ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 15, Issue 4, 2022

Online - 2455-3891
Print - 0974-2441

Research Article

IN VITRO ANTIBACTERIAL ACTIVITY OF GREEN TEA EXTRACT AGAINST MULTIDRUG-RESISTANT BACTERIA

ABHISHEK MEHTA[1]*, GAURAV SAXENA[2], ARTI JAIN[3]

[1]Department of Microbiology, Government Medical College, Datia, Madhya Pradesh, India. [2]Department of Microbiology, Government Medical College, Ratlam, Madhya Pradesh, India. [3]Department of Microbiology, Government Medical College, Vidisha, Madhya Pradesh, India. Email: abhishekmehta623@gmail.com

Received: 24 January 2022, Revised and Accepted: 01 March 2022

ABSTRACT

Objectives: This study aimed to evaluate the antimicrobial activity of ethanolic extract of green tea (*Camellia sinensis*) against multidrug-resistant strains of the pathogenic bacteria: Extended-spectrum-β-lactamase (ESBL) producing Enterobacteriaceae, Carbapenem-resistant Enterobacteriaceae (CRE), and Metallo-β-lactamase (MBL) producing *Pseudomonas aeruginosa* strains.

Methods: In this cross-sectional study, the antibacterial activity of ethanolic extract of commercial green tea against the 23 multidrug-resistant test strains was evaluated by the Agar well diffusion method, and the minimum inhibitory concentration of the extract for the test strains was determined by Agar plate dilution method.

Results: Ethanolic extract of green tea was found to exhibit a remarkably significant antimicrobial activity against the ATCC (American type culture collection) control strains: *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 with slightly higher activity against later as compared to the former. The extract exhibited a significant antibacterial activity against multidrug-resistant bacterial strains. The highest activity was shown against ESBL producing strains, followed by CRE strains and the least activity against MBL producers.

Conclusion: This study strongly depicts that the ethanolic extract of green tea exhibits significant antibacterial activity even against multidrug-resistant strains. Hence, such plant extracts could be a potential source of bioactive lead compounds that could be utilized in developing herbal antimicrobials as an alternative strategy for tackling the problem of antimicrobial resistance.

Keywords: Green tea extract, *Camellia sinensis*, Multidrug resistance, Agar well diffusion, Agar plate dilution, Minimum inhibitory concentration, Extended-spectrum β lactamase, Carbapenem-resistant Enterobacteriaceae, Metallo-β lactamase, *Pseudomonas aeruginosa*.

 DOI: http://dx.doi.org/10.22159/ajpcr.2022v15i4.44220. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

Green tea has been consumed for centuries as a beverage and is well known for its medicinal properties such as anti-oxidant, anti-inflammatory, anti-carcinogenic, and antibacterial activity. In the past few years, several studies have shown that tea extracts exhibiting modest antibacterial activity against a number of pathogenic bacteria attributed mainly to the polyphenolic catechins [1-3].

The emergence of multidrug resistance in bacteria against major antibiotic groups has played havoc in recent years and has posed a serious challenge to clinicians in the prevention, control, and management of infectious diseases. The emergence of multidrug-resistant bacteria in the community as well as in hospital settings is a major public health threat. With most antibiotics being rendered ineffective, clinicians are left helpless with very few alternatives left which are also slipping off their hands.

To tackle this menace, there is an increasing need to develop newer antimicrobial agents particularly from medicinal plant extracts [4-6].

In the Indian subcontinent, very few studies have been conducted to evaluate the antimicrobial activity of green tea extract against multidrug-resistant strains of pathogenic bacteria.

Hence, we had undertaken this cross-sectional study to evaluate the antimicrobial activity of ethanolic extract of commercial green tea against multidrug-resistant strains such as ESBL producing Enterobacteriaceae, Carbapenem-resistant Enterobacteriaceae (CRE), and Metallo-beta-lactamase (MBL) producing *Pseudomonas aeruginosa*.

METHODS

This cross-sectional study was conducted in the Department of Microbiology of a tertiary care teaching hospital of the Central India for a duration of 4 months from August 1, 2015 to November 30, 2015 after obtaining clearance from the Institutional Ethics Committee.

Aim

The aim of the study was to evaluate the antimicrobial activity of ethanolic extract of commercial green tea (*Camellia sinensis*) against multidrug-resistant strains of the pathogenic bacteria: Extended-spectrum-β-lactamase (ESBL) producing Enterobacteriaceae, Carbapenem-resistant Enterobacteriaceae (CRE), and Metallo-β-lactamase (MBL) producing *Pseudomonas aeruginosa* strains.

