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"Combinational evaluation of Bambusa vulgaris Leaf extract with ciprofloxacin against Klebsiella pneumoniae"

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ABSTRACT:
Ciprofloxacin was tested against Pseudomonas aeruginosa using a checkerboard assay in conjunction with a herbal extract of bamboo. All antibiotic combinations studied had predominantly additive combination effects. Only one strain produced synergy, and no antagonistic medication interactions were identified. Experiments on bacterial killing activities supported these findings, with 1:1 acetone extracts with medication being the most effective compared to other combinations.

Key words: Ciprofloxacin, Pseudomonas aeruginosa, antibiotics, bamboo

I. INTRODUCTION:

Intracellular microorganisms must penetrate host cells in order to multiply, therefore they must either be prevented from doing so or detected and eliminated after they have done so. Pathogens that replicate in cellular vesicles, such as viruses and certain bacteria (Chlamydia, Rickettsia, and Listeria), as well as Mycobacteria, can be further divided than those that spread freely throughout the cell [1-6].Neutralizing antibodies whose growth is dependent on TH2 cells can prevent viruses from reaching cells, whereas viruses are treated once within cells. A one-of-a-kind cytotoxic T-cell that identifies and destroys the target cell. Macrophages, on the other hand, are predominantly infected and can be destroyed by pathogen-specific TH1 cells, which activate macrophages to kill the pathogen.Several extracellular infections cause disease by producing specific toxins, which can lead to the production of neutralising antibodies. Intracellular infectious pathogens frequently cause disease by killing the cells that contain them. The precise destruction of virus-infected cells by cytotoxic T-cells not only prevents the virus from spreading, but also eliminates damaged cells. The immunological response to the infectious pathogen may be a key source of pathology in many illnesses.

Streptococcus pneumonia produces pneumonia in the lungs, but it also causes a swiftly fatal systemic illness in the blood.Bacteria that cause cholera, such as Salmonella typhi, which causes typhoid fever, or Vibrio cholera. The HIV virus, which causes AIDS, serves as a reminder to mankind that new infectious agents are still susceptible to being introduced.phytonutrients "is derived from the Greek word "plant" and hence refers to chemical phytochemical substances present in plant foods such as fruits, beans, vegetables, whole grains, peanuts, and so on [7-9].

These chemical components give plant-based goods their distinct appearance, fragrance, and flavour." Because different coloured vegetables and fruits are linked with different phytochemicals, a variety of coloured vegetables and fruits are ingested [10]. Each phytochemical has a distinct effect on our bodies, therefore we should attempt to mix them up as much as possible. These chemicals have been linked to the protection of chronic illnesses such cancer, heart disease, diabetes, and high blood pressure.In recent years, there has been a resurgence of interest in medicinal plants as a source of possible medications. As a result, the objective is to have a better understanding of medicinal plant knowledge as a source of herbal medications [11]. Medicinal herb or phytomedicine is the use of plants for therapeutic and medicinal reasons in order to treat illnesses and improve public health. Secondary t-metabolites, which are known to be medical compounds or medicines for human use, protect plants from therapeutic qualities and antioxidant actions against microbial diseases or infestations.Phytochemicals are active substances with therapeutic characteristics that are categorised as a medication or treatment [12-13]. They are distinguished by their distinct colours, smells, and tastes. Higher quantities of Phytochemicals in the plant are indicated by deep hues and powerful smells. So phytochemicals are naturally present in plants and have biological importance in that they serve an important part in the plant's defence against numerous harmful microorganisms by exhibiting antimicrobial activity through inhibition or killing mechanisms [15-18].

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II. METHODOLOGY:

Fresh Bamboleaves were collected and washed with tap and distilled water before being sun-dried. Cut thin pieces and let them to dry in the sun until totally dry. The dried leaves were crushed into powder using a blender or grinder. Antibacterial substances present in plants are known as secondary metabolites, also known as bioactive compounds and phytochemicals. To dissolve these secondary metabolites, organic solvents such as methanol, chloroform, acetone, petroleum ether, and others are used. Fresh and powdered plant samples were dipped in a 1:10 ratio of polar and nonpolar solvents. The samples were then stored at room temperature for another 48 hours.The solvents were evaporated at 40°C after filtration. The remaining leftovers were dissolved in DMSO and stored at 0°C for further use [15-16]. MRD LifeSciences Pvt. Ltd. in Lucknow sells pathogenic gram-negative bacterial strains like Pseudomonas aeruginosa. Pre-cultured pathogen plates were first resurrected by spreading and streaking them on agar plates, and then the pathogenic strains' broth was prepared and used in various investigations. Using the agar well diffusion technique, the antibacterial properties of Bamboo extracts and modified conventional medications comprising these extracts were evaluated against Pseudomonas aeruginosa.Extracting 20 l from 24-hour-old cultures and putting the samples (500 g/ml) into wells was used to disperse the Pseudomonas aeruginosa. At 37°C, the plates were incubated for 48 hours [16-18]. The MIC test was used to calculate the dosage of various antibiotics used to treat Pseudomonas aeruginosa [19]. To identify phytochemicals or compounds isolated from Dalbergiasissoo, phytochemical tests for flavonoids, saponins, tannins, steroids, terpenoids, and carbohydrates were performed [20,21].

III. RESULTS & DISCUSSIONS

3.1. Collection of plant samples

Table1. Collection of plant samples.

