



SYNERGISTIC ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF *TRIDAX PROCUMBENS* L. AND *PERISTROPHE BICALYCVULATA* RETZ. LEAF EXTRACTS USING DIFFERENT SOLVENT SYSTEMS

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ABSTRACT

Background: The need for novel antimicrobial agents made from natural sources has increased globally due to the quick emergence of pathogens resistant to antibiotics. Traditional medicine has long employed medicinal plants like *Tridax procumbens* L. and *Peristrophe bicalyculata* Retz., but little is known about their combined antimicrobial potential. **Methods:** Methanol and chloroform were used to extract the leaves of *T. procumbens* and *P. bicalyculata* after they had been collected and shade-dried. Using the disc diffusion method, the antimicrobial properties of both single and combined extracts were assessed against fungi (*Aspergillus niger*, *A. fumigatus*, and *A. flavus*), Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*), and Gram-positive bacteria (*Staphylococcus aureus*, *S. haemolyticus*). **Results:** The superior extraction of bioactive phytoconstituents in polar solvents was demonstrated by the noticeably higher antimicrobial activity of methanolic extracts when compared to chloroform extracts. While *T. procumbens* demonstrated strong

inhibition against *S. aureus* (30 ± 1 mm), *P. bicalyculata*'s methanol extract exhibited the highest antibacterial activity against *S. haemolyticus* (37.66 ± 4.50 mm). A synergistic effect was confirmed by the increased inhibitory zones shown by the combined methanolic extracts. Antifungal assays revealed similar trends, with *A. niger* exhibiting the highest sensitivity. **Conclusion:** Strong broad-spectrum antimicrobial activity was shown by the combined methanolic extracts of *P. bicalyculata* and *T. procumbens*. These results point to their potential as natural resources for creating phytotherapeutic agents that fight infections resistant to antibiotics.

KEYWORDS: *Tridax procumbens*; *Peristrophe bicalyculata*; Antimicrobial activity; Synergistic effect; Medicinal plants.

1. INTRODUCTION

Natural substances found in medicinal plants have the potential to prevent or cure various illnesses. Numerous plants have been found to possess antibacterial properties.^[1] Different types of antibiotics have been developed to target specific microorganisms since their discovery.^[2] Plant-based natural products continue to be essential sources of therapeutic agents and have long been used in contemporary drug discovery.^[3] Approximately 2,000 plant species are used medicinally in India, either directly or in contemporary formulations. According to a UNCTAD (United Nations Conference on Trade and Development) survey, 60% of medications are derived from natural sources, whereas 33% of drugs in developed nations are derived from plants UNCTAD/Gatt in 1974. Therefore, it is essential to document and evaluate medicinal plants scientifically, particularly in developing nations.^[4]

The search for novel natural antimicrobial agents has accelerated owing to the growing issue of antibiotic resistance.^[5] According to the World Health Organization (WHO), nearly 80% of people in developing countries receive their primary medical care from traditional plant-based medicine.^[6] Herbal remedies are frequently safer, less expensive, and equally effective in preserving health as synthetic remedies.^[7] However, the emergence of drug-resistant pathogens has created a pressing need for alternative treatments.^[8,9] Long before microorganisms were scientifically identified, plants were prized for their antimicrobial and therapeutic qualities.^[10] Only a small percentage of the more than 400,000 plant species on Earth have been scientifically studied for their bioactivity.^[11]

Secondary metabolites with antimicrobial and antioxidant properties are abundant in medicinal plants.^[12,13] Identifying antimicrobial compounds derived from plants is a promising strategy, as microbial infections continue to be a global health concern.^[14] According to previous studies, some compounds derived from plants, such as carvacrol from thyme and oregano oils, can dramatically reduce the growth of bacteria.^[15] In addition, studies have shown that ethanolic extracts frequently exhibit more potent antibacterial properties than methanolic or aqueous extracts.^[16] Moreover, the combination of antimicrobial and antioxidant activities can enhance therapeutic potential.^[17]

The small annual herb *Tridax procumbens* L., also called coatbuttons or tridax daisy, belongs to the Asteraceae family and is well known for its ability to heal wounds, reduce inflammation, and protect the liver. It has been used to treat bronchial issues, ulcers, diarrhea, and jaundice.^[18] The Acanthaceae family's *Peristrophe bicalyculata* Retz. (kali adhedhi or lisi adhedhi) is a hispid herb frequently used in traditional medicine to treat skin infections, fever, bone fractures, and snakebites.^[19] Additionally, its extracts are used to treat wound healing, malaria, and cough. These two plants show promise as ethnomedical resources with synergistic antimicrobial activity.^[20]

To determine whether *Tridax procumbens* L. and *Peristrophe bicalyculata* Retz. work in concert to increase antibacterial efficacy, this study examined the antimicrobial potential of both species separately and in combination. To ascertain which extraction technique produces the strongest antimicrobial activity, this study focused on comparing plant extracts prepared using various solvents. This study aimed to identify potential synergistic interactions that could enhance the overall antimicrobial spectrum by comparing the effects of each plant separately with their combined response. The ultimate goal of this study is to demonstrate the medicinal potential of these two ethnomedicinal plants as a source of natural antimicrobial agents that could be used in place of or in addition to traditional antibiotics to combat resistant bacterial strains.

2. MATERIALS AND METHODS

2.1 Collection and Identification of Plant Materials

In December 2019, fresh leaves of *Tridax procumbens* L. “Fig. 1” and *Peristrophe bicalyculata* Retz. “Fig. 2” were collected from the St. Joseph's College campus in Tiruchirappalli, Tamil Nadu. Standard botanical references were used to identify and authenticate plants based on their morphological traits. To guarantee surface cleanliness, the

collected leaf samples were rinsed with sterile distilled water after being thoroughly cleaned under running tap water to remove any remaining dust and dirt particles. To maintain the phytochemical integrity of the leaves, they were shade-dried for approximately a week at room temperature. A sterile mixer grinder was used to coarsely powder the leaves after they had completely dried, and they were then placed in airtight containers for further examination.



Fig. 1: *Tridax procumbens* L



Fig. 2: *Peristrophe bicalyculata* Retz.

2.2 Preparation of Plant Extracts

Tridax procumbens and *Peristrophe bicalyculata* leaf powders were used for solvent extraction. One hundred milliliters of the corresponding solvent was combined with 20 g of each powdered sample. To maximize the extraction of bioactive compounds, the mixture was placed in sterile brown glass bottles and left to stand at room temperature for five days, while being shaken occasionally. To remove plant residues, the extracts were filtered through Whatman No. 1 filter paper after incubation. To prevent phytoconstituents from degrading before being used in antimicrobial tests, the filtrates were placed in sterile brown bottles and kept in the dark.^[21]

2.3 Collection of Microorganisms

The Department of Microbiology at K.A.P. Viswanatham Government Medical College in Tiruchirappalli provided bacterial and fungal test organisms. Both Gram-positive and Gram-negative bacteria were isolated: *Klebsiella pneumoniae* and *Escherichia coli* were Gram-negative, while *Staphylococcus aureus* and *Staphylococcus hemolyticus* were Gram-positive. These strains were selected to assess the broad-spectrum antibacterial activity of the plant extracts.

Three pathogenic fungal species, *Aspergillus niger*, *Aspergillus fumigatus*, and *Aspergillus flavus*, were selected for antifungal screening. These fungi are good test organisms for evaluating antifungal potential because they are known to cause opportunistic infections in humans. Prior to experimentation, all microbial strains were kept under suitable culture conditions and subcultured on potato dextrose agar for fungi and nutrient agar for bacteria.

