5	12.5	25.0	3.12	-	25.0	12.5
Syzigium cumini	P. ether	1.56	6.25	1.56	1.56	1.56	3.12	3.12	6.25	12.5	3.12	6.25	3.12
	E. acetate	12.5	12.5	12.5	6.25	25.0	6.25	12.5	25.0	12.5	12.5	12.5	25.0
	Acetone	12.5	6.25	3.12	3.12	3.12	1.56	3.12	3.12	3.12	3.12	1.56	1.56
Trigonella foenum raecum	P. ether	-	-	-	-	-	-	-	-	-	-	-	
	E. acetate	-	-	-	-	-	-	-	-	-	-	-	
	Acetone	-	-	-	-	-	-	-	-	-	-	-	
	Methanol	-	-	-	-	-	-	-	-	-	-	-	
	Ethanol	-	-	-	-	-	-	-	-	-	-	-	
DMSO		-	-	-	-	-	-	-	-	-	-	-	
CH		0.006	0.01	0.01	0.012	0.006	0.012	0.06	0.12	0.03	0.15	0.03	0.06

P = Petroleum, E = Ethyl, CH = Chloramphenicol. (-) Means No Activity, ND = Not determined.

extracts against *E. coli*. Bimlesh et al., (2011) reported minimum 0.05 mg/ml MIC of *Holoptelia integrifolia* leaves extracts in acetone extracts against *S. aureus,* while MIC level 0.5 mg/ml and o.1mg/ml were recorded in petroleum ether and methanol extracts against *E. coli* respectively. Mahesh Chandra et al., (2015) reported the MIC level of *Azadirachta indica* and *S. cumini* in ethanol extracts by 1.56 mg/ml and 0.78 mg/ml respectively against *S. aureus* strains.

Dahia and Purkayastha (2012) reported the MIC of *Azadirachta indica* leaves extracts in Ethanol and methanol by 1.56 mg/ml and 1.56-12.5 mg/ml against *S. aureus* and *E. coli* respectively. Ethanolic and methanolic extracts of *Ocimum sanctum* showed their MIC

ranging from 0.39-3.12 mg/ml and 3.12- 12.5 mg/ml against *S. aureus* and *E. coli* respectively, while methanol extracts showed MIC ranging from 0.78-6.25 and 1.56-12.5 mg/ml against the respective test strains, so the plant extract of *Syzigium cumini* was found to be most active against the MDR strains of *S. aureus* and *E. coli*. Our study also provides a new way of searching antimicrobial activity of various Indian medicinal plants with different solvents against the respective pathogenic test MDR bacterial strains.

CONCLUSION

The findings of this study clarify that the tested plants can be utilized as valuable effective antimicrobial drugs for the treatment *S. aureus* (MRSA and MSSA) of infectious diseases caused by multidrug resistant *S. aureus* (MRSA and MSSA) and *E. coli*. The extract of *Syzigium cumini* was found to be the most effective antimicrobial rather than other plants tested. *Syzigium cumini* leaf extracts may be the drug of choice to treat the *S. aureus* and *E. coli* infections in future.

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