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(RESEARCH ARTICLE)

Antibacterial potential of plant extracts on ESBL and carbapenemase producing pathogens

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Abstract

The extensive spread of antibiotic resistance among pathogens is critically challenging the healthcare system with treatment options. Often, the physicians are left with no choice but to use high doses of antibiotics to combat infectious diseases in spite of the associated toxicity. In an attempt to investigate plant sources as an alternative source of drugs, the current study evaluated the antibacterial activity of extracts obtained from six commonly available plants against 16 pathogens isolated from skin, respiratory and urinary tract. Among these, 14 multi drug resistant pathogens and 7 ESBL producers were identified. Also, 4 isolates showed both ESBL and carbapenemase production whereas 2 *S. aureus* isolates showed ESBL production and resistance to Streptogramins. The plants used in our study included garlic, cinnamon, Indian borage, clove, green tea and onion. The qualitative analysis of antibacterial activity was carried out by well diffusion method for water and methanol extracts of these plants. The results indicated garlic and Indian borage water extracts to be active against both gram negative and gram positive test pathogens. The observed zones of inhibition were in the range of 11-27 mm (Indian borage) and 9-21 mm (garlic) against the test pathogens. However, no synergy was observed when these extracts were combined.

Keywords: β-lactamase; Alternative remedy; Antibacterial activity; Antibiotic resistance

1. Introduction

The successful identification of novel antibiotics and, its increased production and administration, in the last three decades has simultaneously eventuated the emergence of antibiotic resistant pathogens [1]. Over time, the resistance genes have been transmitted through horizontal gene transfer to aid in an increased occurrence of infections caused by drug resistant pathogens. Moreover, the accumulation of multiple resistance genes in a single pathogen has allowed the evolution of Multiple Drug Resistant (MDR) pathogens and superbugs [2].

Among the resistance observed towards several classes of antibiotics, the pathogens showing resistance to β-lactam group of antibiotics are particularly frightening. This is because, the β-lactams are broad spectrum antibiotics widely used for treatment of commonly occurring infectious diseases [3]. The spread of Extended-Spectrum β-Lactamase (ESBL) and carbapenemase (i.e., types of β-lactamases) producing pathogens are reported to occur at an alarming rate, with over 50% drug resistant pathogens identified as ESBL producers in recent years [4]. The ESBLs generally confers resistance to cephalosporins e.g. cefuroxime, cefotaxime and ceftazidime, while the carbapenemases characteristically hydrolyze carbapenem antibiotics like imipenem, ertapenem and meropenem. In addition, they also show a high degree of resistance to most β-lactams and other groups of antibiotics [5, 6, 7].

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The contemplating action for the observed scenario demands our sincere focus towards the use of alternative remedies in combating antimicrobial resistance. Verily, many published studies report the potential of plant extracts as a possible alternative to antibiotics [8, 9, 10]. However, comparatively few documented records including research publications report the efficiency of plant extracts in treatment of infections caused by multi drug resistant pathogens [11, 12].

The plant derived medicines are the pre-eminent choice of pharmaceutical experts as a result of the indelible role of plants in the prevention and treatment of human diseases since a long time [13]. Moreover, it is estimated that around 61% of new drugs developed between 1981 and 2002 were derived from plant products [14]. These drugs are successfully applied in treatment of infectious diseases caused by bacteria, viruses, helminths and protozoa, as well as chronic diseases like cancer, HIV, diabetes and hypertension [15]. Furthermore, the traditional Ayurveda and chinese medicine, that exploits herbal sources of drugs, have a well-built foundation and effective curative potential in treatment of common as well as chronic diseases [16].

Among the various plants known for their antimicrobial potential, the current study narrowed down the list to six commonly available spices and plant products i.e., clove, garlic, cinnamon, onion, green tea and Indian borage, to investigate its potential against ESBL and carbapenemase producing pathogens specifically.

