

Original Article

Evaluation of Medicinal Plant Extracts As Potential B-Lactamase Inhibitors by Studying Their Synergistic Effect with B-Lactam Antibiotics to Combat Antibiotic Resistance

Asst. Prof. Lisa Sam¹ Smit R Kamble²

^{1,2}Sonopant Dandekar Arts, V.S. Apte Commerce & M.H. Mehta Science College

Email-lisasam1616@gmail.com

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*Antibiotic resistance via β -lactamase production is a major threat to worldwide healthcare and underscores the necessity of alternative therapeutic approaches. This research presents a novel approach by assessing the synergistic activity of medicinal plant extracts with β -lactam antibiotics to identify natural β -lactamase inhibitors. Methanolic extracts of *Tinospora cordifolia* (Giloy) and *Calotropis gigantea* (Milkweed) and aqueous extracts of six other medicinal plants, i.e., *Garcinia indica* (Kokum), *Couroupita guianensis* (Kailaspati), *Bryophyllum pinnatum* (Bryophyllum), *Camellia sinensis* (Green Tea), *Carica papaya* (Papaya), and *Trachyspermum ammi* (Ajwain) were screened against *Klebsiella pneumoniae* and *Escherichia coli*. Different in vitro Antibiotic susceptibility tests were performed to find out the zones of inhibition (ZOI). Of the extracts that were tested, *Garcinia indica* and *Tinospora cordifolia* showed considerable inhibition of *Klebsiella pneumoniae* and showed synergy with Penicillin as a beta lactam antibiotic, suggesting the presence of possible β -lactamase inhibitors. Phytochemical analysis indicated the presence of alkaloids, phenols, flavonoids and tannins in the extracts, which are known to possess antimicrobial and enzyme-inhibitory activity. These results indicate that such a synergy between the plant extracts and the β -lactam antibiotics could potentially increase the inhibition of bacteria, providing a natural solution against β -lactamase-associated resistance. Further compound isolation and β -lactamase inhibition assays are required to prove these observations.*

Keywords: Antibiotic resistance, β -lactamase inhibitors, medicinal plant extracts, *Klebsiella pneumoniae*, *Tinospora cordifolia*, *Garcinia indica*, phytochemicals, synergistic effect.

Introduction

Antimicrobial resistance (AMR) is now a major global health issue, risking the efficacy of existing antibiotics and causing rising mortality. According to a study conducted by Murray et al. (2024) and published in The Lancet, estimated yearly deaths from antibiotic-resistant infections will rise from 3.9 million in 2024 to 8.22 million in 2050 if action is not taken quickly. Therefore, there is an urgent need to find alternative antimicrobial approaches. These include plant-derived β -lactamase inhibitors, which have been found to be effective since they can increase the efficacy of antibiotics by inhibiting the resistance mechanisms of bacteria (Hana Mohammed AlSeikh et al., 2020). B-lactam antibiotics include penicillins, cephalosporins, carbapenems, and monobactams that have a typical four-membered β -lactam ring which is responsible for their antibacterial activity. B-lactams act by binding to penicillin-binding proteins (PBPs), and inhibits peptidoglycan cross-linking, causing instability in the cell walls and subsequent lysis of bacteria. Yet, most bacteria have adapted to generate β -lactamase enzymes that degrade the β -lactam ring, making the antibiotics inactive (Bush & Bradford, 2016; Fisher et al., 2005). This has increased the demand for β -



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Address for correspondence:

Asst. Prof. Lisa Sam¹,
Sonopant Dandekar Arts, V.S. Apte Commerce & M.H. Mehta Science College

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Lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam, which irreversibly bind to β -lactamases and inhibit antibiotic degradation, and hence restore antibiotic activity.

Due to the drawbacks associated with synthetic medicines and the increasing resistance to them, plants serve as a source of novel antimicrobial compounds. Plant secondary metabolites, such as alkaloids, flavonoids, tannins and terpenoids, have been reported to inhibit β -lactamase and restrain the growth of resistant microbes. Thus, these compounds augment the potency of antibiotics (Newman & Cragg, 2020; Cushnie & Lamb, 2005; Rajendran et al., 2013). In this study, *Calotropis gigantea*, *Tinospora cordifolia*, *Garcinia indica*, *Couroupita guianensis*, *Bryophyllum pinnatum*, *Camellia sinensis*, *Carica papaya*, and *Trachyspermum ammi* were collected from Palghar, Maharashtra and were screened for β -lactamase inhibitory potential.

