International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 12s,2025
https://theaspd.com/index.php

Combating Multidrug Resistance: A Synergistic Approach Using Medicinal Plant Extracts

Niharika Sharma[1], Shikha Kumari[2]
[1]School of Pharmaceutical and health sciences, Carrier Point University, Hamirpur (H.P.) 176041.
shikha.micro@cpuh.edu.in[1], niharikasharma@gmail.com[2]

Abstract
The emergence and rapid spread of multidrug-resistant (MDR) bacterial pathogens have necessitated the search for alternative antimicrobial agents, particularly from natural sources. This study investigates the synergistic antibacterial activity of three ethnomedicinal plants, Lantana camara (LC) and Cissampelos pareira (CP), Parthenium hysterophorus (CG) against clinically relevant MDR isolates. A total of four MDR strains (BI4 (Pseudomonas aeruginosa), BT3 (Bacillus. pumilus), HMR2 (Cellulosiumicrobium. cellulan), and TD7 (Pseudomonas aeruginosa)), previously isolated from soil samples in hospital-associated environments, and were selected for in vitro antimicrobial screening. Combinatorial extracts of LC and CP (LC + CP) were prepared and evaluated for their antibacterial efficacy using the agar well diffusion method. The LC + CP combination exhibited the highest overall activity with inhibition zones ranging from 16.17 ± 0.27 mm to 24.17 ± 0.27 mm. Based on this preliminary finding, the extract was further assessed for its Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) through broth dilution and agar plating methods, respectively. MIC values ranged from 1.562 to 6.25 mg/ml, while MBC values varied between 3.125 and 12.5 mg/ml. The most notable effect was observed against TD7, which exhibited the lowest MIC and MBC values, indicating high sensitivity to the plant extract combination. MBC/MIC ratios ≤4 across all isolates confirmed the bactericidal nature of the combination. The observed efficacy may be attributed to the synergistic action of phytoconstituents such as flavonoids, alkaloids, and terpenoids. These findings highlight the potential of LC + CP as a promising plant-based antimicrobial strategy against MDR pathogens and warrant further investigation into its phytochemical composition and in vivo efficacy.

INTRODUCTION

The dramatic rise of multidrug-resistant (MDR) bacteria poses a critical threat to global health, as conventional antibiotics become increasingly ineffective (WHO, 2020). In this context, ethnomedicinal plants offer a rich repository of bioactive compounds including alkaloids, flavonoids, and terpenoids known to exhibit significant antibacterial effects (Kumari et al., 2021; Khan et al., 2013). Among these, *Cissampelos pareira* (Menispermaceae) is traditionally used in Ayurvedic medicine and has been reported to contain isoquinoline alkaloids such as hayatinine, magnoflorine, and cissamine, which display potent antimicrobial and cytotoxic activities (Bala et al., 2019; Girma, 2024) Ethnopharmacological evaluations confirm its efficacy against Gram-positive and Gram-negative bacteria, with MIC values ranging from 12.5 to 50 µg/ml for root extracts. Similarly, *Lantana camara* (Verbenaceae) has received attention for its wide-spectrum antimicrobial and antioxidant activity, attributed to phenolic compounds, terpenoids, and essential oils (Bareto et al., 2010). Its ethanolic leaf extracts have produced zones of inhibition of 13–21 mm against *Staphylococcus aureus* and *Pseudomonas aeruginosa*, with MICs around 2–4 mg/ml. *Parthenium hysterophorus* (Asteraceae), although an invasive weed, has demonstrated substantial antimicrobial, antioxidant, and protective effects in vitro; hexane, chloroform, and benzene extracts show strong activity against *Streptococcus mutans*, *Proteus vulgaris*, and *Salmonella typhi* .Evidence suggests that combining plant extracts often enhances antimicrobial potency due to phytochemical synergy, potentially lowering effective concentrations and overcoming resistance (Wagner & Ulrich-Merzenich, 2009). While synergistic interactions between other medicinal plants have been documented, the combined effect of *C. pareira*, *L. camara*, and *P. hysterophorus* against MDR bacteria remains underexplored. The present study aims to evaluate these combinations for antimicrobial and bactericidal efficacy against MDR isolates obtained from hospital-associated soil, with the goal of identifying novel, plant-based strategies to combat antibiotic resistance.