The test strains used in the study were those multidrug-resistant strains that were being isolated, identified, antibiotic resistance type being determined, and henceforth categorized and reported as ESBL/CRE/MBL producing strains from the clinical specimens processed in our bacteriology laboratory as a routine diagnostic protocol for culture sensitivity during past 1 year. Isolates were identified using standard microbiological techniques and were subjected to antibiotic susceptibility testing by the Modified Kirby Bauer Disk Diffusion method as per CLSI 2014 guidelines [7-9].

A total of 23 such MDR strains were being preserved in 10% glycerol at –20°C for academic and research purposes as departmental policy.

The antibacterial activity of ethanolic extract of commercial green tea (*Camellia sinensis*) was evaluated against these 23 beta-lactamase-producing multidrug-resistant bacterial stock strains:

1. ESBL producing Enterobacteriaceae - 10 test isolates
2. Carbapenem-resistant Enterobacteriacae - 8 test isolates
3. Metallo-β-lactamase producing *Pseudomonas aeruginosa* - 5 test isolates

And against two control strains:
1. *Escherichia coli* ATCC 25922 and
2. *Pseudomonas aeruginosa* ATCC 27853.

Among the 10 test strains of ESBL producing Enterobacteriaceae, there were five strains of *E. coli*, three strains of *Klebsiella pneumoniae*, one strain each of *Proteus mirabilis*, and *Enterobacter* spp.

Among eight test strains of CRE, five strains were of *E. coli*, two strains of *K. pneumoniae*, and one strain of *Citrobacter* spp.

Interpretation criteria for screening ESBL, CRE, and MBL producing strains

For ESBL producing Enterobacteriacae

All bacterial isolates belonging to Enterobacteriaceae which were found to be resistant to at least two third generation Cephalosporins, namely, Cefotaxime (30 μg) and Ceftazidime (30 μg) were considered to be probable ESBL producers and further confirmation was done by phenotypic detection of ESBL by Combined Disk Diffusion Test as per CLSI 2014 guidelines [9].

For carbapenem-resistant Enterobacteriacae (CRE)

All bacterial isolates belonging to Enterobacteriaceae and showing resistance to third generation Cephalosporins and/or at least to one of the carbapenems were further screened for CRE by Modified Hodge Test following CLSI 2014 guidelines [9].

For metallo-β-lactamase producing Pseudomonas aeruginosa strains

All *P. aeruginosa* strains showing resistance to Imipenem (10 μg) were further screened for MBL production by phenotypic tests like Imipenem EDTA Combined Disk Test (CDT) [10,11].

Preparation of ethanolic extract of commercial green tea by cold extraction method

The green tea leaves available in the local market as packaged branded commercial green tea were ground to a coarse powder by a pulverizer. 10 g of this powder was soaked in 100 ml of 95% ethanol and left to macerate at room temperature for 5 consecutive days The mixture was subjected to intermittent shaking during this period followed by straining through a sterile muslin cloth and finally filtration through a sterile Whatman No.1 filter paper. The filtrate was thereafter concentrated under reduced pressure in Rotary Vaccum Evaporator (Equitron Roteva-India) at 40°C. Solvent was completely evaporated to yield dry extract powder which was stored in sterile airtight glass containers at 4°C [12-14].

Antibacterial activity test

Agar well diffusion

The antibacterial activity of different concentrations of the green tea extract was assessed by the Agar well diffusion method against different bacterial test isolates and control strains.

The dry extract powder was weighed on a high precision digital balance and reconstituted in 0.1% DMSO (Dimethyl Sulfoxide) which is considered to be an excellent solvent to yield five concentrations of tea extract (12.5, 25, 50, 100, and 200 mg/ml). These were then stored in sterile labeled aliquots in a laboratory refrigerator at 4°C.

Standard suspensions of test strains with turbidity matching optically with the 0.5 McFarland standards were inoculated onto Mueller Hinton Agar (MHA) plates. On the inoculated Agar plates, wells were made using a sterile Cork borer of 6 mm diameter. Total six wells were punched in each plate, five peripheral, and one central keeping at least 30 mm distance between the centers of the adjacent wells. The five peripheral wells were charged with 50 μl of different concentrations of tea extract. The central well designated as negative control was charged with 50 μl of DMSO.

After 24 h of aerobic incubation at 37°C, the plates were observed for the zones of inhibition around the wells. The measurement of zone size diameter around the wells is considered as a crude estimate of the antibacterial activity of the different concentrations of the crude extract [12,13,15].

Determination of minimum inhibitory concentration

Agar dilution method

After deriving a crude idea of antibacterial activity of green tea (GT) extract at different concentrations against the test strains, we proceeded for MIC determination by Agar dilution method.

Ethanolic extract powder was weighed and reconstituted in DMSO to prepare five concentrations (6.25, 12.5, 25, 50, and 100 mg/ml) of extract suspension.

