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SYNERGISTIC ANTIBACTERIAL ACTIVITY OF RICINUS COMMUNIS LEAF EXTRACTS WITH CHLORAMPHENICOL AGAINST STREPTOCOCCUS AUREUS, ENTEROCOCCUS FAECALIS, KLEBSIELLA PNEUMONIAE, AND PROTEUS VULGARIS.

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ABSTRACT- *The study investigated the synergistic antibacterial effect of Ricinus communis extracts combined with chloramphenicol against selected Gram-positive and Gram-negative bacteria. Ethanolic and acetone extracts were evaluated using the agar well diffusion method. The combined treatment showed greater antibacterial activity than plant extracts alone, with the ethanolic extract being more effective than the acetone extract. Notable synergistic effects were observed, particularly against Klebsiella pneumoniae and Proteus vulgaris. These results indicate that Ricinus communis can enhance the efficacy of chloramphenicol and may be useful as an adjunct in antimicrobial therapy.*

Keywords: Synergistic activity, Chloramphenicol, Antibacterial activity.

I. INTRODUCTION:

Rising antibiotic resistance has made bacterial infections a serious global health threat, creating an urgent need for new, effective, and safer antimicrobial agents.

Medicinal plants are valuable alternatives to synthetic drugs because they are easily available, biodegradable, less toxic, and rich in antimicrobial secondary metabolites like alkaloids, flavonoids, tannins, and terpenoids.

Ricinus communis (castor plant) is widely used in traditional medicine, and its leaves are known for treating infections, inflammation, and wounds, suggesting strong antimicrobial potential. Extraction solvents influence antimicrobial activity.

This study evaluates ethanol and acetone leaf extracts of R. communis using the agar diffusion method against Gram-positive and Gram-negative bacteria, comparing their effectiveness with the standard antibiotic chloramphenicol to support the development of plant-based antimicrobials.

Fig: 01 Ricinus communis

II. MATERIALS AND METHODS:

Collection and identification of plant:

The plant leaves were gathered in December 2025 from the Dharmapuri district, and the specimen was verified by a siddha medicinal plants garden (Central Council for Research in Siddha), Ministry of Ayush, Govt. of India.

Preparation:

Collect fresh leaves from the Ricinus communis plant and wash them well with distilled water to get rid of any dirt, dust, or other debris. The cleaned leaves usually dry at room temperature for five to eighteen days. Then, using a mechanical or hand grinder, the dried ones are ground into a coarse or fine powder. This increases the surface area for effective extraction.

Getting plant material out:

Weighing 25 grams of powdered leaves on an analytical balance, we added them to 250 ml of absolute ethanol and acetone in a conical flask at a ratio of 1:10. After that, we started the maceration extraction with an electron magnetic stirrer, which lasted for 6 hours. After that, the extract that had been collected was filtered through muslin cloth. A filtrate of extract was concentrated in a rotary evaporator at 40°C until the crude extracts were further dried to eliminate residual solvents and stored at 4°C until required.

III. PRELIMINARY PHYTOCHEMICAL SCREENING:

To identify the chemical components, phytochemical screening was conducted to detect alkaloids, flavonoids, phenols, tannins, glycosides, steroids, saponins, carbohydrates, and other constituents, adhering to established protocols.

Alkaloid test:

We boiled 0.2 g of the sample with 5 ml of 1% aqueous HCl in a water bath for 45 minutes. We filtered the mixture and put 1 ml of the filtrate evenly into two test tubes, each with two drops of the following reagents.

- **Drangendorff's reagent:** An orange-red solid shows that alkaloids are present.
- **Meyer's reagent:** A precipitate that is creamy white shows that alkaloids are present.

Flavonoids test:

A certain amount, 0.5 g, of the sample was dissolved in ethanol, heated, and then filtered. Three pieces of magnesium chips were added to the filtrate, and then a few drops of concentrated HCl were added. If the colour is pink, orange, red, or purple, it means that flavonoids are present.

Tannins:

Split 0.5 g of the extract into two test tubes, dissolve it in 10 ml of hot saline solution, and wash it three times with petroleum ether. One tube received two to three drops of ferric chloride, while the other tube received two to three drops of the gelatine salts reagent. Tannins are evident from the blackish-blue colour of the first test tube and the hazy appearance of the second test tube.