METHODOLOGY

Collection of plant samples

The leaves of *C. pareira* (CP), *P. hysterophorus* (CG) and *L. camara* (LC) are collected from the Hamirpur district of Himachal Pradesh in the month of july. Plant leaves were then washed and shade dried to prevent the loss of heat sensitive phytocompounds.

Plant extract preparation

Dried plant leaves were grind to make powder. Weigh 20 gram of each plant sample and immerse each in the 100ml of 70% ethanol for 1 week. Then filter the mixtures with Whatman No.1 filter paper and evaporate the access ethanol. Re dissolve the plant extract in 2.5% dimethyl sulfoxide (DMSO) making stock solution of 1g/ml.

Synergistic potential of plant extraction

Antimicrobial activity of plant extracts was evaluated using the agar well diffusion method on Mueller Hinton Agar (MHA) plates. A 100 mg/ml concentration of plant extract was prepared from the stock solution. 3 combinations (LC+CP, LC+CG, CP+CG) were made by taking equal proportion of plant extract (1:1) and make a final concentration of 100mg/ml of the combination. MHA plates were then poured and uniformly inoculated with the isolated MDR bacterial strains to ensure even coverage across the surface. Using a sterile pipette tip, seven wells were carefully made in each agar plate, maintaining appropriate spacing between them. Different concentrations of the herbal extracts were dispensed into the respective wells. The plates were left undisturbed for a short time to allow the extracts to diffuse into the agar. Following this, the plates were incubated at 27°C for a period suitable to support the growth of the test microorganisms. After incubation, the plates were examined for clear zones of inhibition areas where bacterial growth was suppressed by the extracts. The diameters of these zones were measured systematically recorded in tabular form.

Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) of the extract combination

The plant extract combination was further analyzed for its MIC and MBC using the broth microdilution technique. Fresh overnight cultures of the MDR bacterial isolates were cultivated in nutrient broth and standardized to match the 0.5 McFarland turbidity standard, corresponding to roughly 1×10^8 CFU/ml. This suspension was then diluted to achieve a final concentration of 5×10^6 CFU/ml for testing.Serial two-fold dilutions of the plant combination were prepared in Mueller-Hinton Broth (MHB) within sterile 96-well microtiter plates, covering a range of concentrations. Equal volumes of the diluted antimicrobial solution and bacterial suspension were added to each well. Control wells were included: a positive control containing bacteria without the test extract, and a negative control containing only broth. The microplates were incubated at 37°C for 18 to 24 hours. The MIC was identified as the lowest concentration of the test extract at which no visible bacterial growth (i.e., turbidity) was observed. For MBC determination, samples from the wells showing no visible growth (at MIC and higher concentrations) were streaked onto Mueller-Hinton Agar (MHA) plates and incubated at 37°C for another 24 hours. The MBC was determined as the lowest concentration where no bacterial colonies appeared, indicating complete bactericidal action.

Phytochemical analysis of extract combination by Gas-chromatography Mass spectroscopy (GCMS)

Gas Chromatography-Mass Spectrometry (GC-MS) was performed using a Thermo Trace 1300 gas chromatograph coupled with a Thermo Triple Quadrupole 1000 MX mass spectrometer. The system was operated through XCalibur 2.2SP1 software integrated with Foundation 2.0 SP1. For compound separation, a BP-5MS capillary column (30 m × 0.25 mm, 0.25 μm film thickness), consisting of 5% phenyl polysilphenylene siloxane, was utilized. Helium of high purity acted as the carrier gas at a constant flow of 1.2 ml/min. A 3 μl sample was introduced using a split ratio of 1:67. The injector, ion source, and transfer line temperatures were set at 250°C, 230°C, and 240°C, respectively. The oven temperature started at 50°C with a 3-minute hold, then gradually increased by 5°C per minute to 300°C, where it was held for 5 minutes, followed by a further increase to 250°C for an additional 5-minute hold. The total run time was 39.67 minutes. Mass spectral data were collected over a scan range of m/z 40–650, and compounds were identified by comparing their spectra to those in the NIST Coin 2.0 Library.

RESULT AND DISCUSSION

Leaves of the plants *C. pareira*, *P. hysterophorus* and *L. camara* (Fig.1) are collected in the month of july.