Dehydrated Mueller Hinton Agar media powder (Himedia, Bombay) was weighed and reconstituted in distilled water in a flask as per the manufacturer's instructions, followed by autoclaving. The media in the flask is then allowed to cool and its temperature was maintained around 50°C in a water bath. Using the following formula to achieve the desired concentration of the extract in the media, the requisite volume of a given concentration of GT extract suspension was mixed with the molten media and poured immediately into the Petri plates.

$C_1V_1 = C_2V_2$

C_1 = The concentration of extract to be added into the media
V_1 = Volume of extract to be incorporated in the media
C_2 = Target concentration of extract desired in the media
V_2 = Total volume of media along with the added extract per plate (20 ml).

Each culture plate is divided into 18 regions by equally spaced horizontal and vertical lines drawn by a marker on the outer surface of the Petri plate bottom in a Chequerboard pattern. Each region was marked with a number specific for the corresponding strain. 0.5 McFarland standardized inocula of the test strains were prepared and were spot inoculated in their respective specified regions. MHA plates (five sets) each with the specific concentration of GT extract (6.25, 12.5, 25, 50, and 100 mg/ml) and a control plate without any extract were spot inoculated with test strains. The plates were allowed to stand for a while until the inoculum spots were absorbed, followed by aerobic incubation at 37°C for 18–24 h.

The lowest concentration of GT extract corresponding to the complete inhibition of the growth of test strain on the culture media was considered as the minimum inhibitory concentration (MIC) of the extract for that particular strain [16,17].

RESULTS

This study was designed to assess the antibacterial activity of crude ethanolic extract of commercial green tea against beta-lactamase-producing multidrug-resistant stock strains derived from clinical specimens, by Agar well diffusion assay and Agar dilution method.

A crude assessment of the antibacterial activity of GT extract was derived based on the diameters of zones of inhibition formed around the charged wells as depicted in Tables 1-4 and Figs. 1, 2a, 2b, and 3.

Ethanolic GT extract was found to exhibit a remarkably significant antimicrobial activity against the control strains *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 with slightly higher activity against later as compared to the former. This activity followed a dose-dependent pattern.

Table 1: Antibacterial activity of green tea extract against standard control strains

Concentration of GT extract (mg/ml)	Inhibition zone diameter (mm)	
	E. coli (ATCC 25922)	*P. aeruginosa* (ATCC 27853)
200	18	20
100	16	18
50	14	15
25	11	13
12.5	9	11
Negative control	0	0

GT: Green tea, ***E. coli: Escherichia coli,*** *P. aeruginosa: Pseudomonas aeruginosa*

This study depicts that the crude ethanolic extract of green tea exhibits modest antibacterial activity against multidrug-resistant bacterial strains. As evident from the study, the highest activity was seen against ESBL producing strains, followed by CRE strains and the least activity against MBL producers.

Among five ESBL producing strains of *E. coli*, one strain (ESBL-2) has not shown any susceptibility against GT extract even at high concentrations. GT extract exhibited fairly significant activity against two strains (ESBL-3 and 5) even at low concentrations and the activity has shown a gradual increase with increasing concentration.

Among three ESBL strains of *K. pneumoniae*, GT extract exhibited moderate activity against two strains (ESBL-7 and 9) and mild activity against one strain (ESBL-8).

Table 2: Antibacterial activity of green tea extract against extended-spectrum β lactamase producing Enterobacteriaceae strains

Concentration of GT extract (mg/ml)	Inhibition zone diameter (mm.)									
	ESBL-1 *E. coli*	ESBL-2 *E. coli*	ESBL-3 *E. coli*	ESBL-4 *E. coli*	ESBL-5 *E. coli*	ESBL-6 *Enterobacter* spp.	ESBL-7 *K. pneumonia*	ESBL-8 *K. pneumonia*	ESBL-9 *K. pneumonia*	ESBL-10 *P. mirabilis*
200	11	0	22	18	20	13	15	11	16	10
100	8	0	20	12	17	13	14	11	15	0
50	0	0	17	11	14	13	12	11	13	0
25	0	0	16	11	13	11	11	10	10	0
12.5	0	0	13	11	12	11	10	9	10	0

E. coli: Escherichia coli, K. pneumonia: Klebsiella pneumonia, P. mirabilis: Proteus mirabilis, GT: Green tea, ESBL: Extended-spectrum β lactamase

Table 3: Antibacterial activity of green tea extract against Carbapenem-Resistant Enterobacteriaceae strains

Concentration of GT extract (mg/ml)	Inhibition zone diameter in mm							
	CRE-1 *E. coli*	CRE-2 *E. coli*	CRE-3 *E. coli*	CRE-4 *E. coli*	CRE-5 *E. coli*	CRE-6 *K. pneumonia*	CRE-7 *K. pneumonia*	CRE-8 *Citrobacter* spp.
200	13	11	16	12	16	12	16	14
100	11	8	15	11	14	11	14	11
50	0	0	12	9	12	10	13	10
25	0	0	10	0	11	9	11	8
12.5	0	0	0	0	9	0	0	0

