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Antibacterial activities of Indian medicinal plants against nosocomial multidrug-resistant bacteria

Phule DB[1], Manwar AV[2]
[1, 2] Dnyanopasak College of Arts, Commerce, Science, and Technology, College Parbhani Maharashtra, Maharashtra, India

Abstract
Aim: To investigate the antibacterial efficiency of aqueous extract of 12 medicinal plants of India against nosocomial pathogens.
Method: The aqueous extracts of 12 medicinal plants tested solely and in combination with synthetic antimicrobial drugs against, MDR *E. coli, K. pneumomiae, S. aureus, E. aerogenes* and *Pseudomonas aeruginosa* capable of producing vancomycin resistance, β-lactamase carbapenems and Metallo- β-lactamases.
Result: As per MIC assay almost all plant extracts able to inhibit the growth of nosocomial MDR pathogens except *Azadirachta indica* when tested solely in MIC assay. The better MIC recorded (11-50 µg/ml) with the synergistic application of plant extracts with antibiotics thus noted to control MDR pathogens convincingly.
Conclusion: The investigation of plant species available in India showcased capabilities to control MDR nosocomial Gram-positive and Gram-negative bacteria. Favourable antibacterial activities displayed by aqueous extract of medicinal plants once tested solely or in combination with synthetic drugs.

Keywords: medicinal plants, nosocomial pathogens, multidrug-resistant bacteria

Introduction

The microbial species better planned to control by antibiotics, but rising antibiotic resistance is challenging the healthcare sectors to manage resistance problem. The severe spread of multidrug-resistant bacteria expected in both developed and developing countries and prominent with their hospitals and community settings (Eliopoulos *et al.*, 2003) [10]. Till date, we have options for antibiotics; still, the infectious complications increasing the mortality and morbidity among hospitalized patients. The practitioner is now opting second or third-generation antibiotics for treating these patients, but those are bound to have a higher risk of side effects. To address the threatening resistance situation, strategies now invited, mainly those focusing on how drug functions, developed mechanism of resistance and developing new drugs and novel therapeutic strategies. The answer is possible with identifying new compounds available in plant species having the antimicrobial ability and since plants are diverse in chemical composition, making them a rich source of biochemicals (Nascimento *et al.*, 2000) [18]. The success of ancient herbal medicine assists us in developing modern medications. With the hundreds of years of knowledge with traditional medicines, it catalyzes the growth of modern medicines. The medicinal plants are used for centuries for treating human diseases because of their therapeutic values. The numerous bioactive compounds are now antibacterial, antifungal, and antiprotozoal in an activity once utilized locally or systemically (Cowan, 1999) [7]. With the rise in antibiotic resistance in the 20th century, the mindset has shifted towards the use of traditional medicine as alternative medicine. Among plants, antimicrobial plants prominently investigated for potential (Cowan, 1999) [7]. Plants popularized as folk medicines are rich in volatile oils, alkaloids, and polyphenols, while others noted with finished products category as phytomedicines (Kaushik and Goyal, 2008) [14]. They are promising to control many diseases simultaneously assures reduced or no side effects mainly associated with synthetic drugs—the most promising plant biomolecules linked to having antimicrobial activity.

The less prone to the infection itself indicates the success of plant for developed defense system by these molecules (Bolla *et al.*, 2011) [5]. The concept of new antimicrobial defines their features to inhibit the bacterial growth or kill them, without producing any toxicity or minimum in its form (Lee *et al.*, 1998) [15]. Many bioactive compounds inhibit the growth of bacteria by blocking metabolic processes, either by itself or in combination with other drugs (Sivanathan, 2013). To become a successful alternative, medicinal plant bioactive should have alternate target site than a drug molecule. However, many times we lack in such information (Lee *et al.*, 1998) [15]. In today's success stories traditionally, known plants are nominated for alternatives to the synthetic antibacterial and antifungal medications (Elfahmi *et al.*, 2014 [9]; Tan and Chan, 2014 [24]; Panda, 2014 [19]; Akhtar and Mirza, 2018 [2]; Raut and Karuppayil, 2014) [22].

India is one of the countries of Asia having diverse and rich flora and numerous plant species linked with therapeutic values. Still, most of them are not scientifically linked but used traditionally without knowing their exact functioning agents (Nair *et al.*, 2005 [17]; Pavithra *et al.*, 2010 [20]; Samy *et al.*, 1998; Prasannabalaji *et al.*, 2012) [21]. The Infectious Disease Society of America nominated the challenging pathogens which are tough to control such as vancomycin-resistant *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, carbapenem-resistant *Enterobacteriaceae*, Extended -spectrum β-lactamase *Enterobacteriaceae*, mettalo-β-lactamase producing *Pseudomonas aeruginosa* and *Acinetobacter baumanii* (Boucher *et al.*, 2009) [6]. In India also the status of MDR bacterial species is reaching towards log phase with every

data record giving alarming situation to handle in future (Dubey *et al*., 2013 [8]; Jangra *et al.*, 2018) [12].