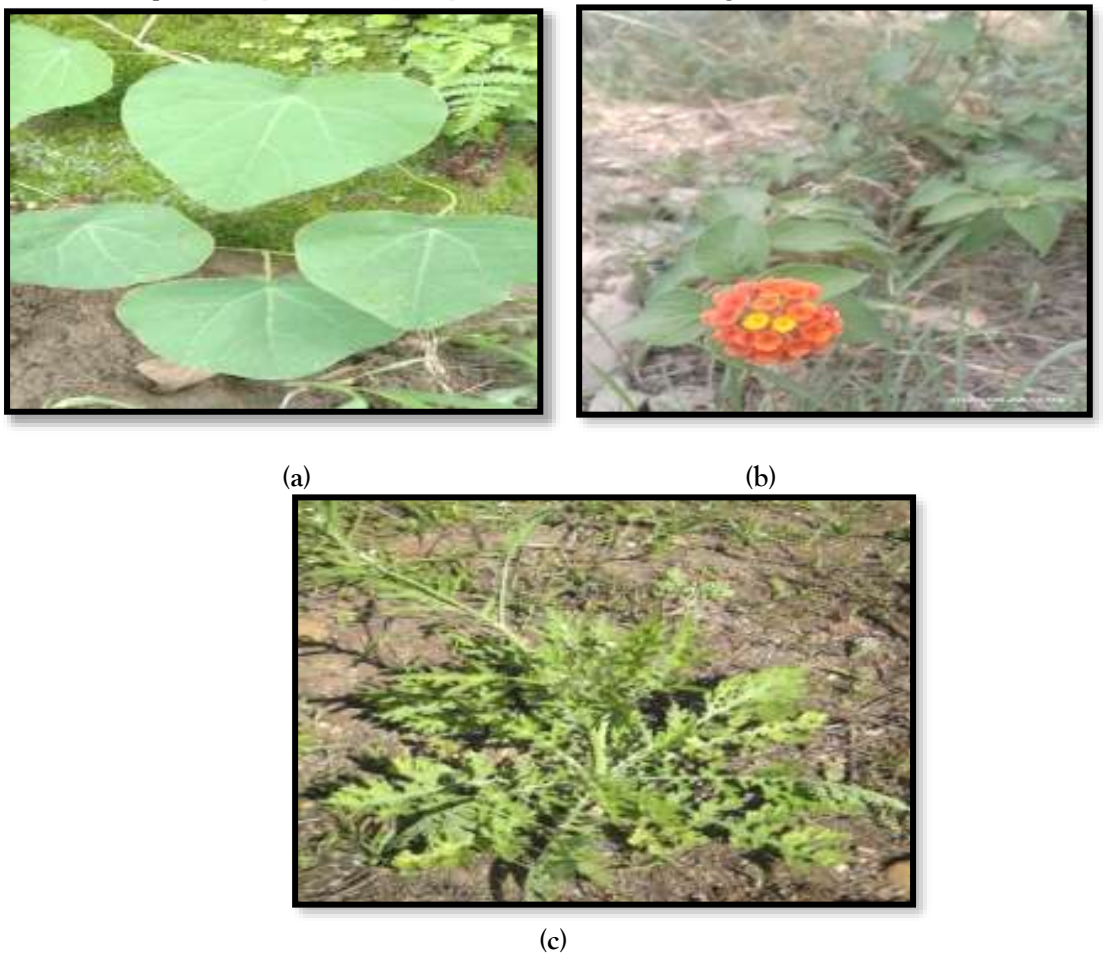

Fig. 1 Picture (a) Showing the leaves of *C. pareira.* (b) Showing the flower and leaves of *L. camara* (c) showing leaves and stem of *P. hysterophorus.*

The comparative analysis of extract yields from the selected medicinal plants revealed significant variation in their phytochemical extraction efficiency. *C. pareira* demonstrated the highest percentage yield of 10%, indicating a greater abundance of extractable constituents. *L. camara* and *P. hysterophorus* yielded 6.0% and 5.0%, respectively, under identical extraction conditions (Table 1). These findings suggest that *C. pareira* may possess a richer profile of bioactive compounds, warranting further phytochemical and antimicrobial investigation. The lower yields observed in *L. camara* and *P. hysterophorus* could be attributed to differences in the chemical composition or solubility of their phytoconstituents.