S. No	Plant sample	Scientific name	Location
1.	 Bamboo leaves	Bambusa indica	Gomtinagar, Lucknow.

1. Antibiogram analysis (Antibacterial sensitivity test)

The extracts were prepared by using the bamboo leaves samples and the organic solvents. Then these extracts were analyzed against K. pneumoniaby using agar well diffusion method and the effective results were obtained. Initially the marketed drug ciprofloxacin, organic solvents and the extracted extracts wereanalyzed against K. pneumoniaand also found that the organic solvents did not show any antibacterial analysis, and by ciprofloxacin 9.4 mm.

The bamboo leaves extracts were also screened against K. pneumoniaand not found effective results, further the antibacterial activities of extracts were enhanced by using the modified drugs by making different combinations of ciprofloxacin and plant extracts in various ratios such as 1:1, 1:4 and 4:1.

a. **Organic solvents**

Table 2. Antibiogram analysis of chloroform, propanol, distilled water (dw), ethanol, acetone and petroleum etheragainst K. pneumonia

Marking	Solvents	Zone of inhibition (in mm)
		K. pneumonia
1.	Acetone	0
2.	Chloroform	0
3.	Ethanol	0

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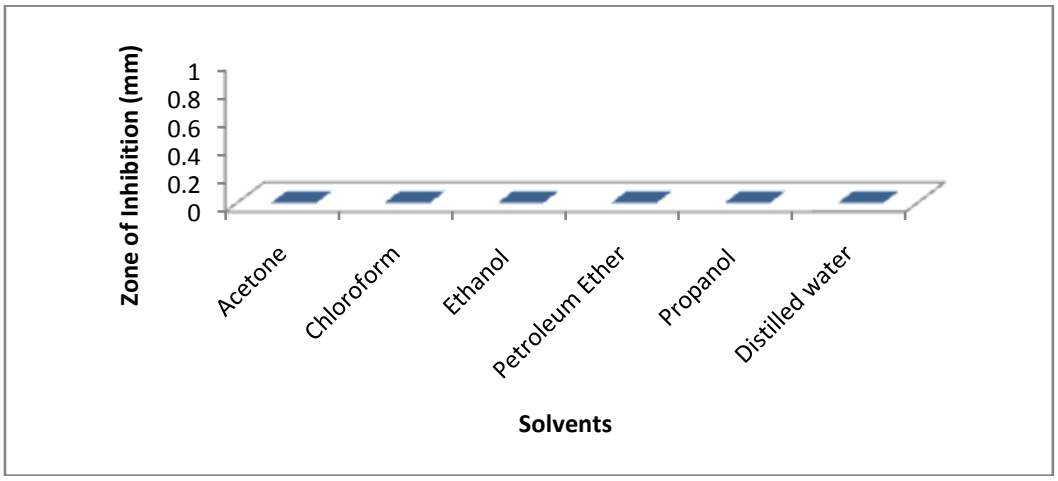
4.	**Petroleum Ether**	0
5.	**Propanol**	0
6.	**Distilled water**	0



Graph 1: Representing the antibacterial analysis of chloroform, propanol, distilled water (dw), ethanol, acetone and petroleum etheragainst K. pneumonia

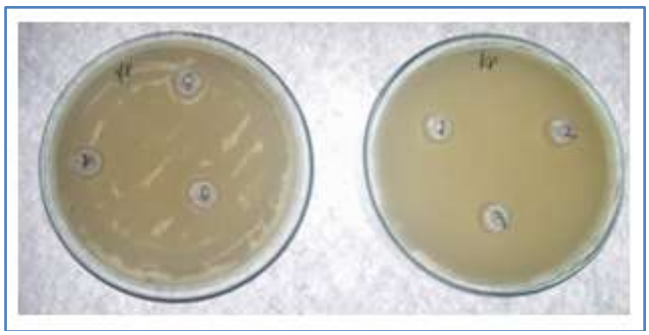


Figure 1: Antibiogram analysis of solventsagainst K. pneumonia

b. **Marketed drug:**

Table 3. Antibiogram analysis of Ciprofloxacin against K. pneumonia

S. No	**Marketed drug**	**Zone of inhibition (in mm)**
		K. pneumonia
1.	**Ciprofloxacin**	9.4 mm

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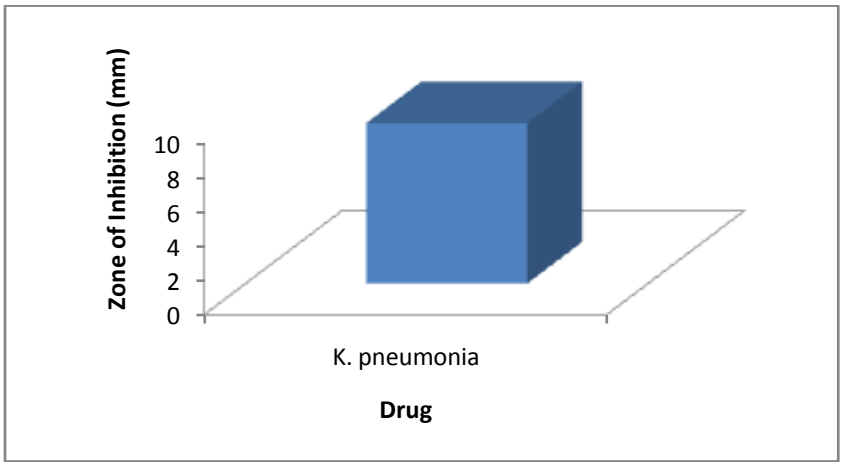

Graph 2: Graphical of antibacterial analysis of Ciprofloxacin against K. pneumonia

c. **Bambusaindica extracts:**

Table 4. Antibiogram analysis of chloroform, propanol, distilled water (dw), ethanol, acetone and petroleum ether extracts of Bambusaindicaagainst K. pneumonia