2.4 Preparation of Culture Media

(i) Nutrient Broth (NB): To prepare the bacterial growth medium, 50 mL of distilled water was used to dissolve 1.3 g of commercially available nutrient broth powder. To guarantee sterilization, the medium was poured into sterile test tubes (10 mL per tube) and autoclaved for 15 min at 121°C. A loopful of bacterial inoculum was aseptically added to each test tube containing nutrient broth once it had cooled to room temperature. To encourage optimal bacterial growth, the cultures were incubated for 24 h at room temperature (approximately 28–30°C).^[22]

(ii) Potato Dextrose Broth (PDB): For fungal culture preparation, 50 mL of distilled water was used to dissolve 1.3 g of premade potato dextrose broth. Similar amounts of the medium were transferred into sterile test tubes (10 mL each), which were then autoclaved for 15 min at 121°C to sterilize them. To ensure adequate fungal growth, a loopful of fungal inoculum was aseptically added to each tube after it had cooled, and the cultures were incubated for 24–48 h at room temperature.^[23]

2.5 Antibacterial Assay (Disc Diffusion Method)

The standard disc diffusion method was used to evaluate the antibacterial activity of the plant extracts. Nutrient agar medium was prepared by dissolving 28 g of premade nutrient agar powder to create nutrient agar medium. The mixture was autoclaved for 15 min at 121°C to sterilize it after heating and constant stirring until it was completely dissolved. Sterile Petri dishes were filled with 15–20 mL of molten medium, which was then left to solidify in an aseptic environment.

Before use, sterile Whatman No. 1 filter paper discs (5 mm in diameter) were prepared and autoclaved. Sterile cotton swabs were used to evenly distribute fresh bacterial cultures on the agar surface. Using sterile forceps, the sterile discs were carefully placed on the inoculated plates after being impregnated with the corresponding plant extracts. Following a 24-hour incubation period at 37°C, the diameter of the zones of inhibition encircling each disc was

measured to assess the antibacterial activity of the plates. The relative potency of the extract against the tested bacterial strains was indicated by the size of the inhibition zone.^[24]

2.6 Antifungal Assay (Agar Diffusion Method)

The antifungal activity was evaluated using potato dextrose agar (PDA) medium and the agar well diffusion method. PDA was prepared by heating 39 g of premade PDA powder until it completely dissolved in 1 L of distilled water. After autoclaving at 121 °C for 15 min, the medium was transferred into sterile Petri dishes (15–20 mL per plate). A sterile L-rod was used to evenly distribute the test fungi (*Aspergillus niger*, *A. fumigatus*, and *A. flavus*) across the agar surface once it had solidified. Sterile discs impregnated with the corresponding plant extracts were placed on the inoculated plates using sterile forceps. Antifungal activity was assessed by measuring the transparent zones of inhibition that developed around the discs after the plates were incubated for 24–48 h at 27°C.^[25]

2.7 Evaluation of Synergistic Antimicrobial Activity

Methanolic and chloroform extracts of *Tridax procumbens* and *Peristrophe bicalyculata* were used to assess their synergistic antimicrobial effects. Sterilized brown bottles containing equal amounts (1:1) of each solvent extract (50 percent *T. procumbens* and 50 percent *P. bicalyculata*) were left at room temperature for 48 h. The standard antibiotic control was chloramphenicol, which was prepared by dissolving 0.1 g in 10 mL of distilled water and allowing it to sit for a full day before use.

As previously mentioned, nutrient agar was prepared for bacteria and potato dextrose agar for fungi, and they were autoclaved for sterilization. Sterilized Petri dishes were filled with 15–20 mL of the medium, which was then allowed to solidify. Bacterial and fungal cultures were evenly distributed on nutrient agar and PDA plates, respectively, using sterile swabs. Using sterile forceps, sterile discs impregnated with a combination of plant extracts and antibiotic controls were carefully positioned on the inoculated plates. The fungal plates were incubated for 48 h at 27°C, and the bacterial plates were incubated for 24 h at 37°C. To evaluate the individual and combined antimicrobial activities of the plant extracts, the zones of inhibition were measured following incubation.^[26]

3. RESULTS

3.1 Antibacterial Activity of Leaf Extracts

***Peristrophe bicalyculata* Retz:** Using the disc diffusion method, the antibacterial activity of leaf extracts from *Peristrophe bicalyculata* was assessed against strains of Gram-positive and Gram-negative bacteria “Fig. 3 & 3.1”. Chloroform and methanol were used as the extraction solvents. Gram-positive *Staphylococcus aureus* and *Staphylococcus haemolyticus* as well as Gram-negative *Escherichia coli* and *Klebsiella pneumoniae* were among the test organisms.

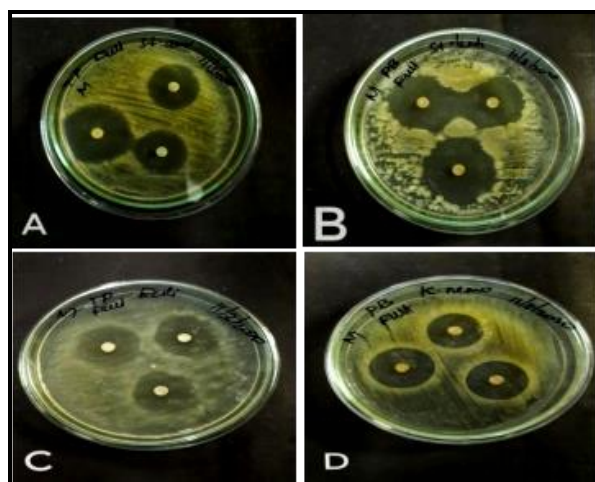


Fig. 3: Antibacterial activity of *Peristrophe bicalyculata* (a.Methanolic extract in *Staphylococcus aureus*, b.Methanolic extract in *Staphylococcus haemolyticus*, c.Methanolic extract in *Escherichia coli*, d.Methanolic extract in *Klebsiella pneumoniae*).

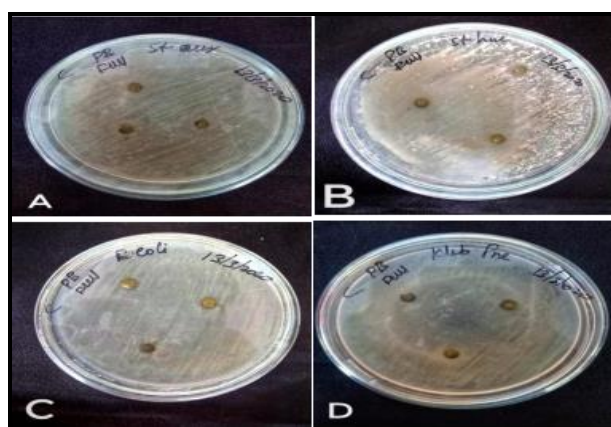


Fig. 3.1: Antibacterial activity of *Peristrophe bicalyculata* (a.Chloroform extract in *Staphylococcus aureus*, b.Chloroform extract in *Staphylococcus haemolyticus*, c.Chloroform extract in *Escherichia coli*, d.Chloroform extract in *Klebsiella pneumoniae*).

Compared to the chloroform extract, the methanolic extract demonstrated a noticeably greater inhibitory effect against every bacterial strain tested. *S. aureus* (30 ± 5.57 mm) was found to

have the second-largest zone of inhibition, after *S. haemolyticus* (37.66 ± 4.50 mm). *E. coli* (25.33 ± 6.11 mm) and *K. pneumoniae* (29.66 ± 1.52 mm) showed moderate inhibition “Table 1”. The chloroform extract demonstrated only weak antibacterial activity, with inhibition zones varying from 6.66 ± 1.15 mm to 10.66 ± 3.51 mm.