Spices not only provide flavour and aroma to the foods but are also rich in potent biomolecules. The secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, and glycosides, among others, majorly contribute to antimicrobial and anti-oxidant properties of plants [17]. Spices are rich sources of these compounds. Cloves are known for its carminative properties and works by increasing the concentration of hydrochloric acid in the stomach to improve peristalsis [18]. It is also widely used as anodyne in dentistry [19]. The sulphur compounds like allin contribute to the strong smell of garlic and it is the major bioactive compound with several medicinal properties [20]. The neuroprotective property of cinnamon is exploited in the management of diabetes in Chinese medicine [21]. Similarly, the tumour inhibitory property of flavonols and organosulfur compounds in onion has been reported [22]. The polyphenols acting as anti-oxidants are present in considerably higher amounts in green tea and its supplements [23]. The anti-mutagenic, antigenotoxic and antibacterial effects of Indian borage leaves are also well documented [24]. Altogether these plants products commonly show anti-inflammatory, antioxidants, antiulcerogenic, antithrombotic antiparasitic, antibacterial and anti-inflammatory activities [18-24]. In an attempt to investigate the efficacy of the extracts obtained from these plants and plant products against ESBL and carbapenemase producing pathogens, the current study was carried out to qualitatively check their antibacterial activity.

2. Material and methods

2.1. Test organisms

A total of 16 test pathogens causing skin, respiratory and urinary tract infections were collected from Saifee Hospital situated in South Mumbai. In addition, 3 representative laboratory cultures were also used. The details of test pathogens are provided in Table 1 below. All the isolates were maintained on Nutrient Agar (NA) slants supplemented with 100 µg/mL of ampicillin and stored at refrigerated conditions.

2.2. Antibiotic resistance profile and detection of β-lactamase production

For carrying out the antibiotic resistance profile of test isolates, they were enriched in sterile nutrient broth for 6-8 h at 37°C. The enriched culture was swabbed on the surface of sterile Mueller and Hinton agar plates and antibiotic discs were aseptically placed over them [25]. The plates were incubated at 37°C for 24 h and the diameters of the zone of inhibition were measured. All media used in the present study were obtained from Hi-media Laboratories Ltd., Mumbai, India.

The resistance of test isolates was tested against 33 antibiotics of different classes commonly used for the treatment of skin, respiratory and urinary tract infections. The antibiotics used in our study included Ticarcillin/Clavulanic acid (Tc 75 µg), Ceftazidime (Ca 30 µg), Cefoperazone/Sulbactam (Cs 50 µg), Meropenem (Mr 10 µg), Ciprofloxacin (Cf 5 µg), Levofloxacin (Le 5 µg), Minocycline (Mi 30 µg), Trimethoprim/Sulphamethoxazole (Tr 75 µg), Ampicillin (As 10 µg), Amoxycillin/Clavulanic acid (Ac 20 µg), Piperacillin/Tazobactam (Pt 100 µg), Cefuroxime (Cu 30 µg), Ceftriaxone (Ci 30 µg), Cefipime (Cpm 30 µg), Ertapenem (Etp 10 µg), Imipenem (I 10 µg), Amikacin (Ak 30 µg), Gentamicin (G 10 µg), Nalidixic acid (Na 30 µg), Nitrofurantoin (Nf 300 µg), Colistin (Cl 10 µg), Aztreonam (Ao 30 µg), Benzyl penicillin (Ban 10 U), Erythromycin (E 15 µg), Linezolid (Lz 30 µg), Daptomycin (Dap 30 µg), Teicoplanin (Te 30 µg), Vancomycin (Va 30 µg), Tetracycline (T 30 µg), Oxacillin (Ox 1 µg), Clindamycin (Cd 2 µg), Rifampicin (R 5 µg) and Tigecycline (Ti 15 µg).

In addition, the production of ESBL was detected with the help of Multi-Ezy MIC™ strips (Hi-Media Laboratories Pvt. Ltd.), which contained a gradient of 3 antibiotics with and without clavulanic acid on either side of the strip. The Multi-Ezy MIC™ strips used in the study contained cefotaxime, ceftazidime, and cefepime (MIX) on one side in a two-fold gradient and the same antibiotics with clavulanic acid (MIX+) on the other side. Table 2 indicates the interpretation criteria for detection of ESBL producers [26]. Reference cultures used in the study were *E. coli* ATCC 25922; used as negative control, and *K. pneumoniae* ATCC 700603; used as positive control, for detection of ESBL production. The detection of carbapenemase production was reported based on the antibiotic sensitivity test, by observing for resistance to carbapenem antibiotics i.e. imipenem, meropenem and/or ertapenem.