S. No	**Extracts**	**Zone of inhibition (in mm)**		
		Fresh Bambusaindica	**Dry Bambusaindica**	
1.	**Acetone**	16	**7.**	14.9
2.	**Chloroform**	14.6	**8.**	18.5
3.	**Ethanol**	19.5	**9.**	18
4.	**Petroleum Ether**	11	**10.**	15
5.	**Propanol**	17	**11.**	16
6.	**Distilled water**	18	**12.**	18

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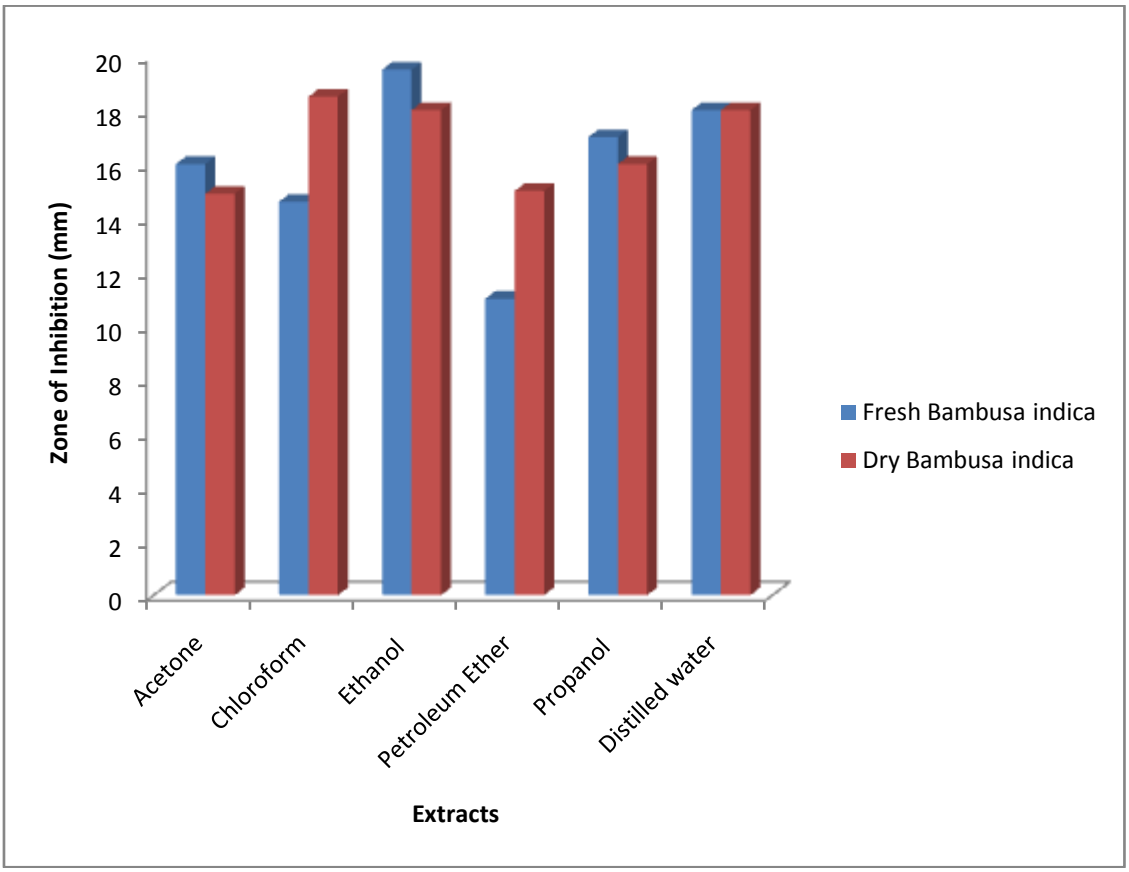

Graph 3: Graphical analysis of antibiogram analysis of chloroform, propanol, distilled water (dw), ethanol, acetone and petroleum ether extracts of Bambusaindicaagainst K. pneumonia

Figure 2: Antibiogram analysis of different Bambusaindicaextracts against K. pneumonia

d. Bambusaindica extracts in combinations with Ciprofloxacin:

Table 5: Antibiogram analysis of combination of dry and fresh Bambusaindicaextracts andCiprofloxacin(1:1) against K. pneumonia

S. No	Extracts	Zone of inhibition (in mm)
A.	**Fresh Bambusaindica + Ciprofloxacin (1:1)**	
1.	**Acetone**	22.1
2.	**Chloroform**	14