Table 1: Antibacterial activity of *Peristrophe bicalyculata* Retz.

Tested Bacteria	Zone of Inhibition (mm)*		
	Methanol Extract	Chloroform Extract	Standard Antibiotic (#)
Gram Positive (+) Bacteria			
<i>Staphylococcus aureus</i>	30 ± 5.57	10.66 ± 3.51	2.7 cm
<i>Staphylococcus haemolyticus</i>	37.66 ± 4.50	7.33 ± 0.57	2.6 cm
Gram Negative (-) Bacteria			
<i>Escherichia coli</i>	25.33 ± 6.11	7.33 ± 1.15	2.2 cm
<i>Klebsiella pneumoniae</i>	29.66 ± 1.52	6.66 ± 1.15	2.5 cm
* Mean of triplicates, # Chloramphenicol, $\pm$ Standard Deviation, - No inhibition			

These results suggest that the methanol extract of *P. bicalyculata* has stronger antibacterial properties, possibly due to the improved solubility and extraction efficiency of the phenolic and flavonoid constituents in polar solvents.

***Tridax procumbens* L:** The antibacterial activity of *Tridax procumbens* leaf extracts was also examined using methanol and chloroform as extraction solvents “Fig. 4 & 4.1”. Both extracts exhibited antibacterial activity against the four tested bacterial species; however, in every instance, the methanolic extract had a greater inhibitory effect.

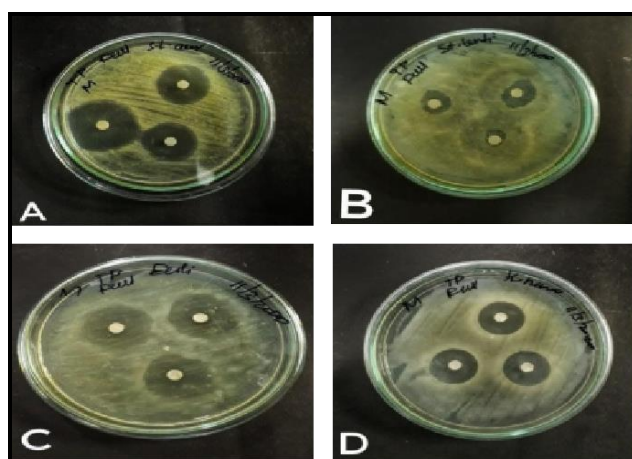


Fig. 4: Antibacterial activity of *Tridax procumbens* L (a.Methanolic extract in *Staphylococcus aureus*, b.Methanolic extract in *Staphylococcus haemolyticus*, c.Methanolic extract in *Escherichia coli*, d.Methanolic extract in *Klebsiella pneumoniae*)

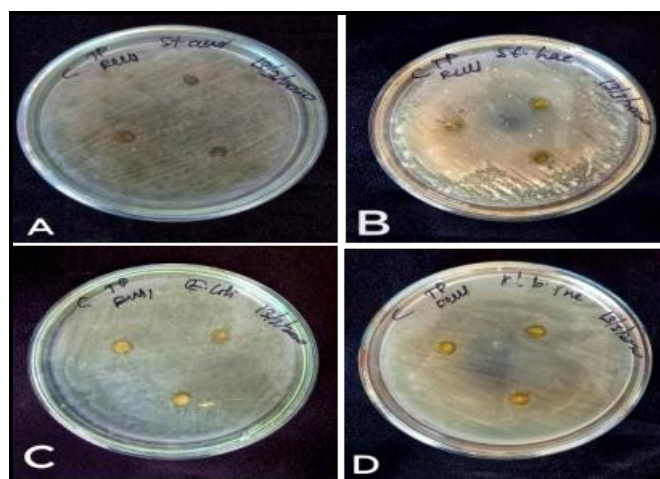


Fig. 4.1: Antibacterial activity of *Tridax procumbens* L (a.Chloroform extract in *Staphylococcus aureus*, b.Chloroform extract in *Staphylococcus haemolyticus*, c.Chloroform extract in *Escherichia coli*, d.Chloroform extract in *Klebsiella pneumoniae*).

The methanol extract exhibited maximum activity against *S. aureus* (30 ± 1 mm), *E. coli* (28 ± 3 mm), *S. haemolyticus* (25.66 ± 4.78 mm), and *K. pneumoniae* (23.66 ± 4.50 mm). In contrast, all strains exhibited negligible inhibition in the chloroform extract, with zones varying from 7.33 ± 0.57 mm to 8 ± 1.73 mm in diameter “Table. 2”. These findings suggest that *T. procumbens* leaves, particularly the polar extracts, contain bioactive substances with broad-spectrum antibacterial potential.

Table 2: Antibacterial activity of *Tridax procumbens* L.

Tested Bacteria	Zone of Inhibition (mm)*		
	Methanol Extract	Chloroform Extract	Standard Antibiotic (#)
Gram Positive (+) Bacteria			
<i>Staphylococcus aureus</i>	30 ± 1	7.33 ± 1.15	2.7cm
<i>Staphylococcus haemolyticus</i>	25.66 ± 4.78	7.33 ± 0.57	2.6cm
Gram Negative (-) Bacteria			
<i>Escherichia coli</i>	28 ± 3	8 ± 1	2.2cm
<i>Klebsiella pneumoniae</i>	23.66 ± 4.50	8 ± 1.73	2.5cm
* Mean of triplicates, # Chloramphenicol, $\pm$ Standard Deviation, - No inhibition			

3.2 Combined Antibacterial Activity of *Peristrophe bicalyculata* and *Tridax procumbens*

The methanolic and chloroform extracts of both plants were mixed in different ratios (50:50, 75:25, and 25:75) and tested against the same bacterial strains to assess any possible synergistic effects. The findings demonstrated that methanolic combinations consistently exhibited higher antibacterial activity than chloroform combinations. The greatest inhibition

against *E. coli* (26.33 ± 4.50 mm) and *S. haemolyticus* (25 ± 2.64 mm) was observed at a 50:50 ratio “Fig. 5 & 5.1”, “Table. 3.”

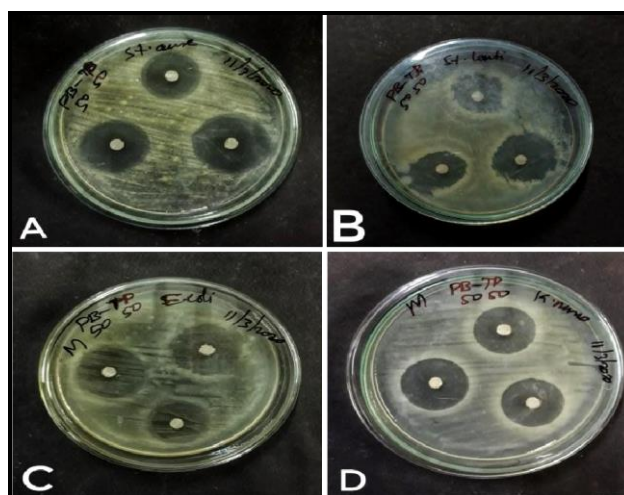


Fig. 5: Combined Antibacterial activity of *Peristroph bicalyculata* and *Tridax procumbens* L. (50/50) (a.Methanolic extract in *Staphylococcus aureus*, b.Methanolic extract in *Staphylococcus haemolyticus*, c.Methanolic extract in *Escherichia coli*, d.Methanolic extract in *Klebsiella pneumoniae*).