CRE: Carbapenem-resistant Enterobacteriaceae, GT: Green tea, *E. coli: Escherichia coli, K. pneumonia: Klebsiella pneumonia*

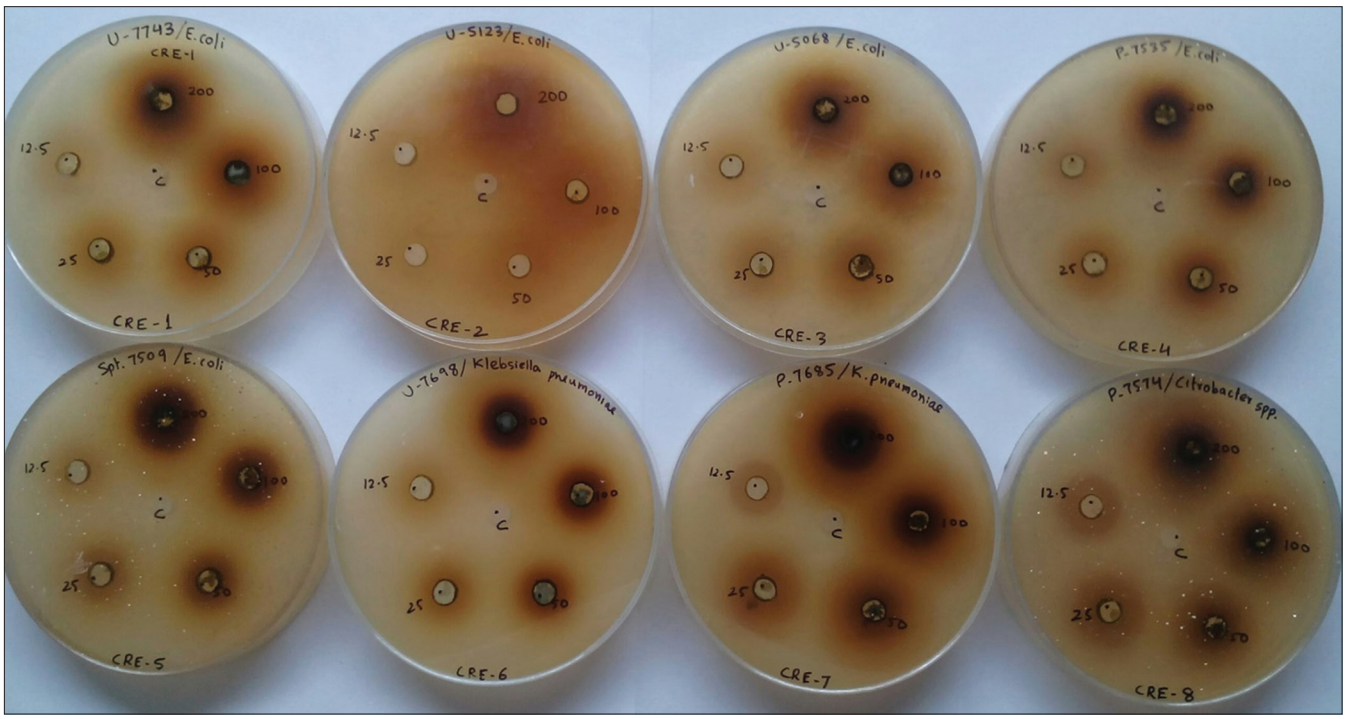

Fig. 1: Antibacterial activity of GT extract against Carbapenem-resistant Enterobacteriaceae strains