Table 1 Test pathogens used in the current study

Reference code	Name of organism	Source
M-01	*Escherichia coli*	Urine
M-125	*Klebsiella pneumonia*	Urine
M-150	*Burkholderia cepacia*	Urine
M-160	*Enterococcus faecalis*	Urine
M-04	*Escherichia coli*	Urine
M-09	*Acinetobacter baumannii*	Respiratory tract
M-40	*Enterobacter cloacae ssp. Dissolvens*	Respiratory tract
M-126	*Pseudomonas aeruginosa*	Respiratory tract
M-10	*Klebsiella pneumoniae*	Respiratory tract
M-139	*Acinetobacter baumannii*	Respiratory tract
M-14	*Escherichia coli*	Skin
M-33	*Staphylococcus epidermidis*	Skin
M-98	*Enterococcus faecium*	Skin
M-132	*Staphylococcus aureus*	Skin
M-141	*Pseudomonas aeruginosa*	Skin
M-109	*Staphylococcus aureus*	Skin
L-1	*Escherichia coli*	Lab culture
L-2	*Staphylococcus aureus*	Lab culture
L-3	*Staphylococcus aureus 6538p*	Lab culture

Table 2 Interpretation of E-Test

Report	Formula	Interpretative Criteria
ESBL positive strain	MIX = ≥ 8 MIX+	When the ratio obtained for MIX and MIX+ is more than or equal to 8 or in the case when, no zone is obtained for MIX and a zone is obtained for MIX+
ESBL negative strain	MIX = ≤ 8 MIX+	When the ratio of the value obtained for MIX in combination with MIX+ is less than 8.
ESBL (non-conclusive)		When no zone of inhibition is obtained on either side. In such cases, resistance may be due to mechanisms other than ESBL production. These have to be further investigated before reporting.

2.3. Plant sample collection

The buds of clove (*Syzygium aromaticum*), bulb of garlic (*Allium sativum*), bark of cinnamon (*Cinnamomum zelanicum*), onion (*Allium cepa*) and green tea (*Camellia sinensis*) were purchased from a local supermarket in Mumbai. The leaves

of Indian borage (*Plectranthus amboinicus*) was collected from a local garden and authenticated by an expert botanist from St. Xaviers College, Mumbai.

2.4. Preparation of extracts

In our study, the plant extracts were prepared using water and methanol. For the preparation of water extract, different weight of plant material was crushed using mortar and pestle and added to 100mL distilled water. They were covered with aluminium foil to avoid volatilization and kept in shaker incubator maintained at 42°C for 48h. Different concentrations of plant material (5-50 mg/mL) were used in our study to check for qualitative antibacterial activity and the concentration showing maximum activity was used for further experiment. The methanol extract was prepared similarly using a soxhlet apparatus. The bioactive components from plants were extracted at 65ºC for 20h. The obtained extract was covered with aluminium foil to avoid volatilization and kept in a rotary shaker at 42°C for 48h. The dried extracts were further diluted using DMSO to obtain a final desired concentration.

2.5. Qualitative evaluation of antibacterial activity of plant extracts by agar diffusion method

The antibacterial activity of plant extracts against test pathogens was assayed by agar well diffusion method. For this purpose, 20 mL of sterile and molten NA butt was cooled to around 40°C, seeded with 0.2mL of test cultures (0.12 O.D540nm) and 0.2 mL Triphenyl tetrazolium chloride (TTC). The mixture was then poured into sterile petri plates. Using a sterile cork borer wells were punched in each plate after solidification of the medium and 50 µL of the extract was added to the wells. The plates were incubated at 37°C for 24 h and observed for zones of inhibition.

2.6. Determination of synergistic activity of plant extracts by agar dilution method

The synergistic activity of plant extracts showing considerable antibacterial activity was evaluated by the agar dilution method. In this method, equal volumes of different plant extracts were mixed thoroughly in a bioassay tube. Similar to the above method, 20mL of sterile and molten NA butt was cooled to around 40°C, seeded with 0.2 mL of test cultures (0.12 $O.D_{540nm}$) and 0.2 mL TTC, and poured into sterile petri plates. Using a sterile cork borer, wells were punched in each plate after solidification of the medium and 50 µL of plant mixture was added to the wells. The plates were incubated at 37°C for 24 h to observe for enhancement of zones of inhibition of the mixture of plant extracts as compared to individual extracts [27].