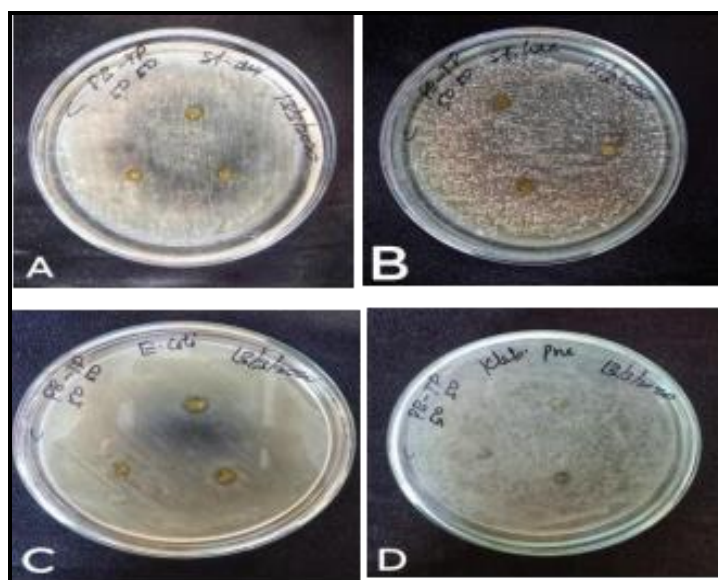


Fig 5.1: Combined Antibacterial activity of *Peristroph bicalyculata* and *Tridax procumbens* L. (50/50) (a.Chloroform extract in *Staphylococcus aureus*, b.Chloroform extract in *Staphylococcus haemolyticus*, c.Chloroform extract in *Escherichia coli*, d.Chloroform extract in *Klebsiella pneumoniae*).

Table 3: Combined Antibacterial Activity of *Peristroph bicalyculata* and *Tridax procumbens* L. (50/ 50).

Tested Bacteria	Zone of Inhibition (mm)*		
	Methanol Extract	Chloroform Extract	Standard Antibiotic (#)
Gram Positive (+) Bacteria			
<i>Staphylococcus aureus</i>	24 ± 1	7.66 ± 2.08	2.7cm
<i>Staphylococcus haemolyticus</i>	25 ± 2.64	8 ± 1	2.6cm
Gram Negative (-) Bacteria			
<i>Escherichia coli</i>	26.33 ± 4.50	8 ± 1	2.2 cm
<i>Klebsiella pneumoniae</i>	25.33 ± 3.51	8.33 ± 1.52	2.5cm
* Mean of triplicates, # Chloramphenicol, ± Standard Deviation, - No inhibition			

K.pneumoniae demonstrated the highest inhibition (28.66 ± 2.08 mm) at a 75:25 ratio “Fig. 6 & 6.1”, “Table. 4”, whereas *S. haemolyticus* showed the highest activity (39.33 ± 4.43 mm) at a 25:75 ratio “Fig. 7 & 7.1”, “Table. 5.”

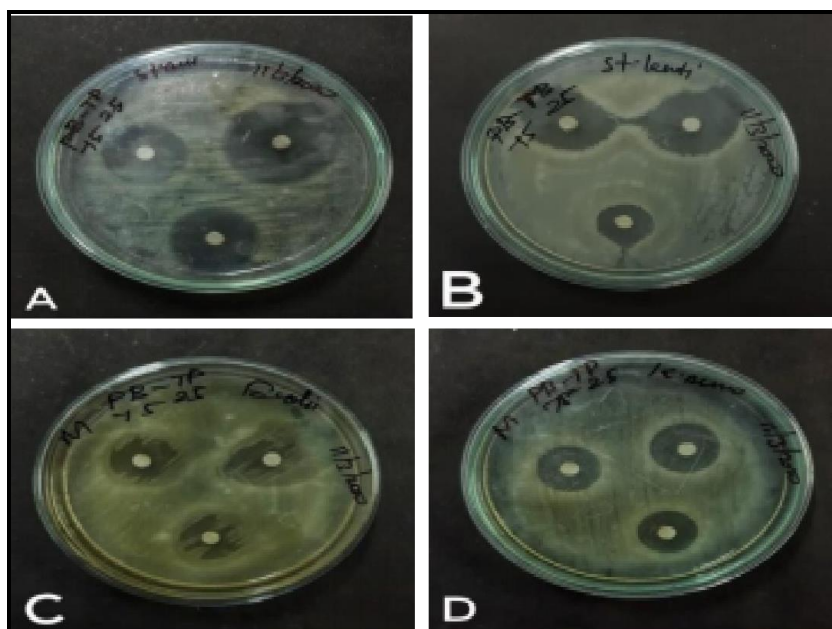


Fig 6: Combined Antibacterial activity of *Peristroph bicalyculata* and *Tridax procumbens* L. (75/25) (a.Methanolic extract in *Staphylococcus aureus*, b.Methanolic extract in *Staphylococcus haemolyticus*, c.Methanolic extract in *Escherichia coli*, d.Methanolic extract in *Klebsiella pneumoniae*).

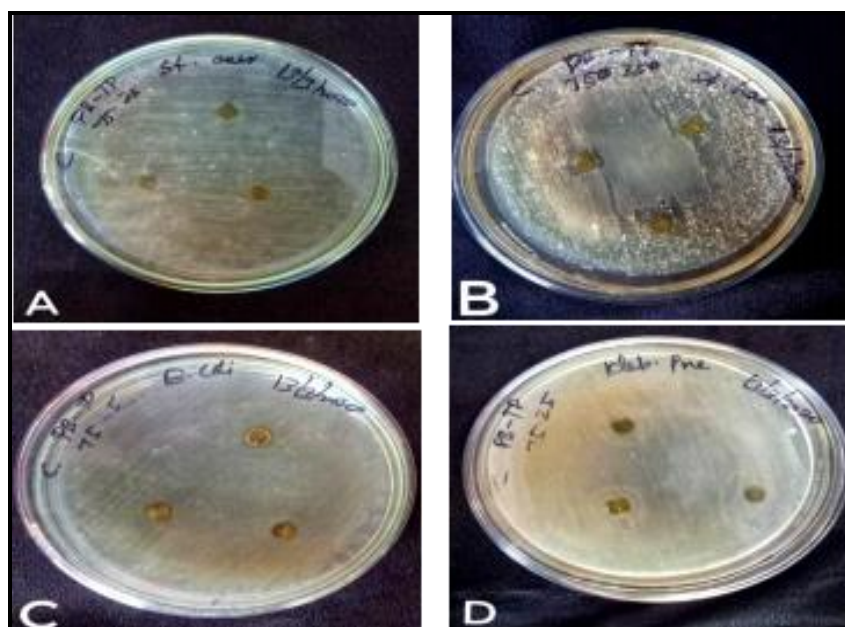


Fig 6.1: Combined Antibacterial activity of *Peristroph bicalyculata* and *Tridax procumbens* L. (75/25) (a.Chloroform extract in *Staphylococcus aureus*, b.Chloroform extract in *Staphylococcus haemolyticus*, c.Chloroform extract in *Escherichia coli*, d.Chloroform extract in *Klebsiella pneumoniae*).

Table 4: Combined Antibacterial Activity of *Peristroph bicalyculata* and *Tridax procumbens* L. (75 / 25).