Table 1. Percentage Yield of Plant Extracts Obtained Through Ethanol Based Maceration

Sr. No.	Name of plant	Assigned Code	Plant material used (g)	Weight of empty plate (g)	Weight of Petri plate with dried extract(g)	Yield of extract (g)	*Percentage Yield (%)
1	*C. pareira*	CP	20	74.36	76.36	2	10
2	*L. camara*	LC	20	74.36	75.56	1.2	6.0

3	*P. hysterophorus*	CG	20	73.36	75.36	1	5.0

*%Yield = Yield of extract/Plant material used × 100

The antimicrobial activity of different plant extract combinations was evaluated against four multidrug-resistant (MDR) bacterial isolates *P. aeruginosa* 1 (BI4), *B. pumilus* (BT3), *C. cellulans* (HMR2) and *P. aeruginosa* 2 (TD7) using the agar well diffusion method. The mean zones of inhibition (in mm) for each extract combination are presented in Table 2 and Figure 2. Among the tested combinations, *L. camara* + *C. pareira* (LC + CP) exhibited the strongest antimicrobial activity across all isolates. The inhibition zones observed were 19.50 ± 1.22 mm (BI4), 16.17 ± 0.27 mm (BT3), 20.00 ± 0.46 mm (HMR2), and 24.17 ± 0.27 mm (TD7). This was closely followed by the LC + CG (*L. camara* + *P. hysterophorus*) combination, which demonstrated moderate inhibitory effects with zones ranging from 9.83 ± 0.27 mm to 19.50 ± 0.46 mm. The CP + CG (*C. pareira* + *P. hysterophorus*) combination showed the least activity, particularly against BI4 and BT3, though it demonstrated notable inhibition against HMR2 and TD7 (17.83 ± 0.27 mm and 24.00 ± 0.46 mm, respectively).

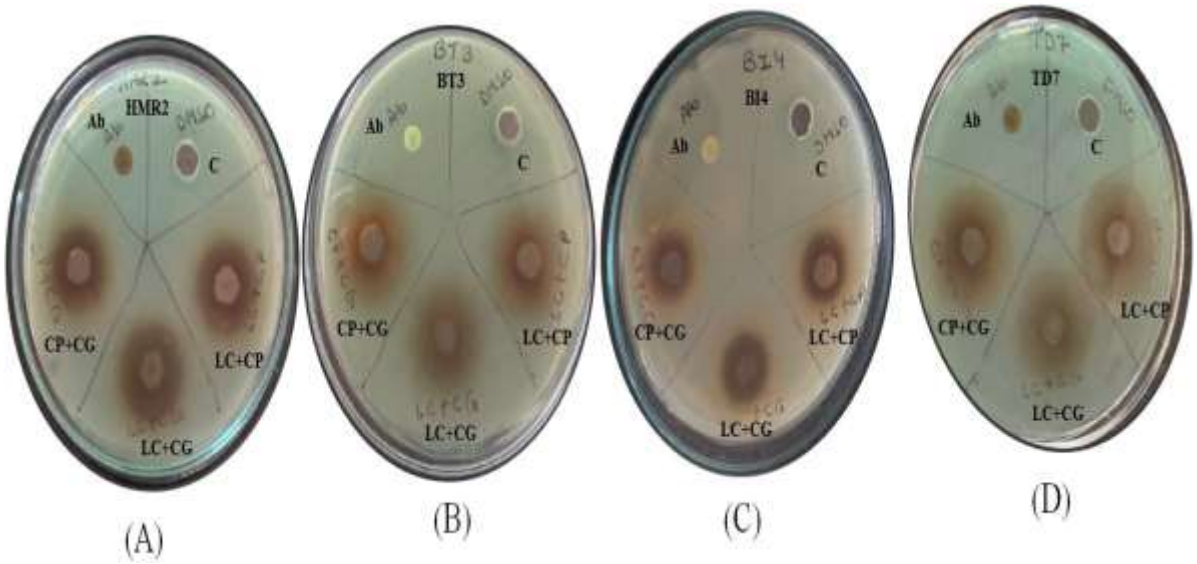

Fig.2. Synergistic activity of selected plants against MDR isolates (A) *C. cellulans* (HMR2) (B) *B. pumilus* (BT3) (C) *P. aeruginosa* (BI4) and (D) *P. aeruginosa* (TD7).

Table 4.17 Synergistic Activity of selected ethanolic extract combinations against MDR isolates.

