IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Impact Factor 8.066 ⁜ Peer-reviewed & Refereed journal ⁜ Vol. 13, Issue 9, September 2026

DOI: 10.17148/IARJSET.2026.13909

Synergistic Effect of Neem-Ginger and Garlic-Clove Extracts in Inhibiting Early Biofilms of *Pseudomonas aeruginosa*

Tadiboina Venkata Poojitha [1], Balla Sowmya [2], Yerra Sai Pravallika [3],

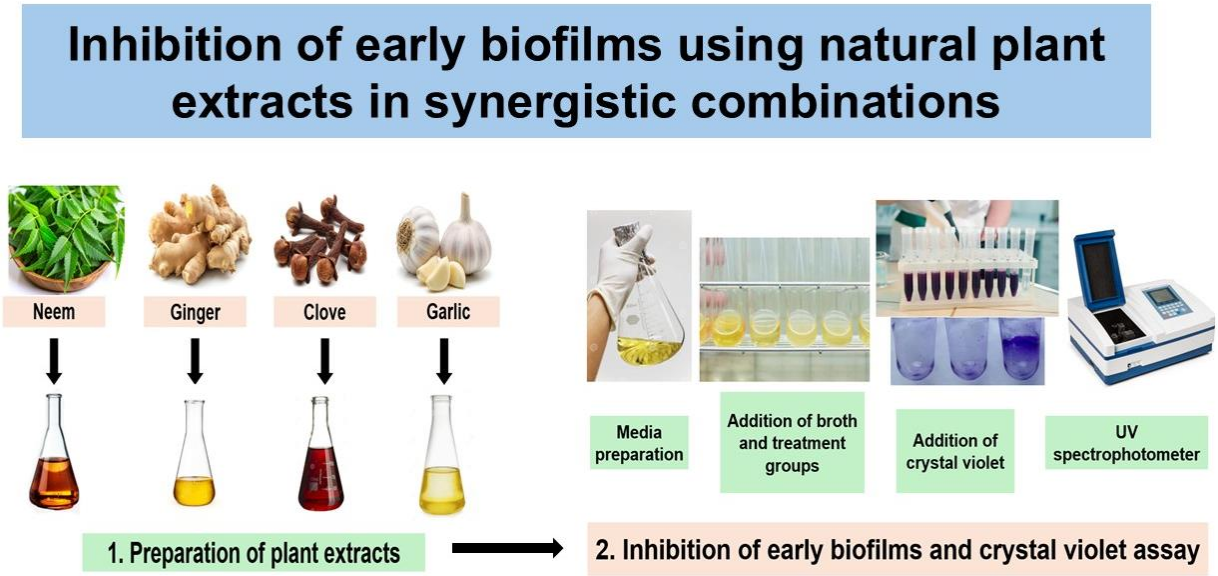
Dharanikota Lakshmi Prasanna [4], Tillapudi Jaya Swaroopa [5], Sanchita Rani Roy[6]

Department Of Chemical Engineering, Andhra University College of Engineering, Andhra University, Visakhapatnam, Andhra Pradesh, India, 530003[1-6]

Abstract: The rod-shaped, gram-negative bacterium *Pseudomonas aeruginosa* is extensively researched in biofilm studies. Because of its highly resistant biofilm-forming capabilities, this bacterium causes persistent infections in patients with cystic fibrosis, urinary infections, infections linked to medical devices, and chronic wounds that are challenging to treat. The current preliminary study uses natural extracts from the solvent extraction process, such as neem, ginger, garlic, and clove, in synergistic combinations to prevent biofilm formation in its early phases. This initial investigation analyses the synergistic effects of neem-ginger and garlic-clove combinations at five concentrations for each combination. The crystal violet assay is used to measure the extract's effectiveness in preventing early biofilm. This observation showed that each combination had a different range of inhibition. Depending on the concentration of the extracts applied, the garlic-clove combination produced consistent inhibition ranging from 32% to 57%, while the neem-ginger combination showed varying inhibition from 0% to 44%.

Keywords: *Pseudomonas aeruginosa*; Biofilm inhibition; Synergistic effect; Crystal violet assay.