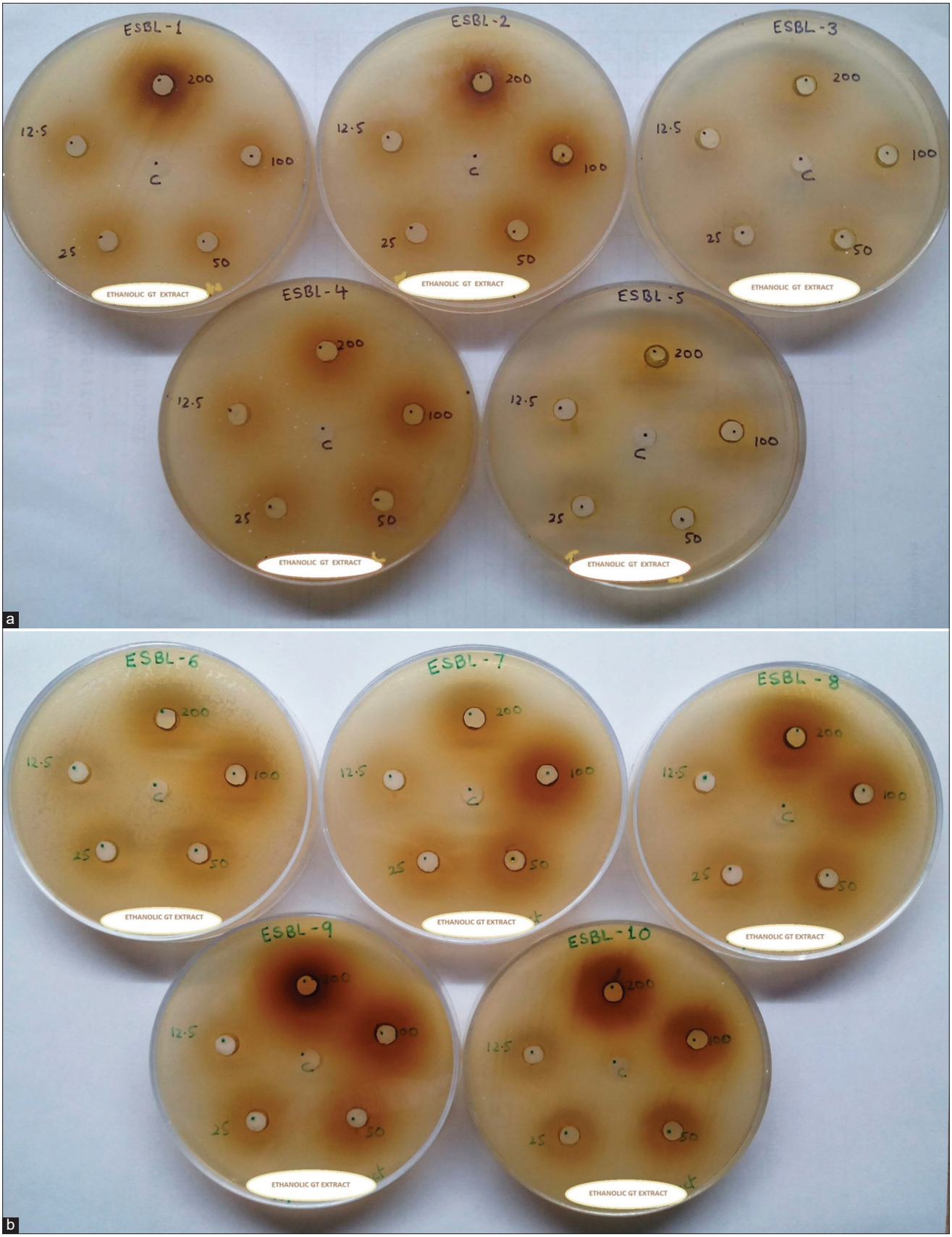

Fig. 2: (a and b) Antibacterial activity of GT extract against ESBL producing Enterobacteriaceae strains

Green tea extract exhibited mild-to-moderate activity against *Enterobacter* spp. (ESBL-6) and minimal activity against *P. mirabilis* (ESBL-10).

In the case of the two strains, ESBL-6 (*Enterobacter* spp.) and ESBL-8 (*K.pneumoniae*), even with increasing concentration of GT extract from