Sr. No.	**Plant Extract combinations**	**Zone of inhibition (mm)**			
		BI4 (*P. aeruginosa*)	**BT3 (*B. pumilus*)**	**HMR2 (*C. cellulans*)**	**TD7 (*P. aeruginosa*)**
1	CP+CG	8.17±0.27	8.00±0	17.83±0.27	24.00 ±0.46
2	LC+CG	14.00 ±0.46	9.83±0.27	19.00 ±0.46	19.50±0.46
3	LC+CP	19.50 ±1.22	16.17±0.27	20.00 ±0.46	24.17±0.27

These results suggest that the synergistic interaction between certain phytochemicals in *L. camara* and *C. pareira* may be responsible for the enhanced antimicrobial effect. *L. camara* is known to contain

triterpenoids, flavonoids, and phenolic compounds with strong antibacterial properties (Ayub et al., 2017), while *C. pareira* has been reported to possess alkaloids like cissamine and hayatine that disrupt microbial cell function (Kumari et al., 2021). A Rwandan study also reported an inhibition zone of 28 mm against *S. aureus* using methanol leaf extracts (Voukeng et al., 2016). Its ethanolic leaf and stem extracts have demonstrated antibacterial activity, with Ajaib et al. (2018) reporting zones between 13–14 mm against *E. coli* and *P. aeruginosa*. The synergistic effect observed in the LC + CP combination may be attributed to the complementary mechanisms of these phytoconstituents, potentially leading to enhanced membrane disruption, enzyme inhibition, or interference with bacterial replication. Previous studies have highlighted that plant extract combinations often exhibit superior efficacy compared to individual extracts, particularly against resistant strains (Sibanda & Okoh, 2007). This phenomenon, known as phytochemical synergy, has been proposed as a promising approach to combat MDR pathogens (Wagner & Ulrich-Merzenich, 2009). The results of the present study align with these findings, reinforcing the therapeutic potential of combining specific medicinal plants to overcome antimicrobial resistance. Additionally, the zone of inhibition values obtained in this study for certain combinations (particularly LC + CP) were comparable to or higher than those reported in previous studies involving crude plant extracts (Holetz et al., 2002), suggesting the strong bioactivity of the tested phytocombinations.The findings of this study are analyzed with regard to both the interaction between the extract combinations and their antimicrobial effectiveness. It has been recommended that MIC values exceeding 1 mg/ml for crude extracts or 0.1 mg/ml for isolated compounds should generally be avoided. Activity is considered highly promising when MICs fall within the range of 0.1 mg/ml for extracts and 0.01 mg/ml for pure compounds (Ríos and Recio 2005). Conversely, Fabry et al. (1998) characterized crude extracts as active if their MIC values were below 8 mg/ml. In the present study, however, MIC and MBC values under 1 mg/ml were regarded as indicative of strong antimicrobial potential. The LC + CP extract combination exhibited antimicrobial activity against all tested MDR strains, with MIC values ranging between 1.562 and 6.25 mg/ml, and MBC values ranging from 3.125 to 12.5 mg/ml**.** Among the tested isolates, TD7 was the most sensitive, exhibiting the lowest MIC (1.562 mg/ml) and MBC (3.125 mg/ml), and suggesting strong susceptibility to the plant combination. BI4 and HMR2 were moderately sensitive (MIC = 3.125 mg/ml), while BT3 was the least responsive (MIC = 6.25 mg/ml, MBC = 12.5 mg/ml). To confirm the bactericidal potential, MBC values were evaluated using the standard agar plating method (Figure 3). Visual observation of plates confirmed complete inhibition of bacterial regrowth at concentrations higher than the MIC, especially in TD7 and BI4 isolates. These results support the bactericidal nature of the LC + CP combination rather than a merely bacteriostatic effect.

Table 4.20 MIC and MBC values for plant extract LC+CP against MDR strains BI4, BT3, HMR2 and TD7.

Sr. No.	MDR isolates	Plant Extracts concentration in mg/ml	
		LC+CP	
		MIC (mg/ml)	MBC(mg/ml)
1.	BI4	3.125	6.25
2.	BT3	6.25	1.25
3.	HMR2	3.125	6.25
4.	TD7	1.562	3.125

According to Holetz et al. (2002), plant extracts with MIC values ≤1 mg/ml are considered highly active, while values between 1-8 mg/ml indicate moderate activity. Therefore, the observed MICs of the LC + CP blend demonstrate moderate but clinically significant antibacterial activity, particularly against TD7

and BI4 isolates. The enhanced antimicrobial potential of the LC + CP combination is likely attributed to the synergistic interaction of their phytochemicals, including alkaloids, flavonoids, terpenoids, and phenolic compounds. Previous studies support the individual antibacterial activity of both plants. *L. camara* extracts have demonstrated MICs of 2–4 mg/ml against Gram-negative bacteria like *E. coli* and *P. aeruginosa*. *C. pareira* has shown MICs in the range of 3.12–6.25 mg/ml in ethanolic extracts against similar pathogens.