Graphical Abstract



1. INTRODUCTION

Microbial biofilms are complex collections of microorganisms that can develop in a range of environments. Bacterial cells can form aggregates in the absence of a solid surface through a process called auto-aggregation, in addition to surface-attached biofilms (Trunk et al.,2018). Additionally, biofilms can take the form of pellicles, which are microbial structures that float at the air-liquid interface and are important for bacterial colonisation and survival (Vaccari et al.,2017). Additionally, biofilms are frequently observed in humans and animals, where a variety of bacteria make up the typical host microbiota. These microbial communities, however, have the potential to become pathogenic and aid in the

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development of diseases under specific circumstances. Because biofilm-associated bacteria are less susceptible to antimicrobial drugs and more tolerant of human immune responses, these infections are particularly challenging to treat (Ciofu et al.,2022). Many tissue-associated infections, such as periodontitis, osteomyelitis, pulmonary infections in patients with cystic fibrosis, infective endocarditis, diseases related to dental plaque, chronic tonsillitis, chronic laryngitis, chronic wounds, and infections of the biliary and urinary tracts, have been linked to biofilm formation (Rather et al.,2021).

Biofilms are frequently found on a variety of surfaces, including living tissues, indwelling medical devices, industrial infrastructures, laboratory equipment, wastewater pipelines, and toilet surfaces, particularly in areas with dampness or watery conditions (Costerton et al.,1999). *Pseudomonas aeruginosa* is an opportunistic bacterium that causes a variety of illnesses and is clinically significant. It is a prevalent cause of infections in burn units and is often linked to chronic respiratory infections in people with cystic fibrosis. Additionally, the bacteria can cause illnesses linked to healthcare by colonising implanted medical devices, including endotracheal tubes and stents. It poses a serious threat to public health due to its virulence and adaptability (Sadikot et al.,2005). The organism has membrane porins that help molecules pass through the cell envelope and are crucial for resistance mechanisms, environmental adaptation, and food uptake. Usually measuring 1.5–3.0 µm in length and 0.5– 0.7 µm in width, the cells are rods that are either straight or slightly bent (Chevalier et al.,2017).

Reversible adhesion, irreversible adhesion, early maturation (maturation I), advanced maturation (maturation II), and finally microbial cell dispersal are the five consecutive stages of biofilm formation (Jiang et al., 2021; de Sousa et al.,2021). When bacteria adhere to the surface of a biomaterial, biofilm development starts. Bacterial cells aggregate to form microcolonies after successful and sustained adhesion. The biofilm structure may break down in unfavourable environmental circumstances, such as variations in temperature, pH, nutrient concentration, or oxygen availability, releasing bacteria into the surrounding environment (Öztürk et al., 2023; Rabin et al.,2015). Numerous factors, including matrix composition, surface properties, growth media, microbial metabolism, intrinsic cellular features, and cell to cell communication signals, influence the formation and stability of biofilms. (Liu et al.,2023, Khatoon et al.,2018). Three main goals are the focus of current management strategies for biofilm-associated infections: preventing biofilm formation, limiting biofilm growth and development, and removing established biofilms by mechanical or chemical means (Vuotto et al.,2019). According to research, 60–80% of human microbial infections are produced by bacteria living in organised biofilm communities, making biofilm-associated illnesses a significant public health concern. (Costerton et al.,1999). One proactive and effective way to mitigate biofilm-associated infections is to prevent biofilm creation during the early phases of bacterial attachment and colonisation. This reduces microbial persistence and improves treatment efficacy. Antimicrobial resistance is expected to have detrimental effects on public health, possibly leading to 10 million deaths per year by 2050, especially from illnesses caused by Gram-negative bacteria (Rachael et al.,2020). Compared to planktonic bacteria, microbial populations encased in biofilms exhibit noticeably higher levels of antibiotic resistance- roughly 10–1000 times higher (Sharma et al.,2019). When compared to their planktonic counterparts, biofilm-associated bacteria show noticeably increased adaptive resistance to antibiotics and disinfectants. Treatment of acute and chronic biofilm-related diseases is becoming more challenging due to the significant global rise in antibiotic resistance (Masak et al., 2014; Li et al., 2017; Costerton et al.,1999).