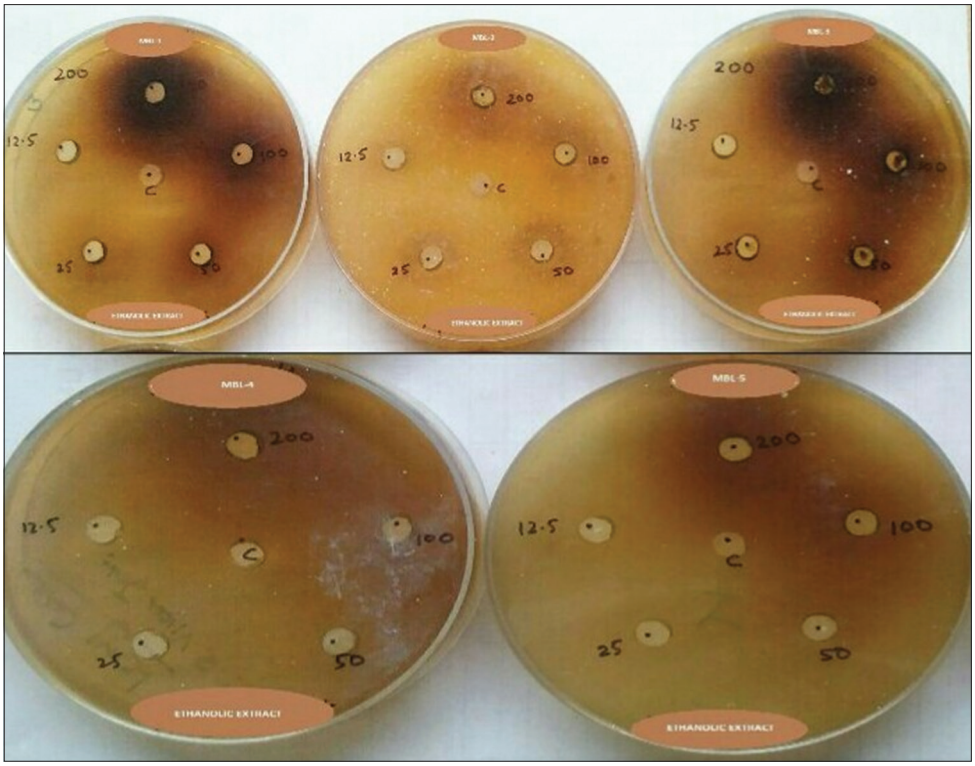

Fig. 3: Antibacterial activity of GT extract against MBL producing *Pseudomonas aeruginosa*

Table 4: Antibacterial activity of green tea extract against Metallo-β-lactamase producing *Pseudomonas aeruginosa* strains

Concentration of GT extract (mg/ml)	Inhibition zone diameter in mm				
	MBL-1	MBL-2	MBL-3	MBL-4	MBL-5
200	12	11	13	12	10
100	10	8	10	11	8
50	8	0	10	9	0
25	0	0	0	0	0
12.5	0	0	0	0	0

GT: Green tea, MBL: Metallo-β-lactamase

50 to 200 mg/ml, there was no increase in inhibition zone diameter (Table 2, Fig. 2a, 2b).

The GT extract has shown mild-to-moderate antibacterial activity against most of the CRE strains (CRE-3, 4, 5, 6, 7, and 8); mild activity against two strains (CRE-1 and 2) and that too only at high concentration (Table 3, Fig. 1).

The extract exhibited a mild activity against all five MBL producing strains of *P. aeruginosa,* only at high concentrations (Table 4, Fig. 3).

Further, the MIC values of the ethanolic GT extract at different concentrations (100, 50, 25, 12.5, and 6.25 mg/ml) against different multidrug-resistant strains were determined by the Agar dilution method as depicted in Table 5 and Fig. 4.

As per the findings of the Agar plate dilution method, MIC values of GT extract came out to be high for the MBL producing strains and most of the CRE strains but relatively much lower for most of the ESBL producing strains except the two strains ESBL-1 (*E. coli*) and ESBL-10 (*P. mirabilis*) with the corresponding MIC values as high as 100 mg/ml (Table 5, Fig. 4). These findings are in line with the findings of the Agar well diffusion method.

DISCUSSION

The emergence of multidrug-resistant strains of pathogenic bacteria esp. β-lactamase producers in hospitals as well as in community settings are the most serious public health threat which is playing havoc in developing countries like India. A large section of the population is increasingly exposed to the dangers of contracting such infections. With most of the first and second-line therapeutic agents rendered ineffective against such strains and a very few reserve drugs left which would meet the same fate in the near future, the management of such infections is posing a serious challenge to the clinicians [4,5,11,12].

To tackle this menace, there is a growing need for extensive research for exploring newer molecules derived from herbal extracts which could be incorporated as an alternative or adjuvant therapeutic agents in the antimicrobial therapy of such infections [18-20].

A conventional antimicrobial drug is a well-defined highly pure molecule. Over time pathogens can develop resistance against such simple molecules. Botanical drugs prepared by processing crude herbal extracts are a mixture of several bioactive compounds and hence harder to develop a resistance against. The antimicrobial activity of green tea is attributed to several phytochemicals the most important being the polyphenolic catechins such as epigallocatechin-gallate (EGCG), epigallocatechin (EGC), epicatechin gallate (ECG), and epicatechin (EC) followed by the tea flavanols like quercitin, kaempferol, and myricetin [21-23].

Many *in vitro* studies [1-4,12-15] conducted throughout the world from time and again had shown that green tea extracts do exhibit significant antimicrobial activity against various pathogenic bacteria but very few studies [19,22,23] were targeted toward multidrug-resistant strains especially the beta-lactamase producers. Our study has focused on this aspect and has successfully demonstrated the antibacterial activity of GT extract against beta-lactamase-producing MDR strains of pathogenic bacteria.