3. Results and discussion

3.1. Antibiotic resistance profile of test isolates

Table 3 represents the antibiotic resistance profile of the test isolates used in our study. Given the widespread cases of antibiotic resistance globally, most of the isolates collected in our study were candidly expected to be MDR (i.e., resistant to 3 or more antibiotics). The presumed observations clearly showed 14 out of 16 test isolates to be MDR. The other 2 isolates i.e. M-160 and M-109 were not MDR strains; however, they showed intermediate resistance to 11 and 17 out of the 33 antibiotics respectively.

Also, among the 16 isolates, 7 isolates (M-01, M-04, M-40, M-126, M-14, M-33, M-141) showed ESBL production, 4 isolates (M-125, M-09, M-10, M-139) showed both ESBL and carbapenemase production whereas 2 *S. aureus* isolates (132 and 109) showed ESBL production and resistance to Streptogramins (Quinupristin-dalfopristin).

Research undertaken by the World Health Organisation (WHO) suggests the highest levels of antimicrobial resistance and infection by β-Lactamase producers (above 50%) in densely populated countries like India and China. This was mainly attributed to the poor quality of antibiotics and its unsupervised use in most of the regions in these countries [28]. In 2014, WHO reported that over 50% population of pathogens like *E. coli*, *K. pneumoniae*, *S. aureus*, and *P. aeruginosa* were evolved as MDR and β-Lactamase producer [11]. Several independent research carried out recently have consistently and perniciously shown a rise in these numbers [29-33]. In a recent study, over 60% *E. coli* strains isolated from horse riding centres in Poland were ESBL producers [34]. In another study, carried out in Ethiopia, over 94.5% gram negative clinical samples collected during a cross sectional study between December 2017 to June 2018 were MDR strains. They further reported 67% and 2% isolates to be producers of ESBL and carbapenemase, respectively. Moreover, more than 70% strains isolated from ICUs were either MDR or ESBL and/or carbapenemase producers [35].