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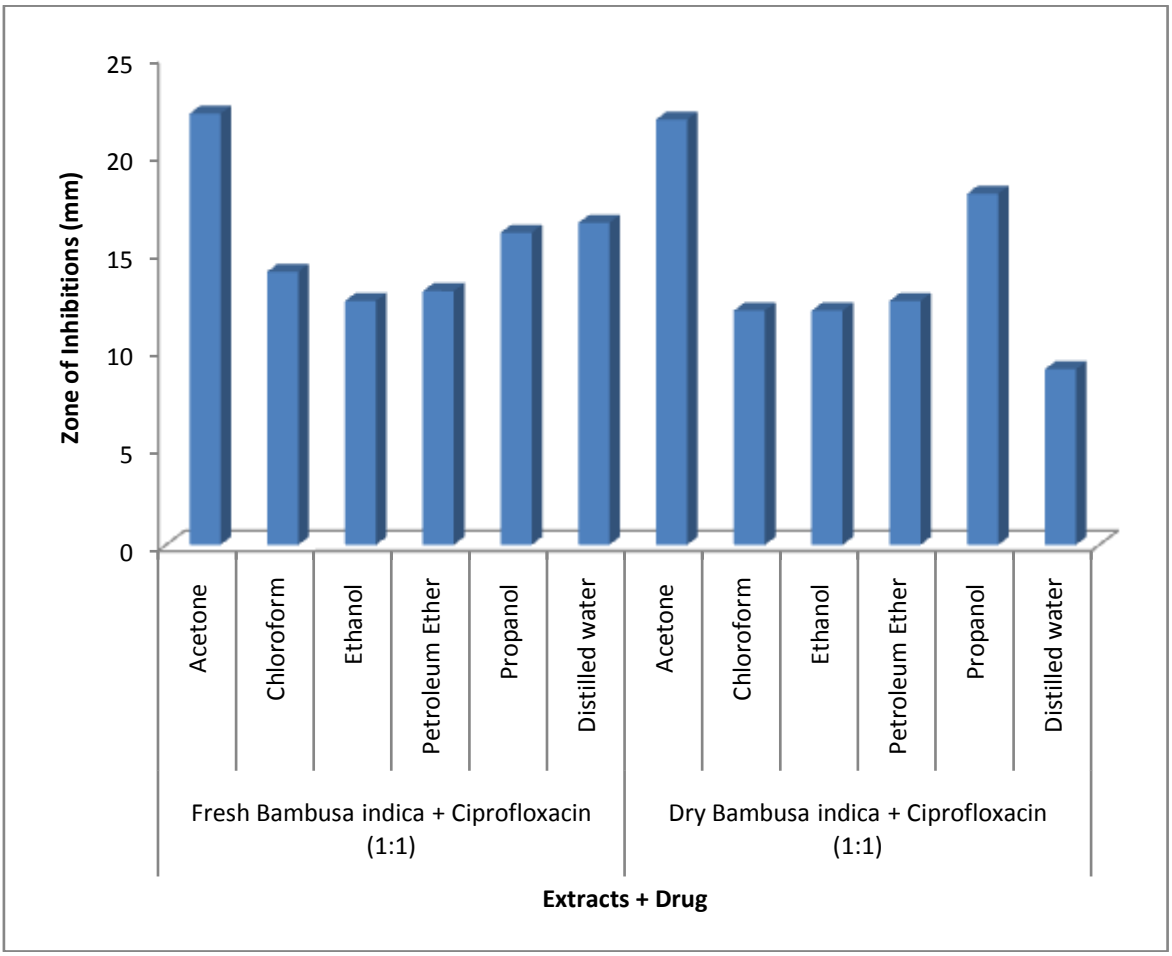
3.	**Ethanol**	12.5
4.	**Petroleum Ether**	13
5.	**Propanol**	16
6.	**Distilled water**	16.5
B.	**Dry Bambusaindica + Ciprofloxacin (1:1)**	
7.	**Acetone**	21.8
8.	**Chloroform**	12
9.	**Ethanol**	12
10.	**Petroleum Ether**	12.5
11.	**Propanol**	18
12.	**Distilled water**	9



Graph 4: Representation of antibiogram analysis of combination of dry and fresh Bambusaindicaextracts andCiprofloxacin(1:1) against K. pneumonia

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Figure 3: Antibacterial analysis of Bambusaindicaextracts andCiprofloxacin(1:1) against K. pneumonia

Table 6: Antibiogram analysis of combination of dry and fresh BambusaindicaandCiprofloxacinextracts (1:4) against K. pneumonia

S. No	Extracts	Zone of inhibition (in mm)
1.	Fresh Bambusaindica + Ciprofloxacin (1:4)	
a.	Acetone	14.5
b.	Chloroform	11
c.	Ethanol	10.5
d.	Petroleum Ether	14
e.	Propanol	14.9
f.	Distilled water	10.5
2.	Dry Bambusaindica + Ciprofloxacin (1:4)	
a.	Acetone	11.5
b.	Chloroform	8
c.	Ethanol	11.7
d.	Petroleum Ether	12.1
e.	Propanol	17.1
f.	Distilled water	13

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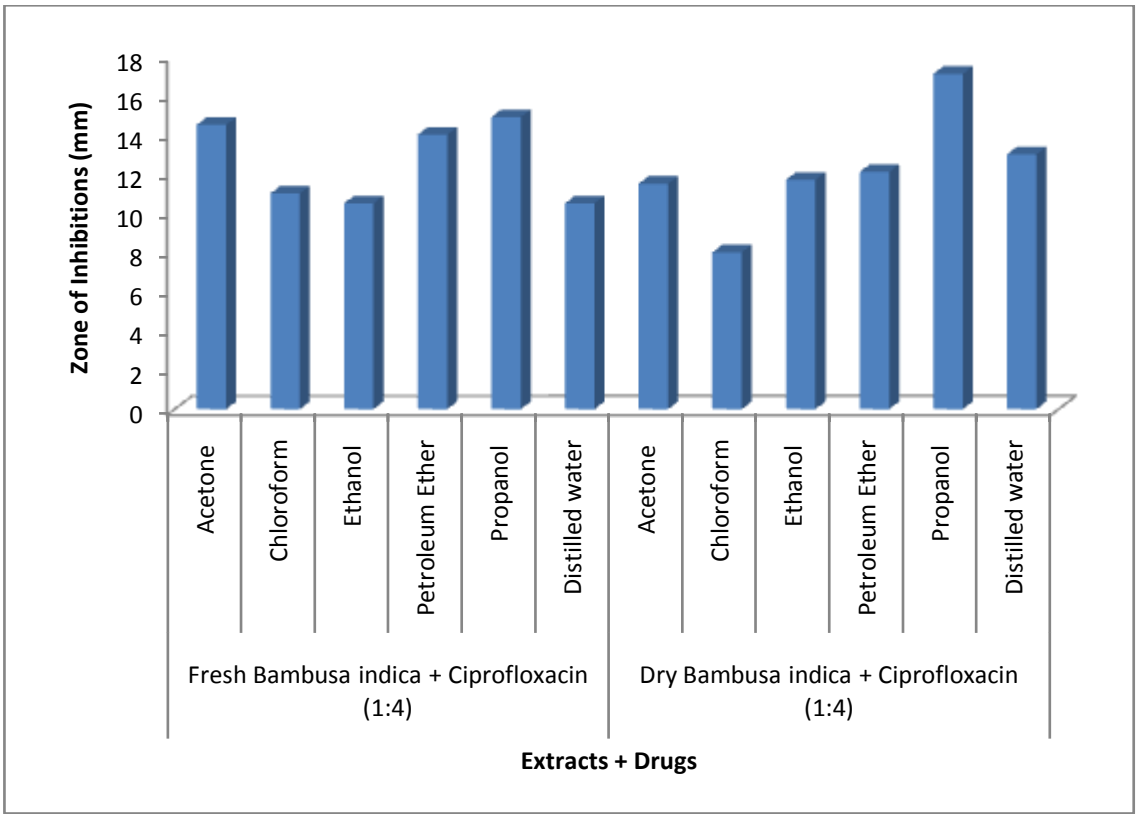