Table 5: Minimum inhibitory concentration values of the ethanolic green tea extract by Agar dilution method

Serial number	Test strain	MIC (mg/ml)	Serial number	Test strain	MIC (mg/ml)	Serial number	Test strain	MIC (mg/ml)
1	ESBL-1	100	11	CRE-1	100	19	MBL-1	100
2	ESBL-2	>100	12	CRE-2	100	20	MBL-2	100
3	ESBL-3	6.25	13	CRE-3	50	21	MBL-3	50
4	ESBL-4	12.5	14	CRE-4	50	22	MBL-4	50
5	ESBL-5	6.25	15	CRE-5	25	23	MBL-5	100
6	ESBL-6	12.5	16	CRE-6	50			
7	ESBL-7	12.5	17	CRE-7	25			
8	ESBL-8	25.0	18	CRE-8	50			
9	ESBL-9	25.0						
10	ESBL-10	100						

MIC: Minimum inhibitory concentration, MBL: Metallo-β-lactamase, CRE: Carbapenem-resistant Enterobacteriaceae, ESBL: Extended-spectrum β lactamase

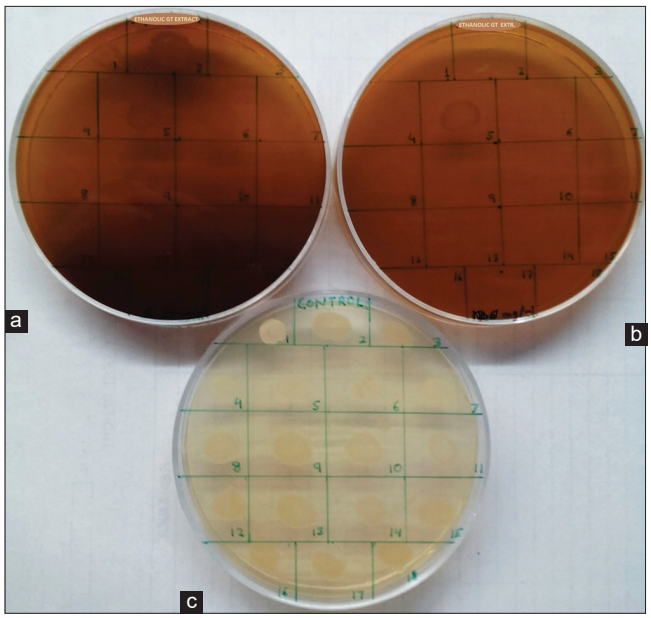

Fig. 4: MIC determination for GT extract by Agar dilution method. (a) GT extract concn. 12.5 mg/ml, (b) GT extract concn. 6.25 mg/ml, (c) Control plate without GT extract

CONCLUSION

It is evident from this study that the crude ethanolic extract of green tea does exhibit antibacterial activity against the multidrug-resistant bacterial strains though this activity may vary with the type of resistance being remarkable against ESBL strains but failing to exhibit significant activity against MBL strains. The picture may be different if the same study is conducted using individual bioactive compounds like epigallocatechin-gallate (EGCG) rather than crude extracts. There is an immense scope of research in this field and such studies had paved the way for the exploration of botanical drugs containing lead bioactive molecules from crude herbal extracts which could bring a new ray of hope in tackling the menace of emerging drug resistance and management of multidrug-resistant infections.

AUTHOR'S CONTRIBUTION

Dr. Abhishek Mehta: Concept, design, and defining intellectual content. Dr. Abhishek Mehta and Dr. Gaurav Saxena: Literature search and data acquisition. Dr. Arti Jain: Data analysis and interpretation. Dr. Abhishek Mehta: Manuscript preparation. Dr. Arti Jain and Dr. Gaurav Saxena: Manuscript Editing and review. All authors: The approval of the final version of manuscript.

ACKNOWLEDGMENT

We are thankful to Ms. Rupal Singh, Scientist-Centre for Scientific Research and Development (CSRD) for the technical assistance.

The study was conducted at Department of Microbiology, People's College of Medical Sciences and Research Centre, Karond Bypass Road, Bhanpur, Bhopal (M.P.). India 462037.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

SOURCE OF FUNDING

None.