Prior to starting laboratory work, a systematic survey was done with five pharmacists in the Boisar area of Palghar district to know local antibiotic usage and resistance patterns. Amoxicillin + Clavulanic Acid, Azithromycin, and Cefixime were the most prescribed antibiotics, primarily because of their broad-spectrum activity against respiratory and urinary infections. Typically faced resistant pathogens were *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus* (including MRSA), and *Streptococcus pneumoniae*. These observations informed the choice of antibiotics and bacterial strains for this work to assess the potential for synergism between plant extracts and Penicillins and Cephalosporins in countering β -lactamase-resistance.

Materials And Methodology

The plant samples of *Tinospora cordifolia* (Giloy) and *Calotropis gigantea* (Milkweed) were collected from Dream city Boisar (east) Palghar district Maharashtra 401501, while the plant samples of *Couroupita guianensis* (Kailaspati), *Garcinia indica* (Kokum), *Camellia sinensis* (Green Tea), *Carica papaya* (Papaya), *Bryophyllum pinnatum* (Bryophyllum) and *Trachyspermum ammi* (Ajwain) were collected from the Botanical garden of SDSM college Palghar Maharashtra 401404. All the plant samples were washed thoroughly with distilled water and were sun dried and then grinded into fine powder. For extraction of *T.cordifolia* and *C.gigantea*, methanol was used as a solvent. Soxhlet apparatus was used for the extraction of bioactive compounds from *C.gigantea* leaves, 50gm dried powder of *C.gigantea* with 400 ml of Methanol was used for the extraction process. After the soxhlet extraction 200 ml of crude extract of *C.gigantea* (250 mg/ml crude) was obtained. Same protocol was implemented for the extraction of bioactive compounds from *T.cordifolia* stem while 2.5 g leaves of *C.guianensis*, *G.indica*, *C.sinensis*, *B.pinnatum*, *C.papaya* and *T.ammi* were macerated in 10 ml of Distilled water and was filtered with Whatman filter paper the crude of each of these exact had concentration of 250 mg/ml. All the extracts were stored in a glass container at 4°C and were used throughout the Studies.

The bacterial cultures of *Escherichia coli* and *Klebsiella pneumoniae* used in this study were subcultured on Sterile NA plates from the laboratory stock cultures maintained by the Department of Biotechnology, SDSM college Palghar. These cultures were stored at 4°C and were used throughout the study. The antibiotics used in the study include: Penicillin G 400 mg (Pentids 400, Abbott Laboratories), Amoxicillin + Clavulanic acid as standard beta lactamase inhibitor (Clamufil 625, Filant Healthcare) and Amoxicillin (Almon 500, Alkem Laboratories Ltd.)

Iodometric test to check presence of beta-lactamase enzyme

The iodometric test was performed to detect β -lactamase production by bacterial isolates. In this method, 0.1 ml of Penicillin G solution was dispensed into a suspension tube, followed by the addition of the test organism to create a milky suspension. Two drops of starch solution were added, mixed, and incubated at room temperature (25°C) for 30–60 minutes. After incubation, one drop of iodine reagent was added and mixed, and color change was observed within 10 minutes. A fading of the blue color indicated β -lactamase activity, while retention of the blue color indicated its absence (Kuo SS, Feng TY, 1989).

Synergistic effect of *C. gigantea* and *T. cordifolia* with penicillin against β -lactamase-producing *E. coli*.

The Paper Disk Diffusion Method was used to assess if *Calotropis gigantea* and *Tinospora cordifolia* enhance penicillin activity against *Escherichia coli* by inhibiting β -lactamase. Disks soaked in crude extract, diluted extract, penicillin, and their combination were placed on inoculated Mueller-Hinton agar and incubated at 37°C for 18–24 hours. An increased Zone of Inhibition indicated β -lactamase inhibition and restored antibiotic efficacy.