Looking at the present scenario, the study aimed to investigate the antibacterial activity of aqueous extract of twelve plant extracts and with selected synergistic combinations of a plant extract with antibiotics better to control of MDR nosocomial pathogens has been reported.

Method

Sampling of nosocomial pathogens

In the present study private hospital of Parbhani, Maharashtra (India) sampled for nosocomial bacterial pathogens. By using selective media (MacConkey agar, Blood agar, EMB agar, MS agar, Hichrome salt agar and CLED) prominent isolation of *E. coli*, *K. pneumoniae*, *S. aureus*, *E. aerogenes* and *P. aeruginosa* carried out further confirmed by 16S rRNA gene sequencing.

Preparation of plant extract:

As per antibiotic sensitivity assay, those nosocomial pathogens isolates registered as multidrug-resistant tested for MIC for plant extract and as a combination of the plant (50 µg/500 µl) with antibiotics (50 µg/500 µl).

In the process, 12 plant species namely *Murraya koenigii*, *Asparagus racemosus*, *Glycyrrhiza glabra*, *Phyllanthus emblica*, *Trigonella foenum-graceum*, *Ocimum tenuiflorum*, *Withania somnifera*, *Terminalia chebula*, *Momordica charantia*, *Azadirachta indica*, *Terminalia bellirica* and *Aegle marmelos*. The whole plant parts were sun-dried and powdered using the grinder and passed through a sieve to get the fine powder.

Plant extract dried powder (100 g) suspended in 95% ethanol at a ratio of 1:10 (w/v) for 72 hours. The preparation filtered and concentrated under reduced pressure using a rotary evaporator set at 40℃. The crude extract air-dried and upon air-drying extract suspended in DMSO, and filtered through Whatman filter paper.

MIC assay

The multidrug-resistant nosocomial pathogens tested for performance of plant extract and synergy effect of extract plus antibiotics by MIC assay.

The bacterial species maintained in nutrient broth at 37℃ for 24-48 hours and used for the MIC assay. The pure effect of antibiotics tested by MIC once its final dose set as 100 µg/ml in an aqueous medium. The synergistic activity of *Murraya koenigii*, *Glycyrrhiza glabra*, *Withania somnifera*, *Azadirachta indica*, and *Aegle marmelos* checked with antibiotics Vancomycin, Amphotericin and Kanamycin.

Process of MIC assay

The nosocomial pathogens maintained on nutrient agar and set at the mid-log stage then frozen at -80℃ to standardize the inoculums. During testing, test compounds set to two-fold dilution and loaded to 96 well plates. The frozen aliquots of bacterial cultures thawed and O.D. set at 600 nm of 0.0025. The broth then added to the plates to reach the final volume of 500 µl (24 well plates). The final preparation allowed to incubate for 144 hours at 37℃ and then supplemented with resazurin 0.025% wt/vol to 1/10 of well volume. With an overnight incubation, the fluorescence of the resazurin metabolite resofurin checked by TECAN infinite M200 microplate reader set at 560 nm and emission at 590 nm. The MIC recorded as the final concentration that preventing resazurin turnover from blue to pink. Then value confirmed by the level of fluorescence determined by the microplate reader.

Result

MIC of Plant Extract

MIC values recorded for the plants tested against nosocomial pathogens and results noted as follows:

Murraya koenigii

As per MIC testing plant, *M. koenigii* prominent to control *P. aeruginosa* (MIC 12 mg/ml) but failed to inhibit *E. coli*, *K. pneumoniae*, *S. aureus* and *E. aerogenes* (Table 1 and Fig. 1).

Asparagus Racemosus

The better success recorded with *A. racemosus* since all nosocomial pathogens controlled with MIC 12-13 mg/ml (Table 1 and Fig. 1).

Glycyrrhiza glabra

The multidrug resistance *S. aureus* registered to be sensitive (12 mg/ml) with plant *G. glabra* while all others remained resistance (Table 1 and Fig. 1).

Phyllanthus emblica

The *P. emblica* recorded with variable MIC values against *P. aeruginosa*, *K. pneumoniae*, *E. aerogenes*, *S. aureus* and *E. coli* with values 12, 16, 17, 20 and 21 mg/ml, respectively (Table 1 and Fig. 1).

Trigonella foenum-graceum

The lowest MIC recorded with *Trigonella foenum-graceum* as 11 mg/ml against *E. aerogenes*, *P. aeruginosa*, further with 12 mg/ml *S. aureus*, a plant better controlled these pathogens (Table 1 and Fig. 1).

Ocimum tenuiflorum

The *O. tenuiflorum* found to be the better controller for all nosocomial pathogens with MIC values as 12, 13, 15, 16 and 16 mg/ml for *P. aeruginosa*, *E. aerogenes*, *E. coli*, *K. pneumoniae*, and *S. aureus*, respectively (Table 1 and Fig. 1).

Withania somnifera

W. somnifera controlled *P. aeruginosa*, *S. aureus* and *K. pneumoniae* with MIC values as 12, 13 and 13 mg/ml, respectively (Table 1 and Fig. 1).