Saponins:

0.5 g of the extract was placed in a sterile test tube to check for saponins. Ten millilitres of distilled water were added, the tube was stoppered, and it was vigorously shaken for approximately thirty seconds. The presence of saponins was subsequently confirmed by the foam that developed when it was allowed to sit for at least an hour.

Phenol:

0.5 g of the crude extract was mixed with 3–4 drops of ferric chloride solution. A bluish-black or blue-green colour means that the test for phenol is positive.

Glycosides:

Add 1 ml of distilled water to the extract and an aqueous solution of NaOH. If the solution turns yellow, the test is positive for glycosides.

Carbohydrates:

Add 2 ml of Benedict's reagent to the extract and heat it in a boiling water bath for 2 minutes. A reddish-brown precipitate shows that carbohydrates are present.

Steroids:

Mix 0.5 g of the sample with 5 ml of 1% lead acetate solution and 10 ml of ethanol in water. The mixture was put in a boiling water bath for two minutes, and then it was cooled and filtered. We took 15 ml of chloroform out of the filtrate twice. Five millilitres of the chloroform layer were allowed to evaporate. After the water had evaporated, 2,3-dinitrobenzoic acid and 1 ml of 1N NaOH were added. If something is red, it means it has steroids in it.

Proteins and amino acids:

We mixed plant extracts with 2 drops of fresh 0.2% ninhydrin solution and heated them to boiling for 1–2 minutes. Then we let them cool. If something is blue, it means it has amino acids, proteins, or peptides in it.

Table 1: Qualitative analysis of phytochemicals presents in the ethanol and acetone extract of Ricinus communis leaf

Phytochemicals	Ethanol extract	Acetone extract
Alkaloids	+	+
Carbohydrates	+	+
Proteins	-	-
Flavonoids	+	+
Phenols	+	+
Glycosides	-	+
Saponins	+	-
Tannins	+	+
Amino acids	+	-
Steroids	+	-

IV. Ricinus communis has antibacterial properties.

Microorganisms for testing:

Four different strains of bacteria were used in the study. The Microbial Type Culture Collection (MTCC) at the Institute of Microbial Technology in Chandigarh, India, provided all of the bacterial cultures. The bacteria that were used were Enterococcus faecalis, Streptococcus aureus, Klebsiella pneumoniae, and Proteus vulgaris.

Test for antibacterial activity

i. **Getting the inoculum ready**
We kept stock cultures on slopes of nutrient agar at 4°C. We made active cultures for the experiment by moving a loopful of cells from the stock cultures to a test tube of Muller-Hinton broth (MHB) for bacteria. The test tube was then incubated without agitation for 24 hours at 37°C. To get optical densities that matched 2.0 X 106 colony-forming units (CFU/ml) for bacteria, the cultures were diluted with fresh Muller-Hinton broth (Smith & Doe 2018).

ii. **Making sterile swabs**
We made and sterilized cotton wool swabs on wooden or plastic applicators by autoclaving or dry heat (only for the wooden swabs). The swabs were put in culture tubes, papers, tins, or other containers to kill germs.

iii. **Cleaning forceps**
You can sterilize forceps by dipping them in alcohol and then burning off the alcohol.

ANTIBACTERIAL ASSAY UTILIZING AGAR WELL DIFFUSION TECHNIQUE:

Antimicrobial activity was tested using the well diffusion method. We tested the antibacterial activity in vitro using HI media's Muller Hinton Agar (MHA) in Mumbai. 15 millilitres of melted medium were added to sterilized petri plates to create the MHA plates. The 0.1% inoculum suspension was uniformly swabbed after the plates were allowed to solidify for five minutes. The inoculum could then be allowed to dry for five minutes. Twenty microliters of the test medication at various concentrations were introduced to wells. The plates were then stored for a full day at 37°C. The size of the inhibition zone that developed around the well was used to gauge the antibacterial activity (NCCLS, 1993). As a positive control, we employed a chloramphenicol disc.