Tested Bacteria	Zone of Inhibition (mm)*		
	Methanol Extract	Chloroform Extract	Standard Antibiotic (#)
Gram Positive (+) Bacteria			
Staphylococcus aureus	24.33 ± 1.52	7.33 ± 0.57	2.7 cm
Staphylococcus haemolyticus	24.33 ± 4.04	7.33 ± 0.57	2.6cm
Gram Negative (-) Bacteria			
Escherichia coli	26.33 ± 3.21	9 ± 1	2.2 cm
Klebsiella pneumoniae	28.66 ± 2.08	12.66 ± 2.51	2.5 cm
* Mean of triplicates, # Chloramphenicol, ± Standard Deviation, - No inhibition			

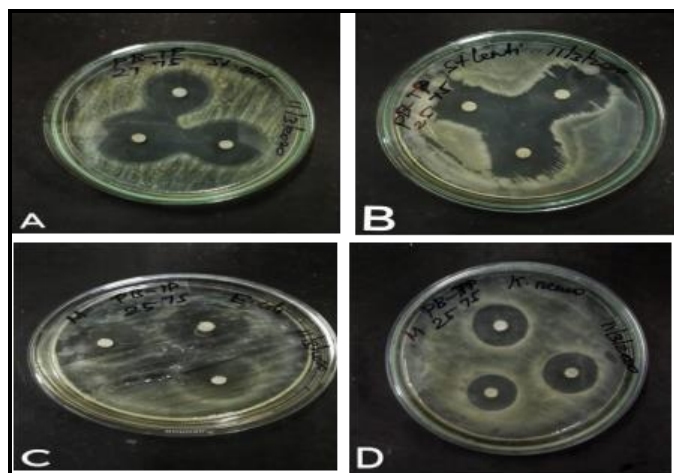


Fig 7: Combined Antibacterial activity of *Peristroph bicalyculata* and *Tridax procumbens* L. (25/75) (a.Methanolic extract in *Staphylococcus aureus*, b.Methanolic extract in *Staphylococcus haemolyticus*, c.Methanolic extract in *Escherichia coli*, d.Methanolic extract in *Klebsiella pneumonia*).

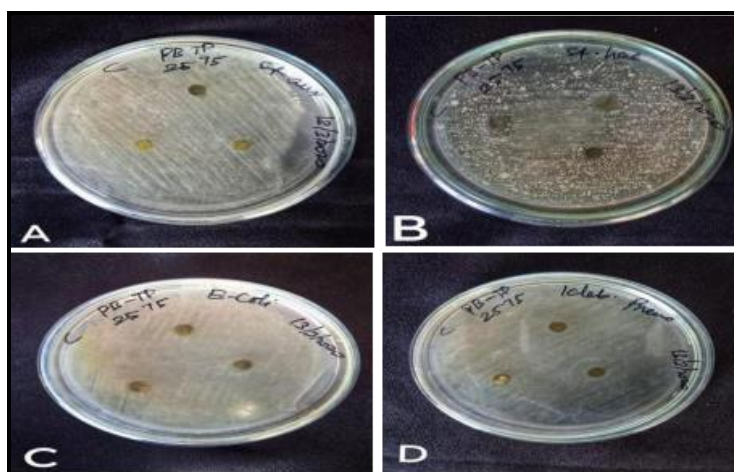


Fig 7.1: Combined Antibacterial activity of *Peristroph bicalyculata* and *Tridax procumbens* L. (25/75) (a.Chloroform extract in *Staphylococcus aureus*, b.Chloroform extract in *Staphylococcus haemolyticus*, c.Chloroform extract in *Escherichia coli*, d. Chloroform extract in *Klebsiella pneumonia*).

Table 5: Combined Antibacterial Activity of *Peristroph bicalyculata* and *Tridax procumbens* L. (25/75)

Tested Bacteria	Zone of Inhibition (mm)*		
	Methanol Extract	Chloroform Extract	Standard Antibiotic (#)
Gram Positive (+) Bacteria			
<i>Staphylococcus aureus</i>	33 ± 6.65	7.33 ± 0.57	2.7cm
<i>Staphylococcus</i>	39.33 ± 4.43	7.66 ± 1.15	2.6cm

haemolyticus			
Gram Negative (-) Bacteria			
Escherichia coli	24.66 ± 4.16	8.66 ± 0.57	2.2 cm
Klebsiella pneumoniae	31 ± 2	7.66 ± 1.15	2.5 cm
*Mean of triplicates, # Chloramphenicol, ± Standard Deviation, - No inhibition			

The methanolic combinations exhibited a stronger and broader inhibition spectrum than the chloroform combinations across all ratios, indicating potential complementary interactions between the bioactive components of both plants, indicating a synergistic enhancement when methanolic extracts of the two plants are combined.

The standard antibiotic control, chloramphenicol, produced inhibition zones ranging from 2.2 to 2.7 cm for every strain tested “Fig. 8”.

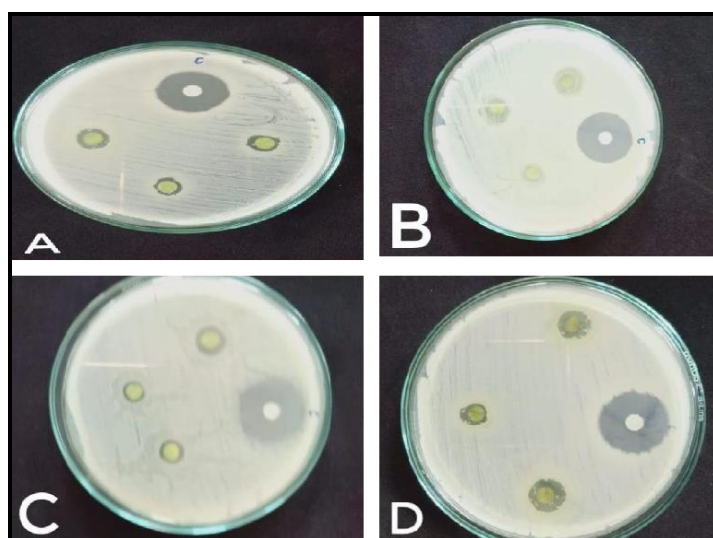


Fig. 8: Chloramphenicol is used as control in this combined activity of *Peristrophe bicalyculata* and *Tridax procumbens* L. leaf extracts against *Staphylococcus aureus*, *Staphylococcus haemolyticus*, *Escherichia coli*, *Klebsiella pneumoniae*.

These results are consistent with research on Acanthaceae species, which showed that methanolic extracts of *Andrographis lineata* and *Justicia nodicaulis* exhibited more potent antibacterial activity than less polar extracts.^[27] While *A. lineata* methanol extract demonstrated significant zones against *S. aureus* and *K. pneumoniae* in comparison to chloroform and petroleum ether extracts, *J. nodicaulis* demonstrated moderate inhibition against *S. aureus* but limited effect on *E. coli*.^[28] Studies on Asteraceae species have also shown that the ethanol extracts of *Vernonia auriculifera* and other members that are high in flavonoids, terpenoids, and tannins have potent antibacterial properties.^[29] The strong

presence of synergistic phytochemicals with antibacterial potential is suggested by the higher inhibition observed in this study, especially by *P. bicalyculata*.

Methanolic extracts from both plants worked in concert to increase antibacterial efficacy, with the 25:75 ratio demonstrating the highest inhibition against *S. haemolyticus* (39.33 ± 4.43 mm). As observed in *V. auriculifera*, where the whole extract outperformed isolated metabolites, this supports claims in the literature that matrices of crude plant extracts offer stronger and wider antibacterial activity due to interactions among several phytocompounds.^[30] In line with structural differences like the outer membrane barrier in Gram-negative species, Gram-positive bacteria were more vulnerable than Gram-negative strains across all treatments.