Terminalia chebula

Plant *T. chebula* registered higher MIC values as compared to other plants with 22, 22, 23, 20 and 28 mg/ml against *E. coli*, *K. pneumoniae*, *S. aureus*, *E. aerogenes* and *P. aeruginosa*, respectively (Table 1 and Fig. 1).

Momordica charantia

M. charantia selectively controlled the growth of *K. pneumoniae*, *E. aerogenes*, and *P. aeruginosa* with MIC value 10, 11 and 12 mg/ml, respectively (Table 1 and Fig. 1).

Azadirachta indica

In a negative response, *A. indica* failed to control the growth of all nosocomial pathogens, and hence MIC could not be recorded (Table 1 and Fig. 1).

Terminalia bellirica

T. bellirica succeeded to control the growth of *E. coli*, *K. pneumoniae*, *S. aureus*, *E. aerogenes* and *P. aeruginosa* with MIC values 24, 18, 19, 20 and 17 mg/ml, respectively (Table 1 and Fig. 1).

Aegle marmelos

The better growth control recorded against *K. pneumoniae* and *P.*
Aeruginosa with MIC value 12 and 11 mg/ml, respectively (Table 1 and Fig. 1).

Table 1: MIC assay of plant extracts against nosocomial pathogens

Sr.No.	Name of plants	Minimum Inhibitory Concentration of extract in mg/ml					
		DP1	DP2	DP3	DP4	DP5	DP6
1	Kadi patta (*Murraya koenigii*)	-	-	-	-	12	-
2	Shatavari (*Asparagus racemosus*)	12	13	12	12	13	12
3	Jeshthamadh (*Glycyrrhiza glabra*)	-	-	12	-	-	-
4	Awala (*Phyllanthus emblica*)	21	16	20	17	12	-
5	Methi (*Trigonella foenum-graecum*)	14	14	12	11	11	14
6	Tulsi (*Ocimum tenuiflorum*)	15	16	16	13	12	-
7	Ashwagandha (*Withania somnifera*)	-	13	13	-	12	18
8	Harda (*Terminalia chebula*)	22	22	23	20	28	-
9	Karela (*Momordica charantia*)	-	10	-	11	12	12
10	Neem (*Azadirachta indica*)	-	-	-	-	-	-
11	Bheda (*Terminalia bellirica*)	24	18	19	20	17	16
12	Belpatra (*Aegle marmelos*)	-	12	-	-	11	-

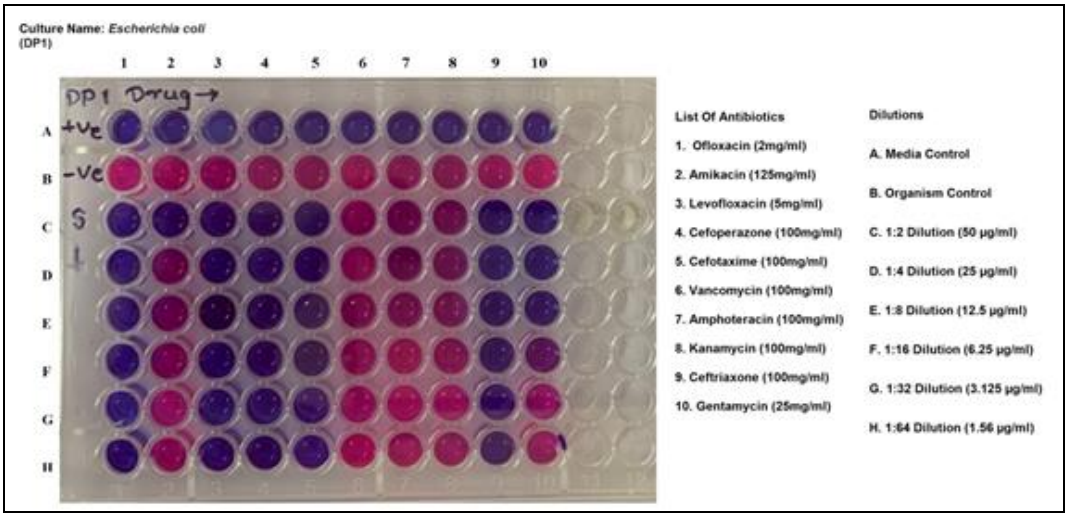

Fig 1a: Minimum inhibitory concentration assay of *Escherichia coli* (DP1) with antibiotics