The potential of plant-derived bioactive chemicals to reduce antibiotic resistance and inhibit the formation of biofilms in pathogenic microbes has drawn a lot of attention in recent years as alternative antimicrobial agents (Costerton et al.,2020). *Pseudomonas aeruginosa*, one of the main ESKAPE bacteria of concern, has evolved resistance to numerous commonly used antibiotics. It is commonly used as a model organism in biofilm studies due to its potent capacity to build biofilms (Kunwar et al.,2021). Plant-derived bioactive compounds have garnered significant attention among the various natural antimicrobial agents studied because of their capacity to disrupt biofilm formation, impede microbial growth, and regulate the synthesis of bacterial virulence factors (Er-Rahmani et al.,2024). Alkaloids, tannins, flavonoids, and polyphenols are examples of plant-derived substances (PDSs) that have garnered a lot of interest as possible substitutes for traditional antibacterial agents. In addition to having direct antibacterial activity, these bioactive substances may also aid in the fight against antibiotic resistance by improving the efficacy of currently available medications and altering pathogenic bacteria's resistance mechanisms (Ojong et al.,2026). Many plants extract antibacterial effectiveness is frequently ascribed to the combined action of several phytochemicals rather than a single active ingredient. While purification of individual components may occasionally lessen the overall effect, synergistic or additive interactions among various constituents can greatly increase biological activity. Garlic and ginger extracts prevented biofilm formation and showed antibacterial effectiveness against *Pseudomonas aeruginosa* that produced ESBL and KPC, according to a prior work by Gharibi et al., underscoring their potential as substitute agents against resistant biofilm-forming bacteria (Gharibi N et al.2020). The previously mentioned trend graph in fig 1 displays data from numerous studies conducted between 2005 and 2026 on the biofilm inhibition of *Pseudomonas aeruginosa* by plant

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extracts. During this time, a significant number of tests were carried out; in 2025, 75 experiments were completed, and research articles were generated regarding the biofilm inhibition of *Pseudomonas aeruginosa* bacteria.

The assessment of the synergistic antibiofilm activities of neem–ginger and garlic–clove extract combinations against *Pseudomonas aeruginosa* is what makes this study novel. To determine the best combination for preventing early biofilm formation, extracts made were examined at five concentration ratios. This method sheds light on the concentration-dependent synergistic interactions of antibiofilm compounds generated from plants. The present study was carried out to investigate the possible synergistic effect of neem–ginger and garlic–clove combinations on biofilm inhibition. Neem (*Azadirachta indica*) possesses bioactive limonoids and other phytochemicals, whereas ginger (*Zingiber officinale*) is a rich source of phenolic constituents like gingerols and shogaols. Garlic *(Allium sativum*) is also rich in organosulfur compounds, especially allicin, and clove (*Syzygium aromaticum*) is characterised by eugenol and other phenolic compounds. The differences in their phytochemical composition justify the screening of these plant extracts in a mixture since compounds with different biological activities could interact to increase the inhibition of bacterial growth, attachment and biofilm formation. Therefore, neem–ginger and garlic–clove combinations were investigated at different proportions to examine their potential inhibitory and synergistic effect on biofilm formation.