Graph 5: Representation of antibiogram analysis of combination of dry and fresh Bambusaindicaextracts andCiprofloxacin(1:4) against K. pneumonia

Table 7: Antibiogram analysis of combination of dry and fresh BambusaindicaandCiprofloxacinextracts (4:1) against K. pneumonia

S. No	Extracts	Zone of inhibition (in mm)
1.	**Fresh Bambusaindica + Ciprofloxacin (4:1)**	
a.	**Acetone**	12.3
b.	**Chloroform**	12.9
c.	**Ethanol**	10
d.	**Petroleum Ether**	11.5
e.	**Propanol**	14.9
f.	**Distilled water**	8.9
2.	**Dry Bambusaindica + Ciprofloxacin (4:1)**	
a.	**Acetone**	11.9
b.	**Chloroform**	9.9
c.	**Ethanol**	10.8
d.	**Petroleum Ether**	12.5
e.	**Propanol**	15
f.	**Distilled water**	16

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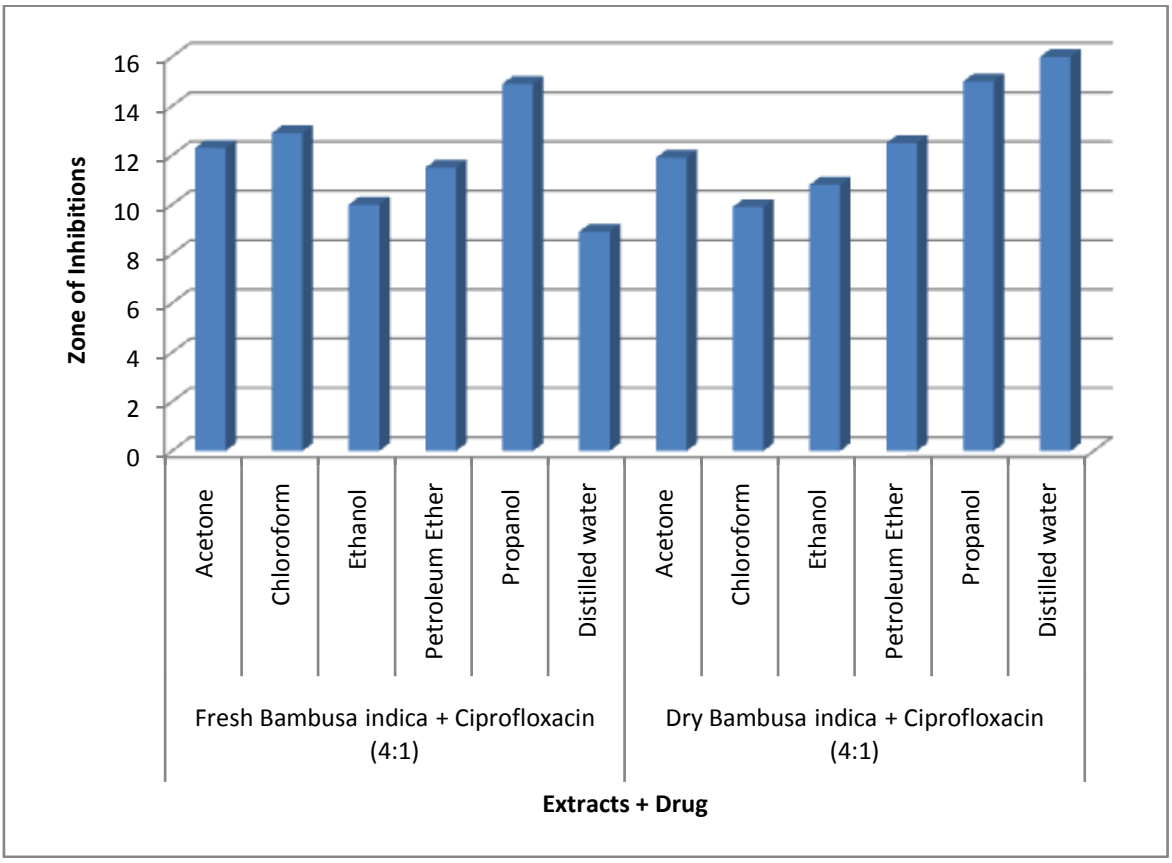