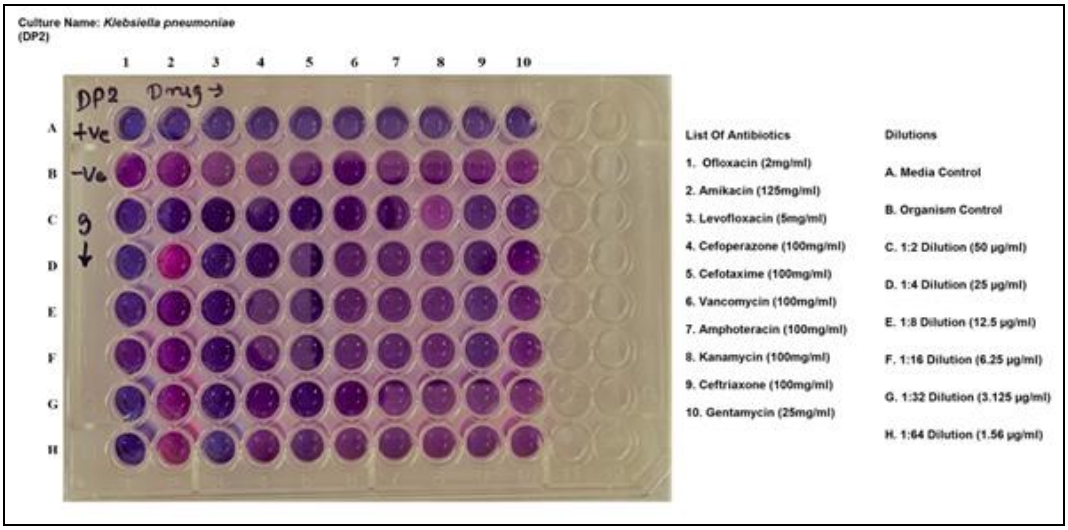

Fig 2: Minimum inhibitory concentration assay of *Klebsiella pneumonia*e (DP2) with antibiotics

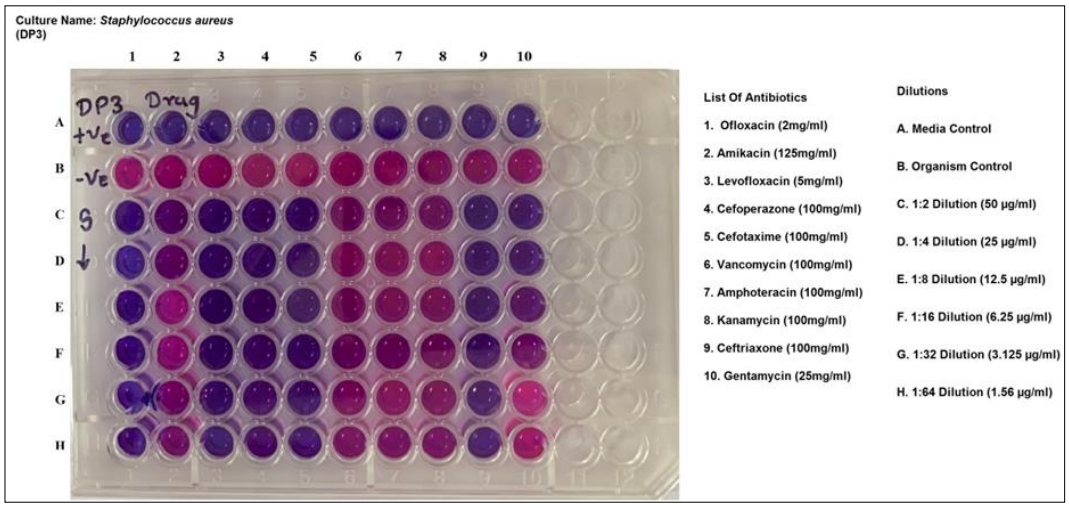

Fig 3: Minimum inhibitory concentration assay of *Staphylococcus aureus* (DP3) with antibiotics

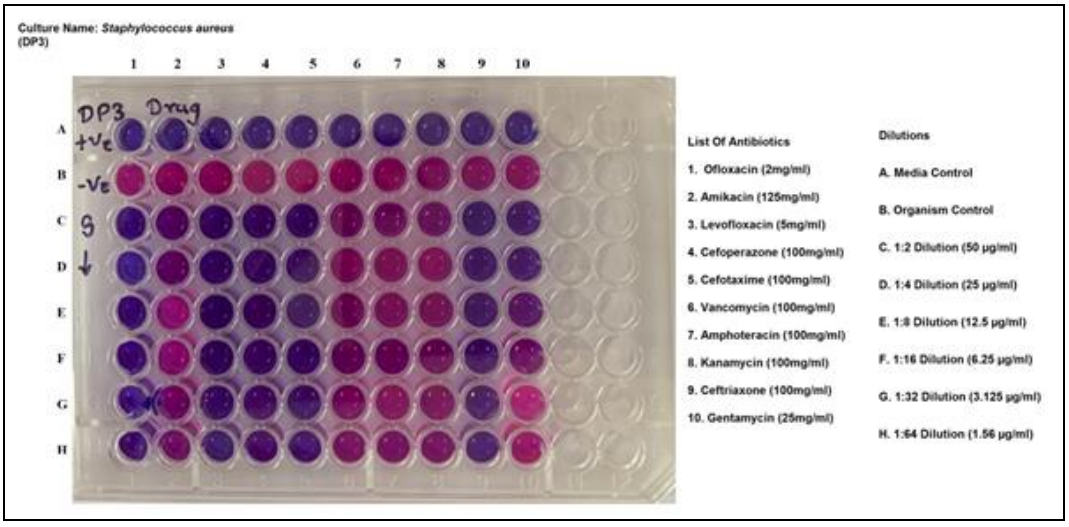

Fig 4: Minimum inhibitory concentration assay of *Enterobacter aerogenes* (DP4) with antibiotics