Table 3 Antibiotic resistance profile of test pathogens

Reference code	Name of organism	Antibiotic resistance profile			β-Lactamase enzyme produced
		Sensitive	Intermediate	Resistant	
M-01	*Escherichia coli*	Ac, Pt, Cu, Ci, Cs, Cpm, I, Etp, Mr, Ak, G, Ti, Nf, Cl	Tc, Ca, Le, Mi, Ao, Ban, E, Lz, Dap, Te, Va, T, Ox, Cd, R	As, Na, Cf, Tr	ESBL
M-125	*Klebsiella pneumonia*	Cl	Tc, Ca, Le, Mi, Etp, Ao, Ban, E, Lz, Dap, Te, Va, T, Ox, Cd, R, Ti	As, Ac, Pt, Cu, Ci, Cs, Cpm, I, Mr, Ak, G, Na, Cf, Nf, Tr	ESBL and Carbapenemase
M-150	*Burkholderia cepacia*	Ca, Mr	Tc, Cs, As, Ac, Pt, Cu, Ci, Cpm, Etp, I, Ak, G, Na, Nf, Cl, Ao, Ban, E, Lz, Dap, Te, Va, T, Ox, Cd, R	Tc, Cf, Le, Mi, Ti, Tr	
M-160	*Enterococcus faecalis*	Ban, G, Cf, Le, E, Lz, Dap, Te, Va, Ti, Nf	Tc, Ca, Cs, Mr, Mi, Tr, As, Ac, Pt, Cu, Ci, Cpm, Etp, I, Ak, Na, Cl, Ao, Ox, Cd, R	T	
M-04	*Escherichia coli*	Cs, Etp, I, Mr, Ak, Ti, Nf, Cl	Tc, Ca, Le, Mi, Ao, Ban, E, Lz, Dap, Te, Va, T, Ox, Cd, R	As, Ac, Pt, Cu, Ci, Cpm, G, Na, Cf, Tr	ESBL
M-09	*Acinetobacter baumannii*	Ti, Cl	Ca, As, Ac, Cu, Etp, Ak, Na, Nf, Ao, Ban, E, Lz, Dap, Te, Va, T, Ox, Cd, R	Tc, Pt, Ci, Cs, Cpm, I, Mr, G, Cf, Le, Mi, Tr	ESBL and Carbapenemase
M-40	*Enterobacter cloacae ssp. Dissolvens*	Pt, Cs, Cpm, Etp, I, Mr, Ak, G, Na, Cf, Ti, Cl, Tr	Tc, Ca, Le, Mi, As, Cu, Ao, Ban, E, Lz, Dap, Te, Va, T, Ox, Cd, R	Ac, Ci, Nf	ESBL
M-126	*Pseudomonas aeruginosa*	Cl	Tr, As, Ac, Pt, Cu, Ci, Etp, Na, Nf, Ao, Ban, E, Lz, Te, Va, T, Ox, Cd, R	Tc, Ca, Cs, Cpm, Dap, I, Mr, Ak, G, Cf, Le, Mi, Ti	ESBL
M-10	*Klebsiella pneumonia*	Ak, G, Cl, Tr	As, Ac, Cu, Ci, Etp, I, Na, Nf, Ban, E, Lz, Te, Va, T, Ox, Cd, R, Ti	Tc, Pt, Ca, Cs, Cpm, Ao, Dap, Mr, Cf, Le, Mi	ESBL and Carbapenemase
M-139	*Acinetobacter baumannii*	Cl	Ca, As, Ac, Cu, Etp, Ak, G, Na, Nf, Ao, Ban, E, Lz, Dap, Te, Va, T, Ox, Cd, R, Ti	Tc, Pt, Ci, Cs, Cpm, I, Mr, G, Cf, Le, Mi, Tr	ESBL and Carbapenemase
M-14	*Escherichia coli*	Pt, Cu, Ci, Cs, Cpm, Etp, I, Mr, Ak, Cf, Ti, Nf, Cl	Tc, Ca, Le, Mi, Tr, Ao, Ban, E, Lz, Dap, Te, Va, T, Ox, Cd, R	Ac, As, G, Na, Tr	ESBL
M-33	*Staphylococcus epidermidis*	Cd, Lz, Dap, Te, Va, Ti, Nf, R	Tc, Ca, Cs, Mr, Mi, As, Ac, Pt, Cu, Ci, Cpm, Etp, I, Ak, G, Na, Cl, Ao	Ban, Ox, Cf, Le, E, T, Tr	ESBL
M-98	*Enterococcus faecium*	Lz, Te, Va, Ti	Tc, Ca, Cs, Mr, Mi, Tr, As, Ac, Pt, Cu, Ci, Cpm, Etp, I, Ak, Na, Cl, Ao, Dap, Ox, Cd, R	Ban, G, Cf, Le, E, T, Nf	-
M-132	*Staphylococcus aureus*	Lz, Dap, Te, Va, Ti, Nf	Tc, Ca, Cs, Mr, Mi, As, Ac, Pt, Cu, Ci, Cpm, Etp, I, Ak, Na, Cl, Ao	Ban, Ox, G, Cf, Le, E, Cd, T, R, Tr	ESBL and Resistant to Streptogramins
M-141	*Pseudomonas aeruginosa*	Dap, I, Mr, Ak, G, Cf, Le, Cl	Tr, As, Ac, Pt, Cu, Ci, Cpm, Etp, Na, Nf, Ao, Ban, E, Lz, Te, Va, T, Ox, Cd, R	Tc, Ca, Cs, Mi, Ti	ESBL
M-109	*Staphylococcus aureus*	Ox, G, Cf, Le, E, Cd, Lz, Dap, Te, Va, T, Ti, Nf, R, Tr	Tc, Ca, Cs, Mr, Mi, As, Ac, Pt, Cu, Ci, Cpm, Etp, I, Ak, Na, Cl, Ao	Ban	ESBL and Resistant to Streptogramins

Key: Ticarcillin/Clavulanic acid (Tc), Ceftazidime (Ca), Cefoperazone/Sulbactam (Cs), Meropenem (Mr), Ciprofloxacin (Cf), Levofloxacin (Le), Minocycline (Mi), Trimethoprim/Sulphamethoxazole (Tr), Ampicillin (As), Amoxycillin/Clavulanic acid (Ac), Piperacillin/Tazobactam (Pt), Cefuroxime (Cu), Ceftriaxone (Ci), Cefipime (Cpm), Ertapenem (Etp), Imipenem (I), Amikacin (Ak), Gentamicin (G), Nalidixic acid (Na), Nitrofurantoin (Nf), Colistin (Cl), Aztreonam (Ao), Benzyl penicillin (Ban), Erythromycin (E), Linezolid (Lz), Daptomycin (Dap), Teicoplanin (Te), Vancomycin (Va), Tetracycline (T), Oxacillin (Ox), Clindamycin (Cd), Rifampicin (R) and Tigecycline (Ti)