Graph 6: Representation of antibiogram analysis of combination of dry and fresh Bambusaindicaextracts andCiprofloxacin(4:1) against K. pneumonia

3. Minimum Inhibitory Concentration test:

Table 8. Minimum inhibitory concentration tests of chloroform, propanol, distilled water (dw), ethanol, acetone and petroleum ether extracts of Bambusaindicawith the combination of Ciprofloxacin (1:1) against K. pneumonia

S. No	Extracts	MIC Value (µg / ml)
1.	Fresh Bambusaindica + Ciprofloxacin (1:1)	
a.	Acetone	10.8
b.	Chloroform	40.4
c.	Ethanol	66.6
d.	Petroleum Ether	66.6
e.	Propanol	40
f.	Distilled water	52
2.	Dry Bambusaindica + Ciprofloxacin (1:1)	
a.	Acetone	10.9
b.	Chloroform	35.9
c.	Ethanol	40.3
d.	Petroleum Ether	60.8
e.	Propanol	15.9
f.	Distilled water	65.2

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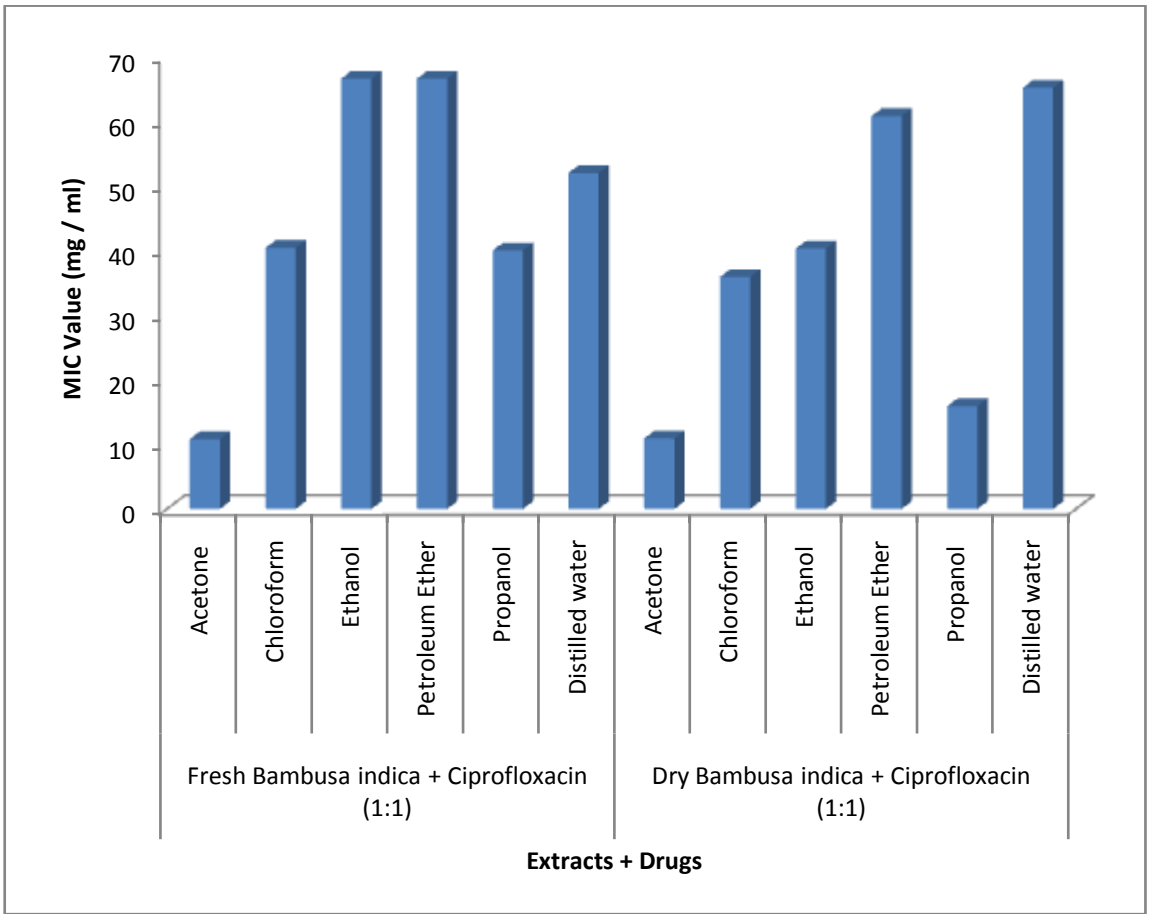

Graph 7: Graphical representation of minimum inhibitory concentration tests of Bambusaindicaextracts with the combination of Ciprofloxacin (1:1) against K. pneumonia

Table 9. Minimum inhibitory concentration tests of chloroform, propanol, distilled water (dw), ethanol, acetone and petroleum ether extracts of Bambusaindicawith the combination of Ciprofloxacin (1:4) against K. pneumonia

S. No	Extracts	MIC Value (µg / ml)
1.	Fresh Bambusaindica + Ciprofloxacin (1:4)	
a.	Acetone	20.8
b.	Chloroform	31.4
c.	Ethanol	45.6
d.	Petroleum Ether	50.6
e.	Propanol	45
f.	Distilled water	50
2.	Dry Bambusaindica + Ciprofloxacin (1:4)	
a.	Acetone	30.9
b.	Chloroform	15.9
c.	Ethanol	30.3
d.	Petroleum Ether	40.8
e.	Propanol	25.9
f.	Distilled water	55.2