3.3 Antifungal activity of Leaf Extracts

***Peristrophe bicalyculata*:** *P. bicalyculata* methanolic extracts demonstrated significant antifungal activity, whereas chloroform extracts did not inhibit the tested fungi. The methanolic extract showed the largest zone of inhibition against *A. niger* (48.33 ± 1.52 mm), followed by *A. fumigatus* (21.33 ± 5.50 mm) and *A. flavus* (7.33 ± 0.57 mm). These results imply that *P. bicalyculata*'s antifungal compounds are primarily methanol-soluble, suggesting that the active ingredients are more polar "Fig. 9 & 9.1", "Table. 6"

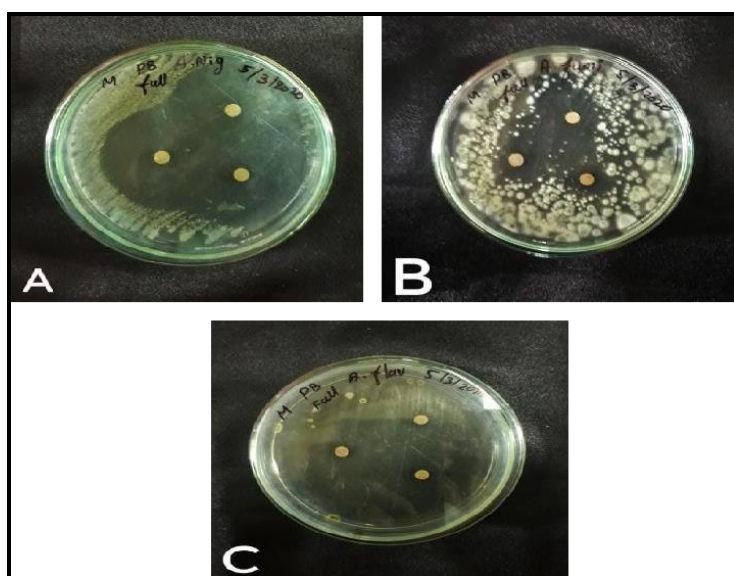


Fig. 9: Antifungal activity of *Peristrophe bicalyculata* (a.Methanolic extract in *Aspergillus niger*, b.Methanolic extract in *Aspergillus fumigates*, c.Methanolic extract in *Aspergillus flavus*).

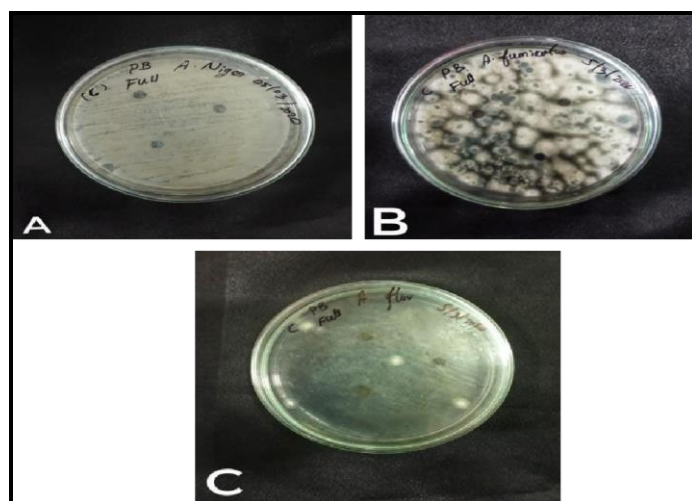


Fig. 9.1: Antifungal activity of *Peristrophe bicalyculata* (a. Chloroform extract in *Aspergillus niger*, b. Chloroform extract in *Aspergillus fumigatus*, c. Chloroform extract in *Aspergillus flavus*).

Table 6: Antifungal activity of *Peristrophe bicalyculata* Retz. Leaf extract.

Tested Bacteria	Zone of Inhibition (mm)*		
	Methanol Extract	Chloroform Extract	Standard Antibiotic (#)
<i>Aspergillus niger</i>	48.33 ± 1.52	-	1.5 cm
<i>Aspergillus fumigatus</i>	21.33 ± 5.50	-	2.7cm
<i>Aspergillus flavus</i>	7.33 ± 0.57	-	2.6cm
* Mean of triplicates, # Ketoconazole, ± Standard Deviation, - No inhibition			

***Tridax procumbens*:** Comparable patterns were observed in *T. procumbens*, where methanolic extracts demonstrated greater antifungal potential than chloroform extracts, which did not exhibit any inhibition. *A. niger* had the largest inhibition zone (49 ± 2 mm), followed by *A. fumigatus* (15 ± 1 mm) and *A. flavus* (7.66 ± 0.57 mm). The findings show that the methanolic extract of *T. procumbens* has strong antifungal effects, particularly against *A. niger*. “Fig. 10 & 10.1”, “Table. 7.”

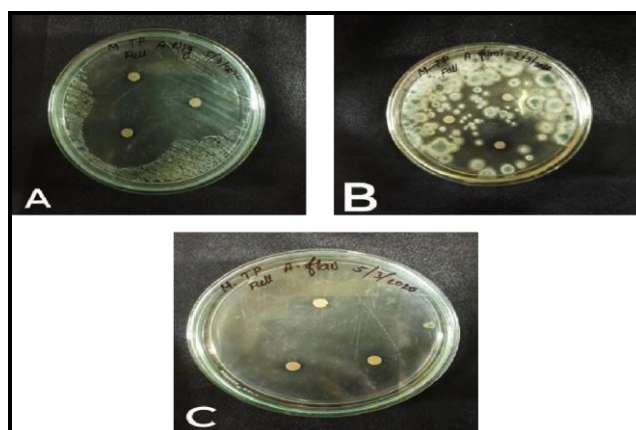


Fig. 10: Antifungal activity of *Tridax procumbens* (a.Methanolic extract in *Aspergillus niger*, b.Methanolic extract in *Aspergillus fumigates*, c.Methanolic extract in *Aspergillus flavus*).

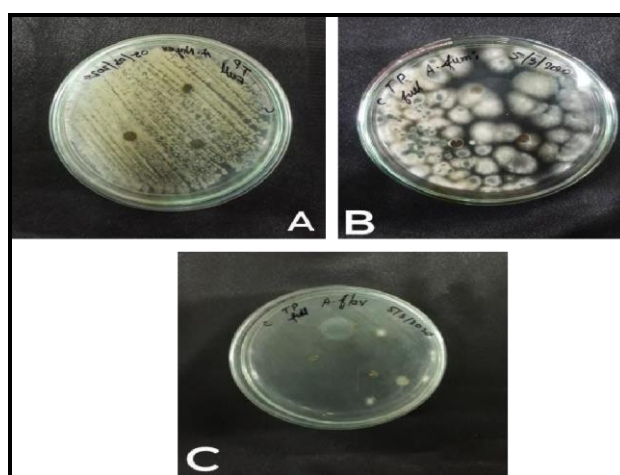


Fig. 10.1: Antifungal activity of *Tridax procumbens* (a. Chloroform extract in *Aspergillus niger*, b.Chloroform extract in *Aspergillus fumigates*, c.Chloroform extract in *Aspergillus flavus*).

Table 7: Antifungal activity of *Tridax procumbens* L. Leaf extract.

Tested Bacteria	Zone of Inhibition (mm)*		
	Methanol Extract	Chloroform Extract	Standard Antibiotic (#)
<i>Aspergillus niger</i>	49 ± 2	-	1.5 cm
<i>Aspergillus fumigates</i>	15 ± 1	-	0.8 cm
<i>Aspergillus flavus</i>	7.66 ± 0.57	-	1.0 cm

* Mean of triplicates, # Ketoconazole, ± Standard Deviation, - No inhibition

3.4 Combined antifungal activity of *P. bicalyculata* and *T. procumbens*

Both plant methanolic extracts showed potent antifungal qualities; therefore, their combined effects were evaluated to determine potential synergistic activity. The same fungal species

was tested against three different methanol and chloroform extract combinations that were prepared in the following ratios: 50:50, 75:25, and 25:75 (v/v).