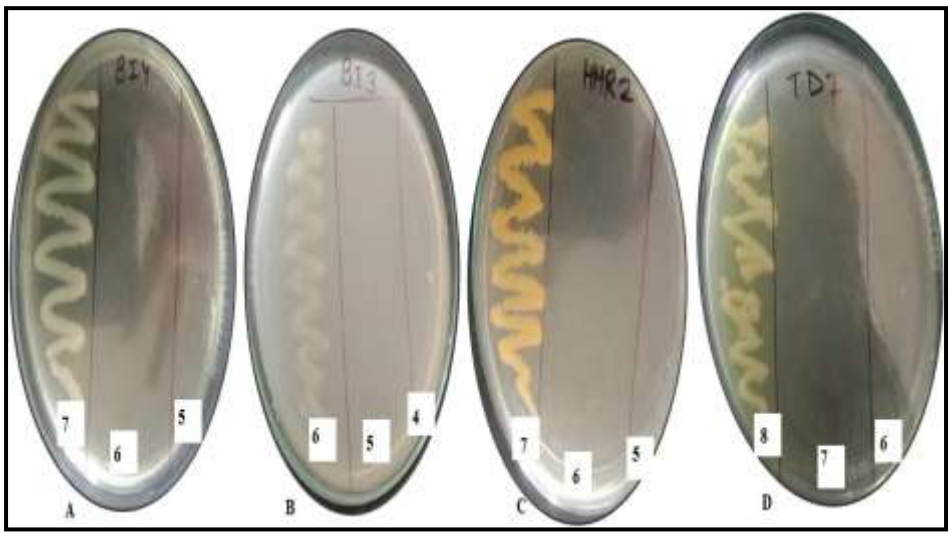

Fig.3. Determination of Minimum Bactericidal Concentration (MBC) of the LC + CP plant extract combination against MDR bacterial isolates.

Moreover, Wagner and Ulrich-Merzenich (2009) have emphasized that plant extract combinations often result in pharmacodynamic synergy through mechanisms such as enhanced membrane disruption, inhibition of efflux pumps, or oxidative damage amplification [4]. The observed MBC/MIC ratios (≤4 for all isolates) further indicate that the LC + CP extract exerts a bactericidal effect, particularly notable in the TD7 strain (ratio = 2). This aligns with accepted microbiological standards that classify antimicrobial agents as bactericidal when MBC/MIC ≤4 (Pankey & Sabath, 2004).

CONCLUSION

The present study demonstrates the significant antimicrobial potential of the synergistic combination of *L. camara* and *C. pareira* (LC + CP) extracts against multidrug-resistant (MDR) bacterial isolates. Among the tested combinations, LC + CP exhibited the highest overall inhibitory zones, confirming enhanced antimicrobial activity attributed to phytochemical synergy. The combination showed broad-spectrum activity against all four MDR isolates, with the strongest inhibition observed against the TD7 strain.Furthermore, MIC and MBC assessments substantiated these findings, with MIC values ranging from 1.562 to 6.25 mg/ml and MBC values between 3.125 to 12.5 mg/ml. The lowest MIC and MBC recorded for TD7 (1.562 and 3.125 mg/ml, respectively) indicate this isolate's heightened sensitivity to the extract combination. The MBC/MIC ratios (≤4) across all isolates affirm the bactericidal nature of the LC + CP extract blend, as per clinical pharmacodynamic standards. These results strongly suggest that combining *L. camara* and *C. pareira* enhances antibacterial efficacy through phytochemical synergy, potentially overcoming resistance mechanisms in MDR strains. The findings support the development of plant-based synergistic therapies as promising alternatives or adjuncts to conventional antibiotics. Further studies involving mechanism of action, phytochemical isolation, cytotoxicity profiling, and in vivo validation are recommended to fully exploit the therapeutic potential of this herbal combination.

Acknowledgement

Authors express their thanks to Division of Microbiology, Career Point University, Hamirpur, H.P.

Conflict of interest

There is no conflict of interest between the authors, all authors contributed directly to the article.

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