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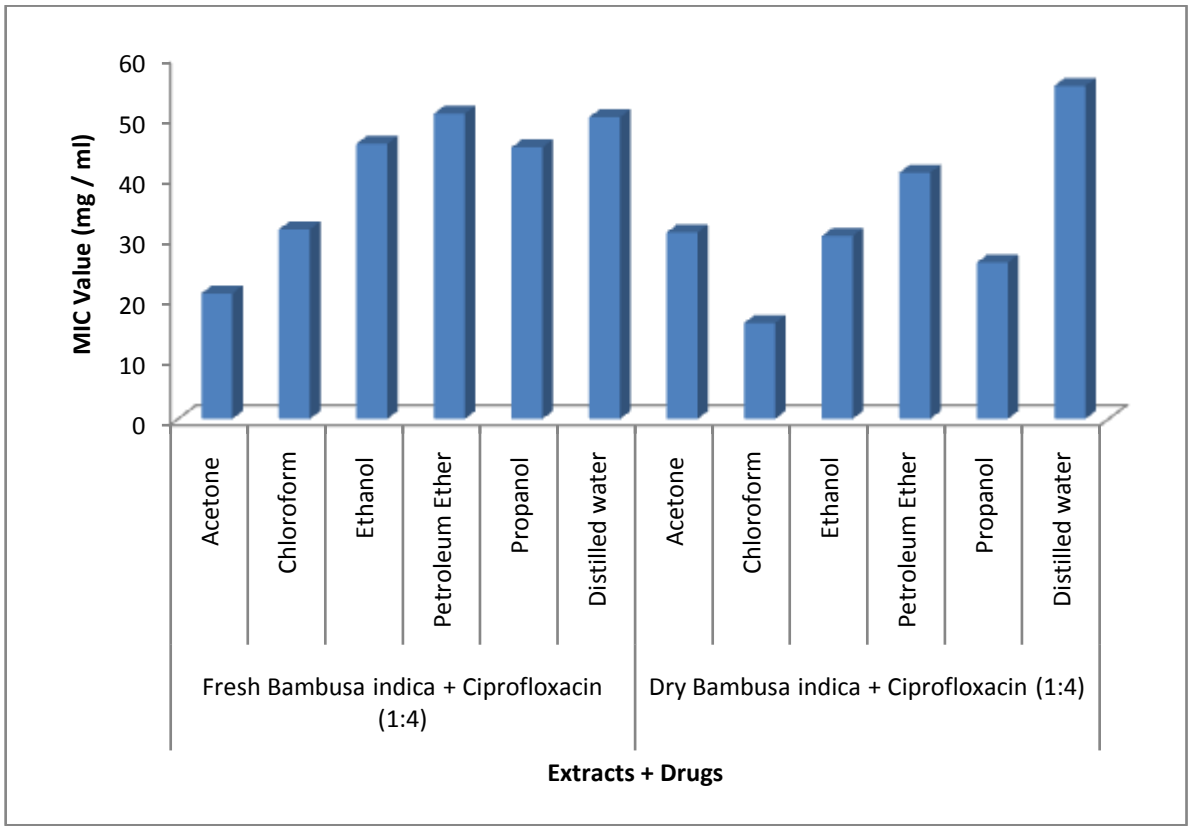

Graph 8: Graphical representation of minimum inhibitory concentration tests of Bambusaindicaextracts with the combination of Ciprofloxacin (1:4) against K. pneumonia

Table 10. Minimum inhibitory concentration tests of chloroform, propanol, distilled water (dw), ethanol, acetone and petroleum ether extracts of Bambusaindicawith the combination of Ciprofloxacin (4:1) against K. pneumonia

S. No	Extracts	MIC Value (µg / ml)
1.	Fresh Bambusaindica + Ciprofloxacin (4:1)	
a.	Acetone	50.8
b.	Chloroform	60.4
c.	Ethanol	86
d.	Petroleum Ether	87
e.	Propanol	83
f.	Distilled water	42
2.	Dry Bambusaindica + Ciprofloxacin (4:1)	
a.	Acetone	30.9
b.	Chloroform	36.9
c.	Ethanol	40
d.	Petroleum Ether	65
e.	Propanol	45.8
f.	Distilled water	64.1

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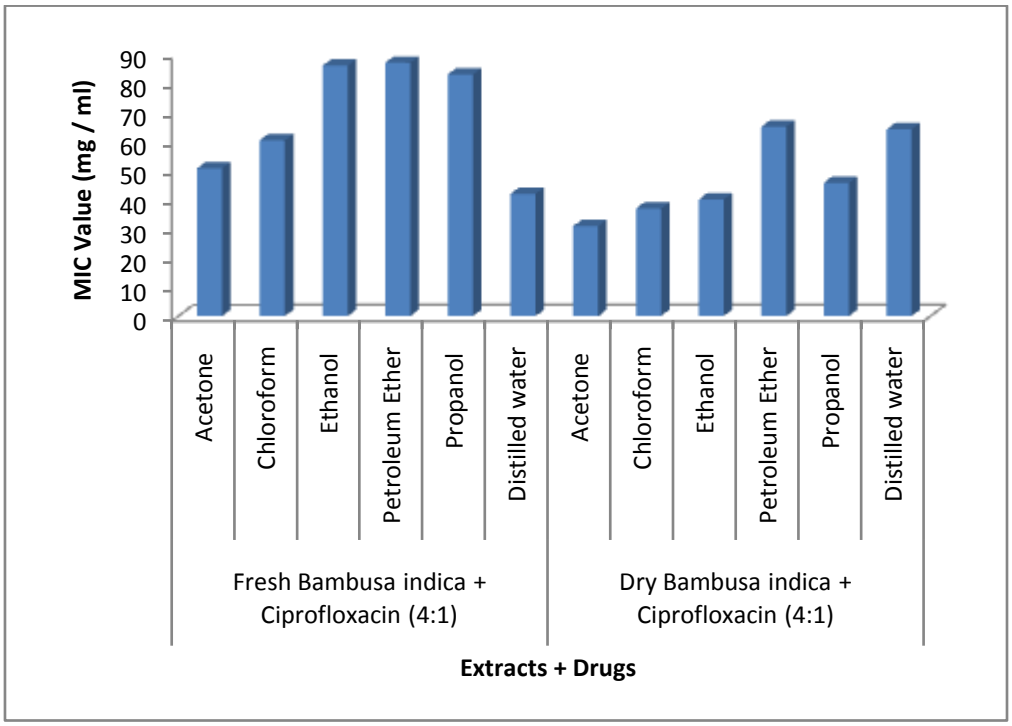