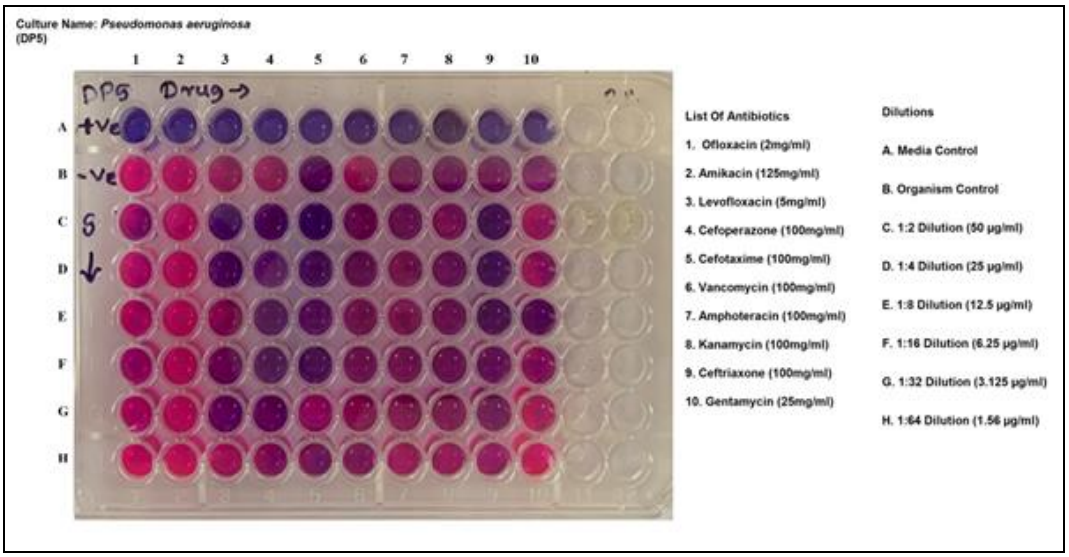

Fig 5: Minimum inhibitory concentration assay of *Pseudomonas aeruginosa* (DP5) with antibiotics

MIC of synergy

To counter the increasing drug resistance, the use of antibiotics with plant extracts advocated to investigate in detail. In the study, the synergy of drug with selected plant extract noted in detail.

E. coli

As per synergistic MIC assay, *E. coli* better controlled by Neem + Kanamycin followed by Jesthamadh + Vancomycin and least by Ashwagandha + Amphotericin with MIC values 12.5, 25, 50 µg/ml, respectively (Table 2a and Fig. 2a).

Table 2: MIC of Synergistic sets of antibiotics plus plant extract against E. coli DP1

Sr. No.	Combinations	Minimum Inhibitory Concentration in µg/ml
1	Kadi patta + Vancomycin	-
2	Jeshthamadh + Vancomycin	25
3	Ashwagandha +Amphotericin	50
4	Neem + Kanamycin	12.5
5	Belpatra + Amphotericin	-

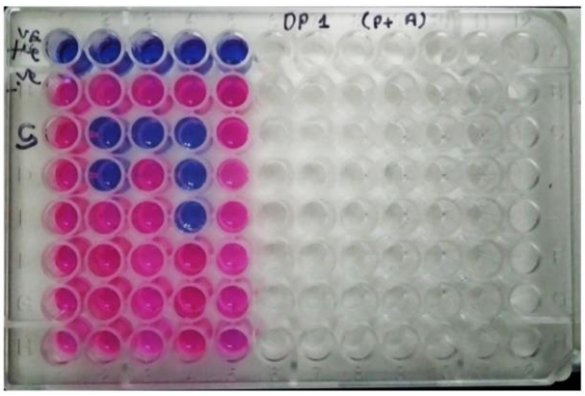

Fig 6: MIC pattern of E.coli DP1 for Antibiotic plus plant extract synergy

K. pneumoniae

The Three combinations Jesthamadh + Kanamycin, Jesthamadh + Vancomycin and Neem + Amphotericin, recorded with MIC 50 µg/ml and other sets failed to record any growth inhibition (Table 2b and Fig. 2b).

Table 3: MIC of Synergistic sets of antibiotics plus plant extract against K. pneumoniae DP2

Sr. No.	Combinations	Minimum Inhibitory Concentration in µg/ml
1	Kadi patta + Amphotericin	-
2	Jeshthamadh + Kanamycin	50
3	Jeshthamadh + Vancomycin	50
4	Neem + Amphotericin	50
5	Kadi patta + Vancomycin	-

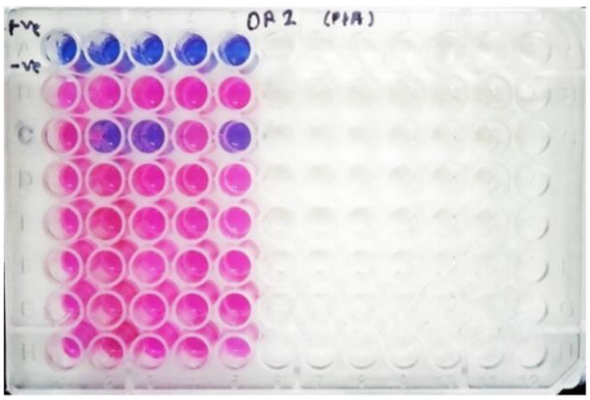

Fig 7: MIC pattern of K. pneumoniae DP2 for Antibiotic plus plant extract synergy

S. aureus

The performance of Karela+Vancomycin noted to be a better performer with MIC 25 µg/ml and then of Karela + Kanamycin and Neem + Kanamycin with MIC 50 µg/ml against *S. aureus* (Table 2c and Fig. 2c).