The 50:50 combination of methanol extracts showed inhibition zones of 48.66 ± 1.15 mm for *A. niger*, 18.33 ± 3.21 mm for *A. fumigatus*, and 7.33 ± 0.57 mm for *A. flavus* “Fig. 11 & 11.1”, “Table. 8.”

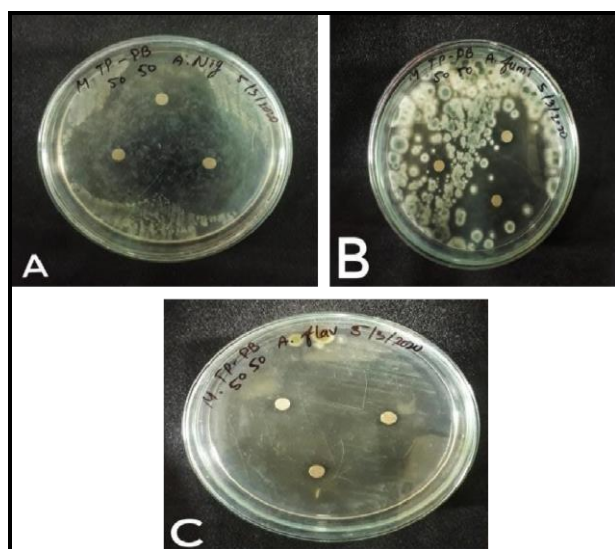


Fig. 11: Combined Antifungal activity of *Peristroph bicalyculata* and *Tridax procumbens* L. (50/50) (a.Methanolic extract in *Aspergillus niger*, b.Methanolic extract in *Aspergillus fumigates*, c.Methanolic extract in *Aspergillus flavus*).

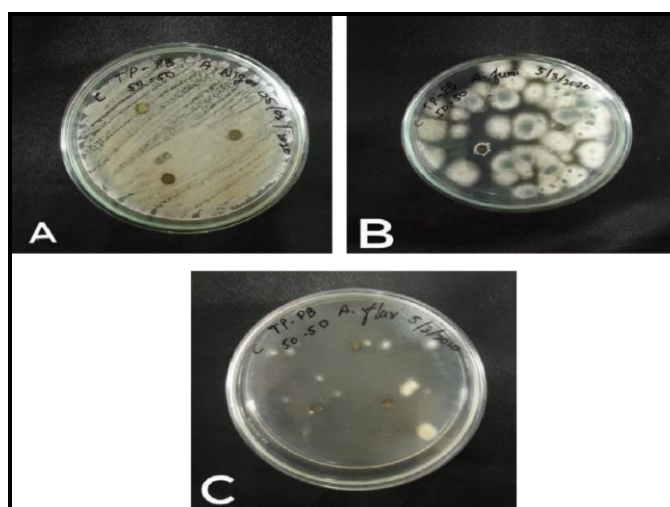


Fig 11.1: Combined Antifungal activity of *Peristroph bicalyculata* and *Tridax procumbens* L. (50/50) (a.Chloroform extract in *Aspergillus niger*, b.Chloroform extract in *Aspergillus fumigates*, c.Chloroform extract in *Aspergillus flavus*).

Table 8: Combined studies of Antifungal activity of *Peristroph bicalyculata* and *Tridax procumbens* (50/50).

Tested Bacteria	Zone of Inhibition (mm)*		
	Methanol Extract	Chloroform Extract	Standard Antibiotic (#)
<i>Aspergillus niger</i>	48.66 ± 1.15	-	1.5 cm
<i>Aspergillus fumigates</i>	18.33 ± 3.21	-	0.8 cm
<i>Aspergillus flavus</i>	7.33 ± 0.57	-	1.0 cm

* Mean of triplicates, # Ketoconazole, ± Standard Deviation, - No inhibition

75:25 Combination: The 75% methanol mixture yielded the largest inhibition zones overall, measuring 53 ± 6.24 mm against *A. niger*, 15 ± 5 mm against *A. fumigatus*, and 8.66 ± 2.08 mm against *A. flavus* “Fig. 12 & 12.1”, “Table 9.”

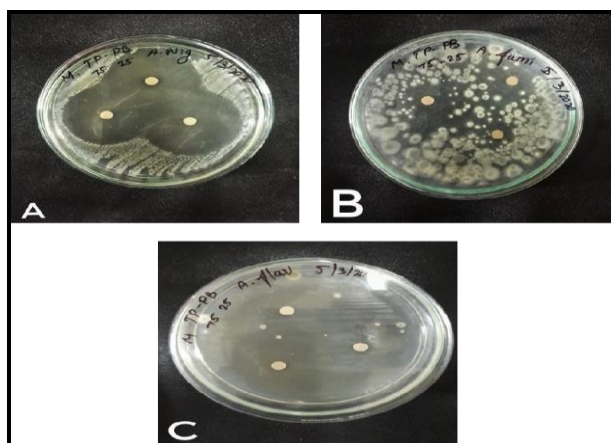


Fig. 12: Combined Antifungal activity of *Peristroph bicalyculata* and *Tridax procumbens* L. (75/25) (a.Methanolic extract in *Aspergillus niger*, b.Methanolic extract in *Aspergillus fumigates*, c.Methanolic extract in *Aspergillus flavus*).

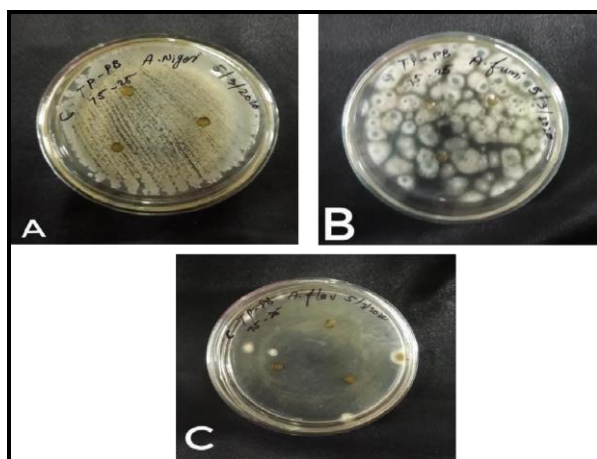


Fig 12.1: Combined Antifungal activity of *Peristroph bicalyculata* and *Tridax procumbens* L. (75/25) (a.Chloroform extract in *Aspergillus niger*, b.Chloroform extract in *Aspergillus fumigates*, c.Chloroform extract in *Aspergillus flavus*).

Table 9: Combined studies of Antifungal activity of *Peristroph bicalyculata* and *Tridax procumbens* (75/25).

Tested Bacteria	Zone of Inhibition (mm)*		
	Methanol Extract	Chloroform Extract	Standard Antibiotic (#)
<i>Aspergillus niger</i>	53 ± 6.24	-	1.5 cm
<i>Aspergillus fumigates</i>	15 ± 5	-	0.8 cm
<i>Aspergillus flavus</i>	8.66 ± 2.08	-	1.0 cm

* Mean of triplicates, # Ketoconazole, ± Standard Deviation, - No inhibition

25:75 Combination: Activity decreased as methanol concentration decreased, displaying 44 ± 2.64 mm (*A. niger*), 15.33 ± 3.21 mm (*A. fumigatus*), and 7 ± 0 mm (*A. flavus*) “Fig 13 & 13.1”, “Table 10”.