Graph 9: Graphical representation of minimum inhibitory concentration tests of Bambusaindicaextracts with the combination of Ciprofloxacin(4:1) against K. pneumonia

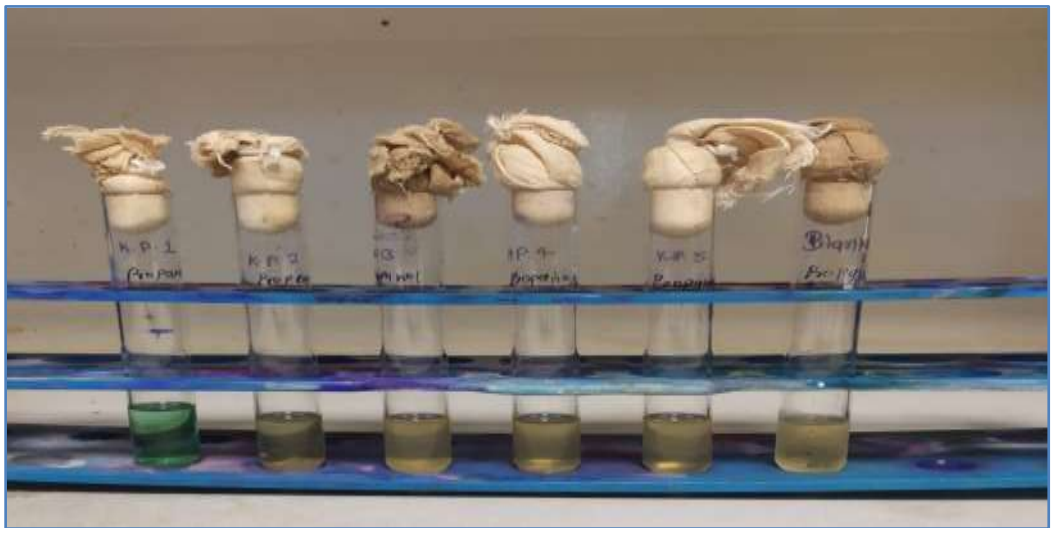

Figure 4: Minimum inhibitory concentration tests of Bambusaindicaextracts with the combination of Ciprofloxacinagainst K. pneumonia

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Phytochemical Screening:

Table 11. Phytochemical tests of chloroform, propanol, distilled water (dw), ethanol, acetone and petroleum ether extracts of freshBambusaindica

Tests	**Extracts of freshBambusaindica**					
Flavonoids	**Acetone**	**Chloroform**	**Ethanol**	**Petroleum Ether**	**Propanol**	**Distilled water**
Carbohydrates	Positive	Positive	Positive	Positive	Positive	Positive
Terpinoids	Negative	Positive	Positive	Negative	Positive	Positive
Steroids	Positive	Positive	Positive	Negative	Negative	Positive
Tannin	Positive	Positive	Negative	Negative	Positive	Positive
Saponin	Positive	Positive	Positive	Positive	Positive	Positive

Table 12. Phytochemical tests of chloroform, propanol, distilled water (dw), ethanol, acetone and petroleum ether extracts of dry Bambusaindica

Tests	**Extracts of dryBambusaindica**					
Flavonoids	**Acetone**	**Chloroform**	**Ethanol**	**Petroleum Ether**	**Propanol**	**Distilled water**
Carbohydrates	Positive	Positive	Positive	Positive	Positive	Positive
Terpinoids	Negative	Positive	Positive	Positive	Positive	Positive
Steroids	Positive	Positive	Positive	Positive	Negative	Positive
Tannin	Positive	Positive	Negative	Negative	Positive	Positive
Saponin	Positive	Positive	Positive	Positive	Positive	Positive

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To carry out such a scheme, we consume a large amount of medications or antibiotics, which have a direct impact on the immune system and allow it to heal in a reasonable amount of time; nevertheless, these antibiotics have certain negative effects on us. The widespread usage of such high-dose antibiotics that are supposed to treat us today is instead making us sick. Mother Nature has blessed us with the costly treasures of certain medical plants without affecting us by providing us flowers, fruit to eat, and wood to make useful fuel for a cheaper price for aesthetic beauty.All of creation points us in the direction of natural medicine, and we must seek it out for the benefit of human beings. Entering this final path, I chose a medicinal plant, Bambosaindica, for its antibacterial activity, and finally, acetone extract of Bambosaindica with the combination of ciproflaxin in (1:1) shows maximum antibacterial activity, giving the highest ZOI, but other five sols do not.

IV. CONCLUSION:

The study has come to the conclusion that the leaves of Bambosaindica plants are an excellent source of antibacterial compounds and may be used as a natural medication. Bambosaindica was studied to see whether it has antibacterial action against human infections. Purification of Bambosaindica secondary metabolites will aid in the identification of key compounds that will be beneficial to human welfare.

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