Table 4: MIC of Synergistic sets of antibiotics plus plant extract against S. aureus DP3

Sr. No.	Combinations	Minimum Inhibitory Concentration in µg/ml
1	Karela + Kanamycin	50
2	Karela + Vancomycin	25
3	kadi patta + Amphotericin	-
4	Belpatra + Vancomycin	-
5	Neem + Kanamycin	50

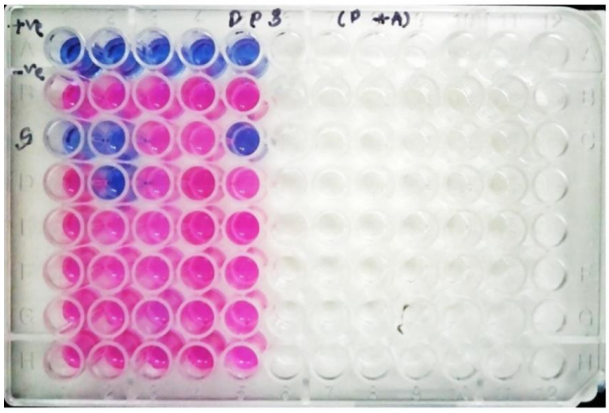

Fig 8: MIC pattern of *S. aureus* DP3 for Antibiotic plus plant extract synergy

E. aerogenes

E. aerogenes prominently controlled by Ashwagandha plus amphotericin with MIC 12.5 µg/ml followed by Kadipatta + amphotericin (MIC 25 µg/ml) (Table 2d and Fig. 2d).

Table 5: MIC of Synergistic sets of antibiotics plus plant extract against E. aerogenes DP4

Sr. No.	Combinations	Minimum Inhibitory Concentration in µg/ml
1	Ashwagandha + Vancomycin	-
2	Ashwagandha + Amphotericin	12.5
3	Neem + Vancomycin	50
4	Jeshthamadh + Kanamycin	50
5	kadi patta + Amphotericin	25

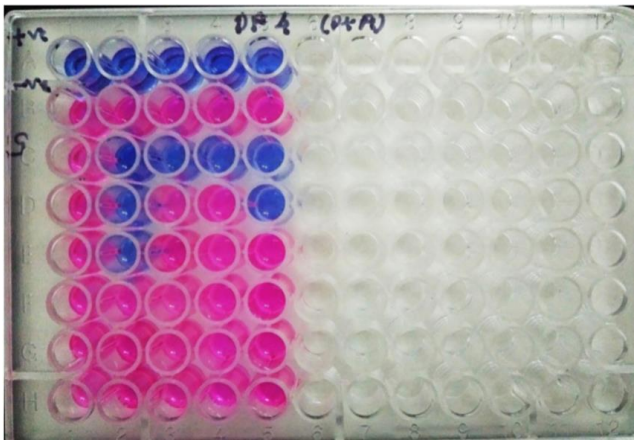

Fig 9: MIC pattern of *E. aerogenes* DP4 for Antibiotic plus plant extract synergy

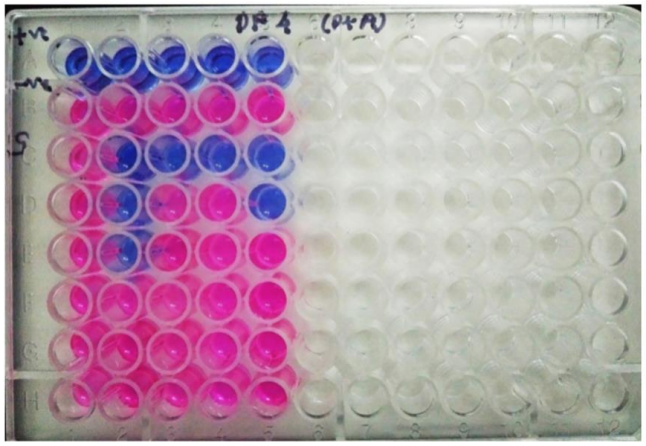

Fig 10: MIC pattern of *E. aerogenes* DP4 for Antibiotic plus plant extract synergy

P. aeruginosa

P. aeruginosa better controlled with Jesthamadh + Vancomycin with MIC value 25 µg/ml followed by Methi plus amphotericin (50 µg/ml) (Table 2e and Fig. 2e).