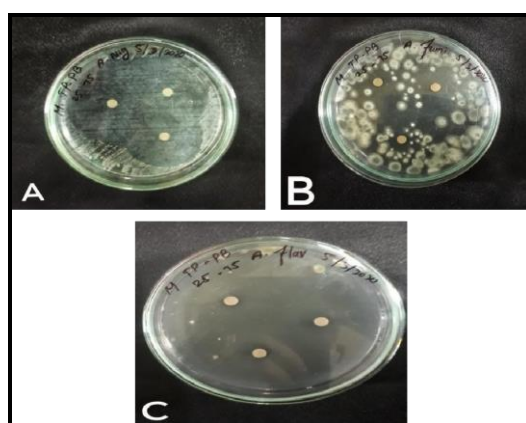


Fig. 13: Combined Antifungal activity of *Peristroph bicalyculata* and *Tridax procumbens* L. (25/75) (a.Methanolic extract in *Aspergillus niger*, b.Methanolic extract in *Aspergillus fumigates*, c.Methanolic extract in *Aspergillus flavus*).

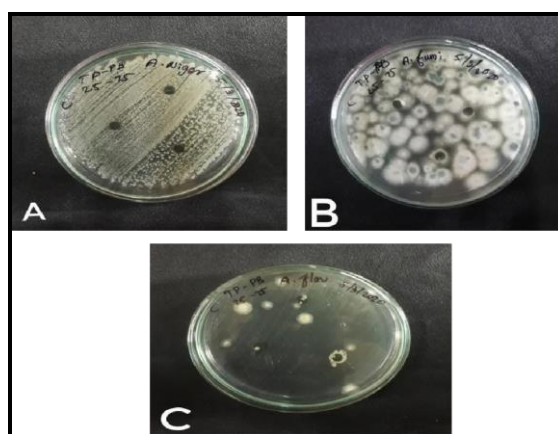


Fig. 13.1: Combined Antifungal activity of *Peristroph bicalyculata* and *Tridax procumbens* L. (25/75) (a.Chloroform extract in *Aspergillus niger*, b.Chloroform extract in *Aspergillus fumigates*, c.Chloroform extract in *Aspergillus flavus*).

Table 10: Combined studies of Antifungal activity of *Peristrophe bicalyculata* and *Tridax procumbens* (25/75).

Tested Bacteria	Zone of Inhibition (mm)*		
	Methanol Extract	Chloroform Extract	Standard Antibiotic (#)
<i>Aspergillus niger</i>	44 ± 2.64	-	1.5 cm
<i>Aspergillus fumigates</i>	15.33 ± 3.21	-	0.8 cm
<i>Aspergillus flavus</i>	7 ± 0	-	1.0 cm

* Mean of triplicates, # Ketoconazole, ± Standard Deviation, - No inhibition

A. niger was the most sensitive fungal strain across all ratios, whereas *A. flavus* was the least sensitive. In all combinations, the chloroform fractions exhibited no antifungal activity. In these combination studies, ketoconazole was used as the standard reference medication “Fig. 14”.

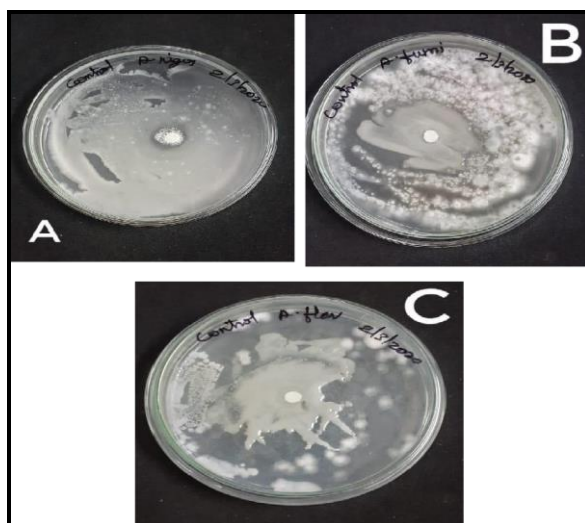


Fig. 14: Ketoconazole is used as control in this combined activity of *Peristrophe bicalyculata* and *Tridax procumbens* L. leaf extracts against *Aspergillus niger*, *Aspergillus fumigates*, *Aspergillus flavus*.

These findings are consistent with the methanolic antifungal activity observed in *Nassauvia dentata* (Asteraceae), where higher concentrations of methanolic extracts significantly inhibited *Rhizoctonia solani* by up to 77%.^[31] The presence of tannins, coumarins, alkaloids, and flavonoids in *N. dentata* supports the idea that phenolic compounds and conjugated aromatic systems aid in membrane disruption, protein denaturation, and free radical-mediated fungal growth inhibition. *A. niger*'s high susceptibility in this study is also consistent with filamentous fungi's sensitivity to phenolic-rich extracts, which has been documented in polyphenol-dominant taxa in the past.

In contrast, methanolic extracts of *Erigeron floribundus* were ineffective against dermatophytes, whereas its dichloromethane extract demonstrated strong antifungal activity, with MIC values as low as 0.25 mg/mL.^[32] This divergence demonstrates the variation in phytochemical polarity within the Asteraceae family. *E. floribundus* was linked to non-polar lipophilic substances like essential oils and prenylated flavonoids, but the methanol-active pattern of the current investigation points to a preponderance of polar antifungal metabolites, most likely flavonoids, glycosides, and phenolic acids.

The outcomes unequivocally show that *P. bicalyculata* and *T. procumbens* methanol extracts have strong antifungal activity, particularly against *A. niger*. Compared to the individual extracts, the combination of methanolic extracts increased the inhibitory effect, with the strongest synergistic effect observed at a ratio of 75:25.

4. CONCLUSION

The results of this investigation offer compelling proof that the leaf extracts of *Tridax procumbens* and *Peristrophe bicalyculata* have broad-spectrum antimicrobial properties, especially when methanol is used for extraction. Both plants showed significant inhibitory effects against gram-positive and gram-negative bacterial pathogens, as well as pathogenic fungi, with *Staphylococcus haemolyticus* and *Aspergillus niger* showing the highest susceptibility. The superior performance of methanolic extracts over chloroform extracts suggests that polar phytochemicals, which are effectively extracted in polar solvents, such as flavonoids, phenolic acids, tannins, and alkaloids, are the main active ingredients responsible for antimicrobial activity.

In the synergistic evaluation of the combined extracts, an increased antimicrobial efficacy was observed, indicating additive or complementary effects between the bioactive compounds of the two species. This interaction may decrease the likelihood of microbial resistance development and increase the antimicrobial spectrum. These results demonstrate the potential of *T. procumbens* and *P. bicalyculata* as viable candidates for developing plant-based antimicrobial formulations or natural preservatives.

These findings support the ethnomedicinal use of both species in conventional healing methods and highlight their applicability in contemporary phytotherapeutic research from a pharmacognostic perspective. Future research should focus on chromatographic isolation, bioassay-guided fractionation, and structural characterization of active molecules. A deeper

understanding of their pharmacological potential is possible through mechanistic investigations involving molecular docking, enzyme inhibition, and in vivo efficacy evaluations.

In light of the growing multidrug resistance, this study supports the sustainable investigation of medicinal plants as substitutes for synthetic antimicrobials, while also reaffirming the therapeutic value of Asteraceae and Acanthaceae family members as repositories of bioactive metabolites.

Author Contributions

Conceptualization, Karthick S. and Vanathi Sundarapandiyan; methodology, Karthick S. and Vanathi Sundarapandiyan; validation, Vanathi Sundarapandiyan, Karthick S., and Anand S.P.; investigation, Karthick S, Vanathi Sundarapandiyan, Subasri Kumar; resources, Vanathi Sundarapandiyan; data curation, Karthick S, Subasri Kumar; writing—original draft preparation, Vanathi Sundarapandiyan; writing—review and editing, Vanathi Sundarapandiyan and Anand S.P.; visualization, Karthick S.; supervision, Anand S.P.; project administration, Vanathi Sundarapandiyan; funding acquisition, not applicable. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

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