REFERENCES

1. Tiwari TP, Bharti SK, Kaur HD, Dikshit RP, Hoondal GS. Synergistic antimicrobial activity of tea and antibiotics. Indian J Med Res 2005;122:80-4. PMID 16106094
2. Fanaki NH, Kassem MA, Fawzi MA, Dabbous SE. Influence of aqueous green tea extract on the antimicrobial activity of some antibiotics against multi resistant clinical isolates. Egypt J Microbiol 2008;17:449-60.
3. Stephen KM, Francis NW, Robert KK. *In vitro* antimicrobial and synergistic properties of water-soluble green and black tea extracts. Afr J Microbiol Res 2014;8:1527-34. doi: 10.5897/AJMR2014.6655
4. Radji M, Agustama RA, Elya B, Tjampakasari CR. Antimicrobial activity of green tea extract against isolates of methicillin-resistant *Staphylococcus aureus* and multidrug-resistant *Pseudomonas aeruginosa*. Asian Pac J Trop Biomed 2013;3:663-7. doi: 10.1016/S2221-1691(13)60133-1, PMID 23905026
5. Reygaert WC. The antimicrobial possibilities of green tea. Front Microbiol 2014;5:434. doi: 10.3389/fmicb.2014.00434, PMID 25191312
6. Taylor PW, Hamilton-Miller JM, Stapleton PD. Antimicrobial properties of green tea catechins. Food Sci Technol Bull 2005;2:71-81.
7. Collee JG. Marr W. Mackie and McCartney practical medical microbiology. In: Collee JG, Fraser AG, Marmion BP, Simmons A, editors. Culture of Bacteria. 14th ed. London: Churchill Livingstone; 2006. p. 113-29.
8. Collee JG, Miles RS. Watt B. Mackie and McCartney practical medical microbiology. In: Collee JG, Fraser AG, Marmion BP, Simmons A, editors. Tests for the Identification of Bacteria. 14th ed. London: Churchill Livingstone; 2006. p. 131-49.
9. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing; 24th Informational Supplement. CLSI Document M100-S24. Wayne PA: Clinical and Laboratory Standards Institute; 2014.
10. Chaudhari MS, Javadekar TB, Ninama G, Pandya N. A study of metallo-β-lactamase producing *Pseudomonas aeruginosa* in clinical samples of SSG hospital. Natl J Med Res 2011;1:60-3.
11. Mehta A, Prabhu T. Detection and characterization of metallo-β-lactamases producing Pseudomonas aeruginosa clinical isolates at a tertiary care hospital of Bhopal, India. Int J Res Med Sci 2016;4:4084-8. doi: 10.18203/2320-6012.ijrms20162938
12. Mehta A, Saxena G, Mani A. Comparative analysis of antibacterial activity of aqueous, ethanolic, methanolic and acetone extracts of commercial green tea and black tea against standard bacterial strains. Int J Curr Microbiol Appl Sci 2016;5:145-52.
13. Archana S, Abraham J. Comparative Analysis of antimicrobial activity of leaf extracts from fresh green tea, commercial green tea, and black tea on pathogens. J Appl Pharm Sci 2011;1:149-52.
14. Kumar A, Thakur P, Patil S, Chandani P. Antibacterial activity of green tea (*Camellia sinensis*) extracts against various bacteria isolated from

environmental sources. Recent Res Sci Technol 2012;4:19-23.
15. Tariq AL, Reyaz AL. *Camellia sinensis* leaves a new treatment against urinary tract infection caused by *Pseudomonas fluorescens* and *Serratia* sp. Int J Pharm Sci Res 2013;4:1546-50.
16. Clinical and Laboratory Standards Institute. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically; Approved Standard. 10th ed. CLSI document M07. Vol. A10. Wayne, PA: Clinical and Laboratory Standards Institute; 2015.
17. Islam MA, Alam MM, Choudhury ME, Kobayashi N, Ahmed MU. Determination of minimum inhibitory concentration (MIC) of cloxacillin for selected isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) with their antibiogram. Bangl J Vet Med 2008;6:121-6. doi: 10.3329/bjvm.v6i1.1350
18. Gopal J, Muthu M, Paul D, Kim HD, Chun S. Bactericidal activity of green tea extracts: The importance of catechin-containing nanoparticles. Sci Rep 2016;6:19710. doi: 10.1038/srep19710
19. Rathod S, Williamson M. Antibacterial activity of green tea extract in combination with cefotaxime on diarrhea-causing ESBL producing *E. coli*. Int J Pharm Pharm Sci 2015;7:258-62.
20. Hamilton-Miller JM. Antimicrobial properties of tea (*Camellia sinensis* L.). Antimicrob Agents Chemother 1995;39:2375-77. doi: 10.1128/AAC.39.11.2375, PMID 8585711
21. Yam TS, Hamilton-Miller JM, Shah S. The effect of a component of tea *Camellia sinensis* on methicillin resistance, PBP2 synthesis and beta-lactamase production in *Staphylococcus aureus*. J Antimicrob Chemother 1998;42:211-6. doi: 10.1093/jac/42.2.211, PMID 9738838
22. Jazani NH, Shahabi SH, Ali AA. Antibacterial effects of water soluble green tea extracts on multi-antibiotic resistant isolates of *Pseudomonas aeruginosa*. Pak J Biol Sci 2007;10:1544-6. doi: 10.3923/pjbs.2007.1544.1546, PMID 19069973
23. Farooqui A, Khan A, Borghetto I, Kazmi SU, Rubino S, Paglietti B. Synergistic antimicrobial activity of *Camellia sinensis* and Juglans regia against multidrug-resistant bacteria. PLoS One 2015;10:e0118431. doi: 10.1371/journal.pone.0118431, PMID 25719410