Evaluation of Antibacterial and β -Lactamase Inhibitory Potential of Selected Plant Extracts Using the Agar Cup Method

To assess antibacterial and synergistic activity, *E. coli* and *K. pneumoniae* cultures were adjusted to 0.5 McFarland standard and mixed with sterile molten Nutrient Agar, then poured into Petri plates. After solidification, three wells were made per plate with a st. cork borer and filled with 100 μ l of: crude extract alone, crude extract + penicillin (1:1), and crude extract + amoxicillin (1:1). This was repeated for all six plant extracts. Control plates contained penicillin alone, amoxicillin alone, and distilled water. Plates were incubated at 37°C for 24 hours, and zones of inhibition were measured to evaluate antimicrobial and synergistic effects.

Evaluation of Synergistic Action between Plant Extracts and Penicillin Using the Perpendicular Strip Method

The Perpendicular Strip Method was employed to evaluate the synergistic interaction between selected plant extracts and penicillin against *Escherichia coli* and *Klebsiella pneumoniae*. Plant extracts from *Garcinia indica*

(Kokum), *Calotropis gigantea*, *Tinospora cordifolia*, *Couroupita guianensis* (Kailaspati), and *Carica papaya* were chosen based on promising results from previous agar cup assays, suggesting potential β -lactamase inhibitory activity. Nutrient Agar (NA) plates were inoculated with a uniform lawn of bacterial culture using sterile cotton swabs. Two sterile filter paper strips, Strip A was dipped in penicillin (5 mg/ml) and placed horizontally, while Strip B, dipped in plant extract, was placed perpendicularly across it, intersecting at the center. After incubation at 37°C for 24 hours the Zone of Inhibition (ZOI) was observed and measured to assess possible synergistic effects, additive interaction, or antagonism between the plant extract and antibiotic. (Langeveld, W., Veldhuizen, E., & Burt, S. (2013)).

Qualitative Analysis of Phytochemicals

Qualitative phytochemical analysis was performed to detect the presence of bioactive compounds such as alkaloids, flavonoids, phenols, and tannins in the selected plant extracts. These phytochemicals are known for their antimicrobial, antioxidant, and enzyme inhibitory activities, which may enhance the efficacy of antibiotics when used in combination. Qualitative tests for tannins, flavonoids, alkaloids and phenols were done. Identifying these compounds helps establish a correlation between their presence and the synergistic effects observed in earlier tests.

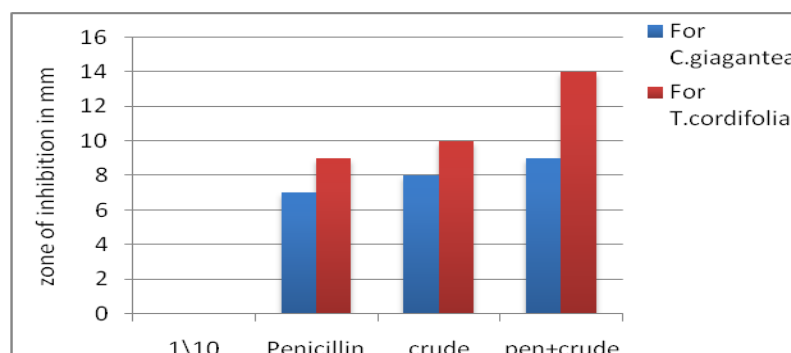
Observation



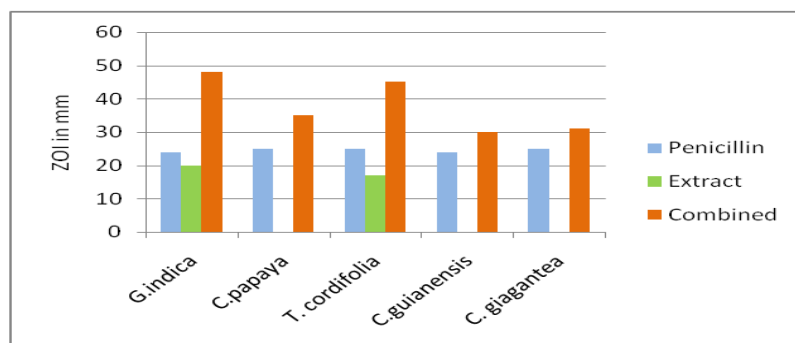
Perpendicular strip assay assessing the synergistic effect of methanolic extract of *Garcinia indica* with penicillin against *K.pneumoniae*. Strip A is impregnated with penicillin, while strip B contains the plant extract



Perpendicular strip assay assessing the synergistic effect of methanolic extract of *T.cordifolia* with penicillin against *K.pneumoniae*. Strip A is impregnated with penicillin, while strip B contains the plant extract



Graph 1: Graph showing the individual and the combined effect of the plant extract and penicillin against *E.coli*



Graph 2: Graph showing the individual and combined effect of the plant extracts with penicillin and amoxicillin.