Table 10: MIC of Synergistic sets of antibiotics plus plant extract against *P. aeruginosa* DP5

Sr. No.	Combinations	Minimum Inhibitory Concentration in µg/ml
1	Jeshthamadh + Vancomycin	25
2	Jeshthamadh + Amphotericin	-
3	Methi + Kanamycin	50
4	Neem + Amphotericin	-
5	Methi + Kanamycin	-

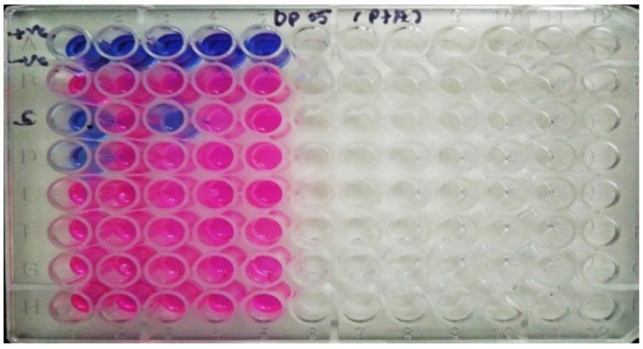

Fig 11: MIC pattern of *P. aeruginosa* DP5 for Antibiotic plus plant extract synergy

Discussion

The crude extracts of plants growing in India controlled the growth of nosocomial Gram-positive and Gram-negative pathogens. Here plants *Murraya koenigii, Asparagus racemosus, Glycyrrhiza glabra, Phyllanthus emblica, Trigonella foenum - graceum, Ocimum tenuiflorum, Withania somnifera, Terminalia chebula, Momordica charantia, Terminalia bellirica* and *Aegle marmelos* reported useful to control nosocomial pathogens except *Azadirachta indica.* The range antibacterial activity with them noted as 11 µg/ml to 50 µg/ml, which could be stated as a success.

In a similar study, *MRSA* and *VRSA* species better controlled by leaf extracts of *Piper betle* but noted high MIC ranging from 19-15 µg/ml while we recorded as low as 11 µg/ml (Valle *et al.*, 2015).

The present study noted the success of plant extracts controlled multidrug-resistant clinical pathogens causing nosocomial infection once tested by MIC assay. In a similar approach, *Musa paradisiaca* alos able to control *E. coli, P. aeruginosa* and *Citrobacter sp.* with MIC value ranged between 15.63-250 µg/ml (Karuppiah and Mustaffa, 2013). Similarly, plants *Cynodon dactylon* (Marasini *et al.*, 2015); *Hibiscus sabdariffa L. calyces* (Abdallah, 2016); *Quercus infectoria* (WA, *et al.*, 2014); and *Salvadora Persia* (Al-Ayed *et al.*, 2016) noted to control MDR nosocomial pathogens.

In the present study, synergy recorded for plant extracts with antibiotics via MIC assay. MDR *E. coli* better controlled by Neem + Kanamycin (12.5 µg/ml); *K. pneumoniae* by Jesthamadh + Kanamycin, Jesthamadh + Vancomycin and Neem + amphotericin (50 µg/ml). Similarly, *S. aureus* prominently controlled by MIC 25 µg/ml of Karela + vancomycin. Further, *E. aerogenes* best controlled by Ashwagandha + amphotericin (12.5 µg/ml) and lastly *P. aeruginosa* with MIC 25 µg/ml controlled by Jesthamadh + Vancomycin.

It is essential to find out promising antibacterial agent via artificial or natural way and sometimes by combination. The use of synergy remedy of drug and plants noted to a promising approach (Betoni *et al.*, 2006) and justified by the present study. Similar to the present study, *S. aureus* noted to better Controlled by using 13 antimicrobial drugs with eight plant extracts in synergy (Betoni *et al.*, 2006). The methanolic extract of *Punica granatum* with ciprofloxacin or erythromycin controlled the growth of *P. aeruginosa, S. aureus,* respectively, once used in synergy (Elmanama *et al.*, 2011). In this study, almost all plant extracts showcased the ability to control MDR nosocomial pathogens once used solely or in combination with drugs.

Conclusion

The study put forward another evidence of plant extract controlling MDR nosocomial pathogens once used solely or in combination with antimicrobial drugs. By involving these combinations or plants, better therapeutic remedies could be possible in coming time. The study suggested using a synergistic approach to tackle nosocomial infection, especially by involving plant species available in India.

References

1. Abdallah EM. Antibacterial activity of Hibiscus sabdariffa L. calyces against hospital isolates of multidrug resistant Acinetobacter baumannii. Journal of Acute Disease. 2016; 5(6):512-516.
2. Akhtar N, Mirza B. Phytochemical analysis and comprehensive evaluation of antimicrobial and antioxidant properties of 61 medicinal plant species. Arabian Journal of Chemistry. 2018; 11(8):1223-1235.
3. Al-Ayed MSZ, Asaad AM, Qureshi MA, Attia HG, lMarrani AH. Antibacterial activity of Salvadora persica L. (Miswak) extracts against multidrug resistant bacterial clinical isolates. Evidence-based complementary and alternative medicine: eCAM, 2016.
4. Betoni JEC, Mantovani RP, Barbosa LN, Di Stasi LC, Fernandes Junior A. Synergism between plant extract and antimicrobial drugs used on Staphylococcus aureus diseases. Memórias do Instituto Oswaldo Cruz. 2006; 101(4):387-390.