ANTIBACTERIAL SYNERGISTIC ACTIVITY OF RICINUS COMMUNIS LEAF:

S.NO	MICRO ORGANISMS	ZONE OF INHIBITION (ETHANOL)	ZONE OF INHIBITION (ACETONE)	ZONE OF INHIBITION (CHLORAMPHENICOL) RATIO (1:1)
Gram-positive bacteria				
1	Enterococcus faecalis	20	13	22
2	Streptococcus aureus	17	12	24
Gram negative bacteria				
3	Klebsiella pheumoniae	21	10	31
4	Proteus vulgaris	22	13	29

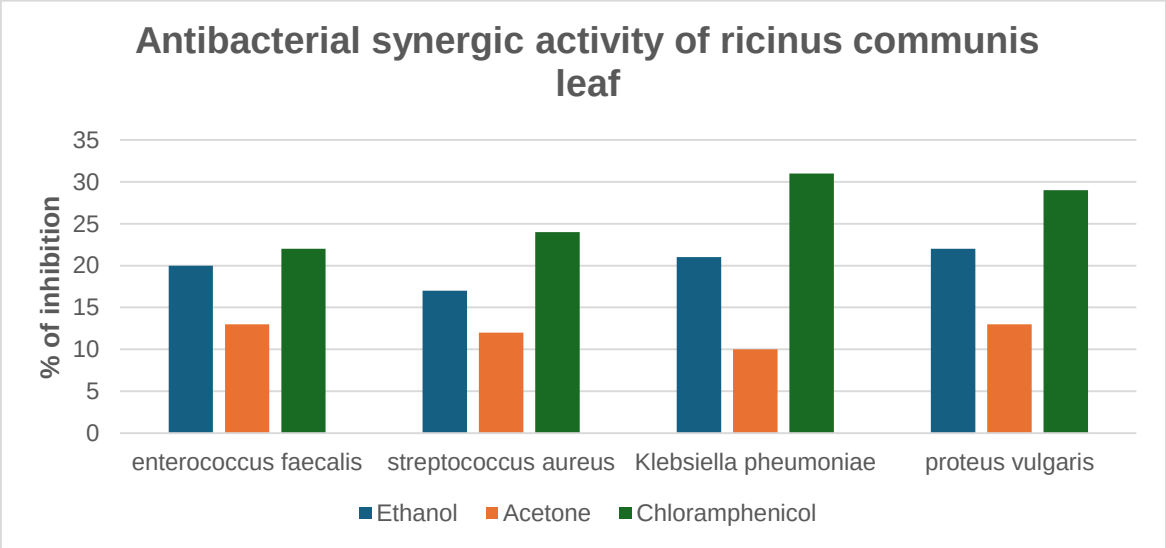

Fig. 02 : % of inhibition

ZONE OF INHIBITION

ETHANOL EXTRACT:

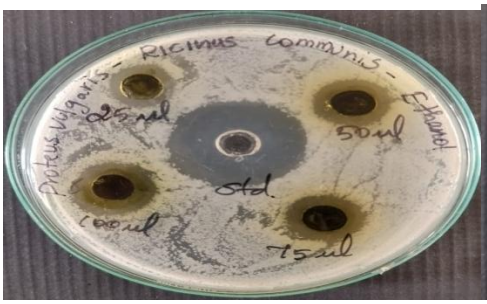

Fig. 03 Proteus vulgaris (ethanol)

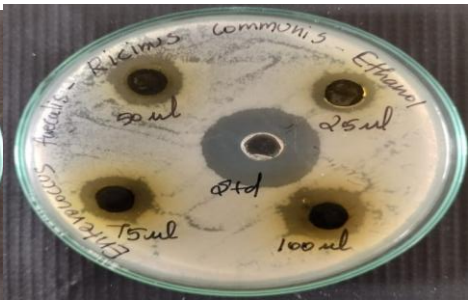

Fig. 04 Enterococcus faecalis (ethanol)

Fig. 05 Streptococcus aureus (ethanol)

Fig. 06 Klebsiella pneumoniae (ethanol)

ACETONE EXTRACT:

Fig. 07 Streptococcus aureus (acetone)

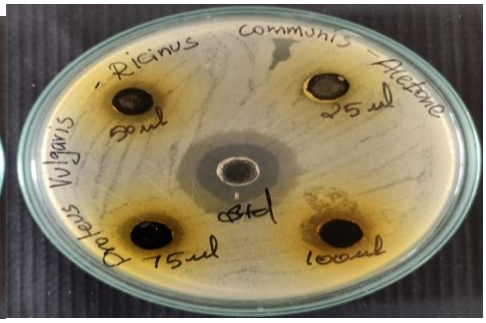

Fig. 08 Proteus vulgaris (acetone)

Fig. 09 Enterococcus faecalis (acetone)