3.2. Preliminary study of methanol and water extracts of plant products on Lab cultures

Table 4 represents the preliminary antibacterial activity of methanol (10 mg/mL) and water extracts (10 mg/mL) of plant products on representative gram positive (*S. aureus* and *S. aureus* 6538p) and gram negative (*E. coli*) lab cultures. As observed in the table, the undiluted solvent extracts, except for onion, were ineffective against test cultures. The methanol extract of onion showed activity against gram positive cultures only. The undiluted water extracts of clove, garlic, cinnamon and onion showed considerable zones of inhibition against one or more lab cultures. Significant zones of inhibition of plant extracts were observed against lab cultures when the solvent extracts were diluted 1:1 with DMSO. From these observations it was concluded that the viscosity of the concentrated extracts may have prevented the diffusion of extracts in the media, hence resulting in no zones of inhibition. Also, in the current study, garlic and green tea extracts showed antibacterial activity against all three lab cultures (i.e. both gram positive and gram negative).

Table 4 Antibacterial activity of methanol and water extracts of plant products on lab cultures

Plant extracts	**Zone of inhibition (mm)**		
	S. aureus	***S. aureus* 6538p**	***E. coli***
Clove			
Solvent extract (undiluted)	-	-	-
Water extract (undiluted)	28	17	-
Solvent extract (diluted)	29	29	-
Water extract (diluted)	15	16	-
Garlic			
Solvent extract (undiluted)	-	-	-
Water extract (undiluted)	17	24	17
Solvent extract (diluted)	15	15	21
Water extract (diluted)	-	-	-
Cinnamon			
Solvent extract (undiluted)	-	-	-
Water extract (undiluted)	20	13	-
Solvent extract (diluted)	19	15	12
Water extract (diluted)	16	12	-
Onion			
Solvent extract (undiluted)	13	11	-
Water extract (undiluted)	10	-	-
Solvent extract (diluted)	9	-	-
Water extract (diluted)	-	-	-
Green tea			
Solvent extract (undiluted)	-	-	-
Water extract (undiluted)	-	-	-
Solvent extract (diluted)	21	23	14.5
Water extract (diluted)	19.5	14	14
Indian borage			
Solvent extract (undiluted)	-	-	-
Water extract (undiluted)	-	-	-
Solvent extract (diluted)	14.5	-	-
Water extract (diluted)	14	-	-

The broad spectrum antibacterial activity of garlic and green tea is also reported previously by many researchers [36-39]. Similar to our findings, Farjana et al. [40] reported antibacterial activity of green tea against *S. aureus*, but no activity

against *E. coli*. The bioactive components in medicinal plants work by mechanisms of inhibition of cell wall synthesis, accumulation in bacterial membranes causing energy depletion, increased cell permeability, membrane disruption and change of the structure and function of key cellular constituents. These mechanisms result in mutations, cellular damage and ultimately cellular death. In general, gram positive bacteria are more sensitive to antibacterial agents of natural or synthetic origin as compared to gram negative bacteria. This is due to the complex cell wall structure of the gram negative bacteria that prevent the easy diffusion of molecules inside the cell [41]. In this study, both solvent and water extracts showed significant antibacterial activity. However, water extracts are much more practical, economical as well as acceptable in pharmaceutical applications. Hence further studies were carried out using 5 mg/mL concentration of water extracts.

3.3. Qualitative study of methanol and water extracts of plant products on test organisms

Table 5 represents the qualitative antibacterial activity of water extracts (5 mg/mL) of plant products on test pathogens. As predicted from the preliminary results on lab cultures, garlic and green tea extracts were expected to give considerable antibacterial activity against test pathogens. This is because the plant extracts are a blend of several bioactive components. These components may act synergistically against pathogens. Moreover, the MDR pathogens are rarely exposed to these agents and hence seldom acquire resistance towards plant extracts [42, 43]. Hence consistent results can be generally expected from plant extracts against drug sensitive as well as resistant pathogens. However, interestingly, the green tea extracts were ineffective on most of the test pathogens used in our study. Instead, Indian borage showed significant zones of inhibition in the range of 11-27mm against all test pathogens except two (M-01 and M-150). Garlic extracts also showed considerable antibacterial activity with zones of inhibition in the range of 9-21 mm against all test pathogens except *P. aeruginosa* isolates (M-126 and M-141).