5. Bolla JM, Alibert-Franco S, Handzlik J, Chevalier J, Mahamoud A, Boyer G, *et al*. Strategies for bypassing the membrane barrier in multidrug resistant Gram-negative bacteria. FEBS letters. 2011; 585(11):1682-1690.
6. Boucher HW, Talbot GH, Bradley JS, Edwards JE, Gilbert D, Rice LB, *et al*. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clinical infectious diseases. 2009; 48(1):1-12.
7. Cowan MM. Plant products as antimicrobial agents. Clinical microbiology reviews. 1999; 12(4):564-582.
8. Dubey D, Rath S, Sahu MC, Pattnaik L, Debata NK, Padhy RN, *et al*. Surveillance of infection status of drug resistant Staphylococcus aureus in an Indian teaching hospital. Asian Pacific Journal of Tropical Disease. 2013; 3(2):133-142.
9. Elfahmi WH, Kayser O. Jamu: Indonesian traditional herbal medicine towards rational phyto pharmacological use. Journal of Herbal Medicine. 2014; 4(2):51-73.
10. Eliopoulos GM, Cosgrove SE, Carmeli Y. The impact of antimicrobial resistance on health and economic outcomes. Clinical infectious diseases. 2003; 36(11):1433-1437.
11. Elmanama AA, Alyazji AA, Abu-Gheneima NA. Antibacterial, antifungal and synergistic effect of Lawsonia inermis, Punica granatum and Hibiscus sabdariffa. Antibacterial, antifungal and synergistic effect of Lawsonia inermis, Punica granatum and Hibiscus sabdariffa, 2011, 7.
12. Jangra MS, Chhabra M. Cycloserine induced suicidal tendencies and kanamycin induced ototoxicity in Indian MDR-TB patient: a case report. Current drug safety. 2018; 13(3):211-213.
13. Karuppiah P, Mustaffa M. Antibacterial and antioxidant activities of Musa sp. leaf extracts against multidrug resistant clinical pathogens causing nosocomial infection. Asian Pacific journal of tropical biomedicine. 2013; 3(9):737-742.
14. Kaushik P, Goyal P. *In vitro* evaluation of Datura innoxia (thorn-apple) for potential antibacterial activity. Indian Journal of Microbiology. 2008; 48(3):353.
15. Lee CK, Kim H, Moon KH, Shin KH. Screening and isolation of antibiotic resistance inhibitors from herb materials-resistance inhibition of volatile components of Korean aromatic herbs. Archives of pharmacal research. 1998; 21(1):62-66.
16. Marasini BP, Baral P, Aryal P, Ghimire KR, Neupane S, Dahal N, *et al*. Evaluation of antibacterial activity of some traditionally used medicinal plants against human pathogenic bacteria. BioMed research international, 2015.
17. NAIR R, KALARIYA T, Chanda S. Antibacterial activity of some selected Indian medicinal flora. Turkish Journal of biology. 2005; 29(1):41-47.
18. Nascimento GG, Locatelli J, Freitas PC, Silva GL. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. Brazilian journal of microbiology. 2000; 31(4):247-256.
19. Panda SK. Ethno-medicinal uses and screening of plants for antibacterial activity from Similipal Biosphere Reserve, Odisha, India. Journal of Ethnopharmacology. 2014; 151(1):158-175.
20. Pavithra PS, Janani VS, Charumathi KH, Indumathy R, Potala S, Verma RS, *et al*. Antibacterial activity of plants used in Indian herbal medicine. International Journal of Green Pharmacy (IJGP), 2010, 4(1).
21. Prasannabalaji N, Muralitharan G, Sivanandan RN, Kumaran S, Pugazhvendan SR. Antibacterial activities of some Indian traditional plant extracts. Asian Pacific Journal of Tropical Disease. 2012; 2:S291-S295.
22. Raut JS, Karuppayil SM. A status review on the medicinal properties of essential oils. Industrial crops and products. 2014; 62:250-264.
23. Sivananthan M. Antibacterial activity of 50 medicinal plants used in folk medicine. Int. J. Biosci. 2013; 3(4):104-121.
24. Tan YP, Chan EWC. Antioxidant, antityrosinase and antibacterial properties of fresh and processed leaves of Anacardium occidentale and Piper betle. Food Bioscience. 2014; 6:17-23.
25. Valle Jr DL, Andrade JI, Puzon JJM, Cabrera EC, Rivera WL. Antibacterial activities of ethanol extracts of Philippine medicinal plants against multidrug-resistant bacteria. Asian pacific journal of tropical biomedicine. 2015; 5(7):532-540.
26. WA WNA, Masrah M, Hasmah A, NJ NI. *In vitro* antibacterial activity of Quercus infectoria gall extracts against multidrug resistant bacteria. Tropical Bio medicine. 2014; 31(4):680-688.