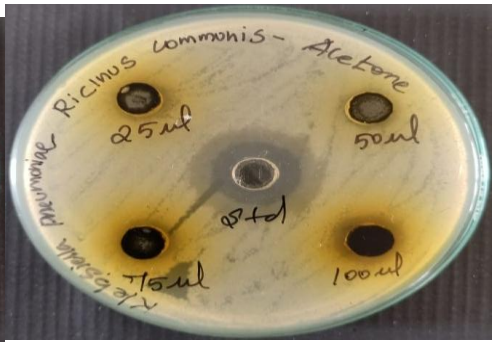

Fig. 10 Klebsiella pneumoniae (acetone)

CHLORAMPHENICOL:

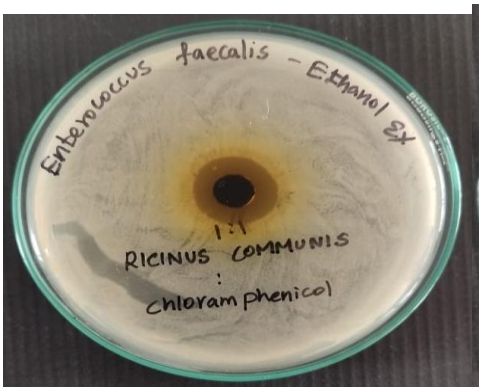

Fig. 11 Enterococcus faecalis (ethanol extract and chloramphenicol)

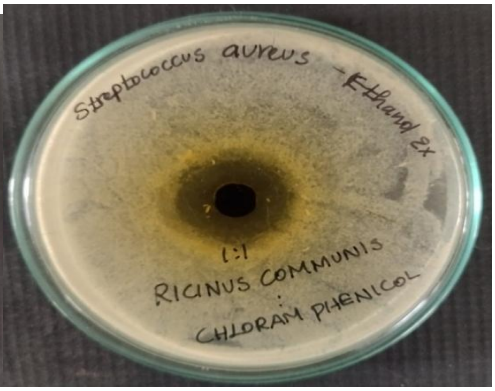

Fig. 12 Streptococcus aureus (ethanol extract and chloramphenicol)

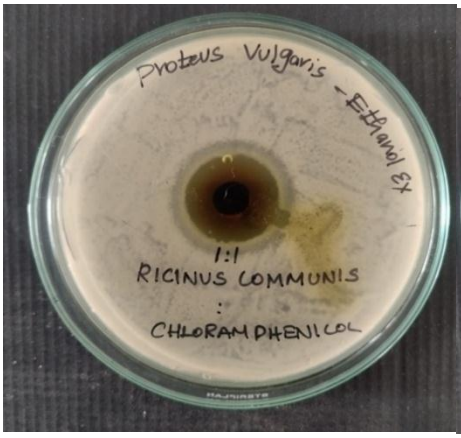

Fig. 13 Proteus vulgaris (ethanol extract and chloramphenicol)

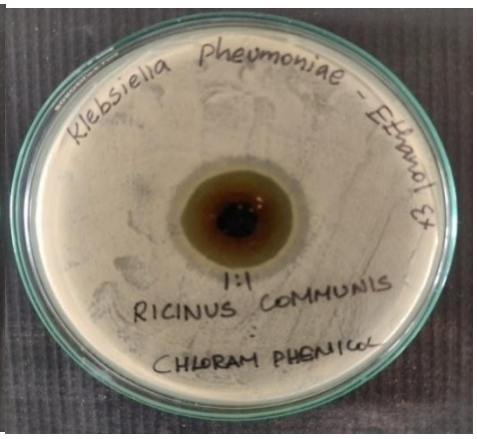

Fig. 14 Klebsiella pneumoniae (ethanol extract and chloramphenicol)

V. CONCLUSION:

The study demonstrated that *Ricinus communis* leaf extracts possess significant antibacterial activity against both Gram-positive and Gram-negative bacteria. Phytochemical screening confirmed the presence of bioactive compounds responsible for this activity. The ethanolic extract showed better antibacterial efficacy than the acetone extract. When combined with chloramphenicol, the plant extract exhibited enhanced antibacterial effects, indicating a synergistic interaction. Notable synergism was observed against *Klebsiella pneumoniae* and *Proteus vulgaris*. These findings suggest that *Ricinus communis* can serve as a potential adjunct in antimicrobial therapy.

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