Table 5 Antibacterial activity of water extracts of plant products on test pathogens

Reference code	Organism	Zones of inhibition (mm)					
		Clove	Garlic	Cinnamon	Onion	Green tea	Indian borage
M-01	*Escherichia coli*	-	17	-	-	-	-
M-04	*Escherichia coli*	-	15	-	-	-	10
M-09	*Acinetobacter baumannii*	-	13	-	-	-	12
M-10	*Klebsiella pneumonia*	18	11	-	-	-	27
M-14	*Escherichia coli*	-	18	-	-	-	20
M-33	*Staphylococcus epidermidis*	-	20	-	-	-	20
M-40	*Enterobacter cloacae ssp dissolvens*	-	9	-	-	-	14
M-98	*Enterococcus faecium*	16	16	-	20	-	17
M-109	*Staphylococcus aureus*	17	20	-	-	-	13
M-125	*Klebsiella pneumonia*	-	19	-	-	-	11
M-126	*Pseudomonas aeruginosa*	-	-	-	-	-	12
M-132	*Staphylococcus aureus*	16	19	-	-	11	19
M-139	*Acinetobacter baumannii*	-	20	-	-	-	14.5
M-141	*Pseudomonas aeruginosa*	-	-	-	-	-	17
M-150	*Burkholderia cepacia*	11	12	-	-	-	-
M-160	*Enterococcus faecalis*	-	21	-	12	20	15

The antibacterial activity of garlic is attributed mainly to allicin, di allyl thiosulphinic acid or diallyl disulphate [44]. Allicin, particularly acts by targeted inhibition of RNA synthesis and partial inhibition of DNA and protein synthesis [45]. It is also postulated that bioactive components in garlic extract inhibit succinic dehydrogenase by inactivation of thiol group [46]. The presence of carvacrol and camphor as major constituents could contribute to the antibacterial activity of Indian borage [47]. Other components like alpha-terpinene, gamma-terpinene and o-cymene present in low concentrations in Indian borage, may be responsible for its antibacterial activity [48].

A study reported appreciable antibacterial activity of Indian borage on foodborne pathogens namely *S. aureus*, *B. cereus*, *E. coli* and *Y. enterocolitica* [49]. Moreover, the anti-biofilm efficacy of Indian borage has also been reported against *Streptococcus pyogenes* isolated from pharyngitis patients [50]. The garlic extract has shown significant reduction of Salmonella in commercial mayonnaise and *E. coli* in ground meat [51]. Another study reported antibacterial activity of garlic against streptomycin-resistant *S. aureus* and *E. coli* [52].

3.4. Determination of synergistic activity of garlic and Indian borage extracts

Water extract of Indian borage and garlic, when used together, did not show enhancement of zones of inhibition compared to their individual zones observed in Table 5.

Previous studies have reported synergistic activity of *Camellia sinensis* and *Juglans regia* on MDR pathogens [53]. The synergistic activity of garlic and antibiotics has also been reported in another study [54]. To the best of our knowledge, the synergistic activity of Indian borage with other plants or antibiotics has not been reported.

4. Conclusion

The increasing antibiotic resistance among pathogens towards commonly used antibiotics is increasingly creating a challenge for medical practitioners as well as patients. Today, any case of infection requires closed monitoring since the fear of existing antimicrobials becoming ineffective is quite possible in near future. Our study clearly demonstrates the extent of spread of MDR pathogens. In order to cope with this scenario several published studies have reported the use of rare or less common plant sources and plant products as an alternative to antibiotics. However, it will definitely be more feasible and economical to use common plants as sources of antibiotics, as attempted in the current study. The preliminary studies clearly show the efficacy of garlic as well as Indian borage against ESBL as well as carbapenamase producers. Further studies on this subject with respect to safety and pharmacokinetics, may lead the way towards hopeful outcome against the battle of antibiotic resistance.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

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