Results and Discussion

1. Iodometric test to check presence of beta-lactamase enzyme

The iodometric test confirmed β -lactamase production in both *Klebsiella pneumoniae* and *Escherichia coli*, with all tubes showing complete discoloration by 8 minutes. Among them, *E. coli* showed the highest β -lactamase activity with rapid discoloration, followed by *K. pneumoniae*.

2. Paper disk method to test for the synergy between *C. gigantea* and *T. cordifolia* with Penicillin against *E. coli*.

	Zone of inhibition (in mm)	
	<i>C. gigantea</i>	<i>T. cordifolia</i>
1\10	0	0
crude	8	10
pen	7	9
pen+crude	9	14

3. Agar Cup Method to test the antibacterial and synergistic action of aqueous extracts of additional six plant extracts with penicillin and amoxicillin.

Plant Extract	Zone of inhibition in (mm)		
	Crude (C)	C+ Penicillin	C + Amoxicillin
<i>Bryophyllum pinnatum</i>	1	17	18
<i>Couroupita guianensis</i>	9	19	19
<i>Garcinia indica</i>	15	16	16
<i>Camellia sinensis</i>	8	18	18
<i>Carica papaya</i>	3	19	18
<i>Trachyspermum ammi</i>	3	17	18

The control results showed that distilled water had no effect (nil), penicillin had a 16 mm inhibition, and amoxicillin had a 17 mm inhibition against *K. pneumoniae*.

	Zone of inhibition in (mm)		
	Extract	Penicillin	Combined
<i>Garcinia indica</i>	20	24	48
<i>Carica papaya</i>	0	25	35
<i>T. cordifolia</i>	17	25	45
<i>Couroupita guianensis</i>	0	24	30
<i>C. gigantea</i>	0	25	31

4. Evaluation of Synergistic Action Between Plant Extracts and Penicillin Using the Perpendicular Strip Method

Plant Extract	A (Penicillin)	B (Extract)	C (Combination)	A + B	Interpretation
<i>Garcinia indica</i>	24 mm	20 mm	48 mm	44 mm	Synergistic (A+B < C)
<i>Tinospora cordifolia</i>	25 mm	17 mm	45 mm	42 mm	Synergistic (A+B < C)

Enhanced Antibacterial Activity of Penicillin in Combination with Plant Extracts

Against *E.coli* the aqueous crude extracts of the plant did not show any zone of inhibition suggesting the multidrug resistance nature of the bacteria.

5. Phytochemical analysis

	Alkaloids	Flavonoids	Tannins	Phenols
<i>Garcinia indica</i>	+	-	+	-
<i>Carica papaya</i>	+	+	+	-
<i>T. cordifolia</i>	+	+	+	+
<i>Couropita guianensis</i>	+	-	-	-
<i>C. giagantea</i>	+	+	+	+

Key: + Presence, - Absence

Conclusion

This research proves the efficacy of aqueous extracts of *Garcinia indica* and the methanolic extract of *Tinospora cordifolia* as β -lactamase inhibitors with the ability to augment the action of β -lactam antibiotics against resistant strains of bacteria, especially *Escherichia coli* and *Klebsiella pneumoniae*. The iodometric assay validated β -lactamase production in *E. coli*, and *K. pneumoniae*, attesting to the clinical significance of inhibiting this enzyme. The agar cup and perpendicular strip tests conducted in vitro confirmed a significant synergistic increase in the zone of inhibition upon blending plant extracts with antibiotics.

Phytochemical testing established the existence of alkaloids, phenols, flavonoids and tannins in the plant extracts substances known to be antimicrobial and enzyme-inhibitory. These results validate the postulation that secondary metabolites from plants inhibit β -lactamase activity, thus maintaining antibiotic effectiveness. Isolation of the active compounds and determination of their modes of action of the bioactive compounds present in *Garcinia indica* and *Tinospora cordifolia* should be the focus of future studies. Enlarging this study to cover a larger number of resistant strains and in vivo testing will be key processes toward creating new therapies to tackle antimicrobial resistance.

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