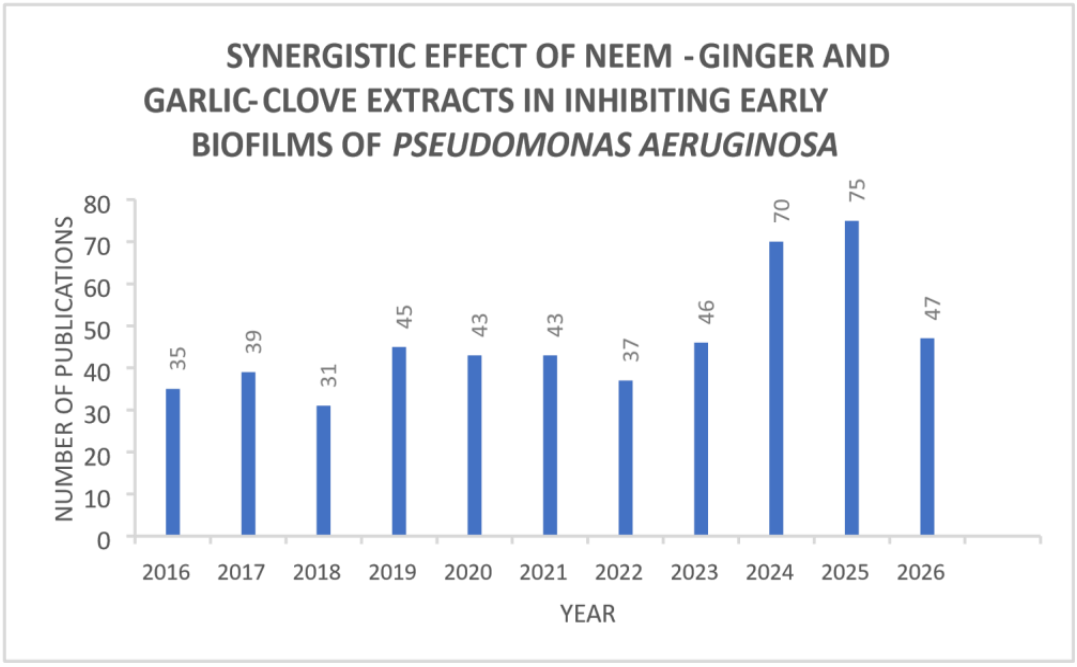

Fig 1: Trend graph of natural plant extracts in inhibiting biofilms of *Pseudomonas aeruginosa*

2. MATERIALS AND METHODS

2.1. Study Objective and Experimental Design

This study was conducted to determine the antibiofilm properties of some natural plant extracts such as neem, ginger, garlic and clove in a synergistic manner on widely studied bacteria for biofilm work, *Pseudomonas aeruginosa*. This preliminary study was carried out in laboratory conditions for three months to determine their effects on early biofilm formation.

2.2. Chemicals and Reagents Required

The procedure made use of the required chemicals and reagents, such as LB broth (Luria-Bertani), distilled water, methanol, ethanol, DMSO (dimethyl sulfoxide), crystal violet, and ethanol obtained from Merck. These substances are mainly used for encouraging bacterial growth, preparing broths, cleaning, aiding dissolution during extractions, staining biofilms, and measuring optical density (OD).

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2.3. Bacterial Strain Collection and Storage

The commercial product *Pseudomonas aeruginosa* (MTCC NO 1688, risk group N/A, HSN code 30029030), a potent biofilm-forming bacterial species, was acquired from the lab and cultivated on LB broth by incubating it at 37°C for 24 hours. It was then stored at 4°C for use in the experiment.

2.4. Plant Materials

Table 1: Plant parts and solvents used for the extraction process.

Plant	Scientific Name	Plant Part Used	Solvent Used for Extraction
Neem	*Azadirachta indica*	Leaves	Methanol
Ginger	*Zingiber officinale*	Rhizome	Ethanol
Clove	*Syzgium aromaticum*	Flower buds	Ethanol
Garlic	*Allium sativum*	Bulb	Methanol

2.5. Preparation of Plant Extracts

The plant parts such as neem leaves, ginger rhizome, garlic bulbs and clove buds mentioned in Table 1 were collected from local markets.

2.5.1 Extraction from Neem Leaves

Neem leaves were oven dried for eight hours at 45 °C after being cleaned with distilled water. Using a blender, the dried leaves were ground to a fine powder. After soaking 10g of powder in 100ml of methanol for 48 hours, the mixture was filtered. The crude thick extract of neem leaves is prepared by subjecting the filtrate to high temperatures to evaporate solvent.

2.5.2 Extraction from Ginger Rhizome

Ginger rhizomes were cleaned with distilled water, sliced into tiny pieces, and then dried in an oven at 45 °C for eight hours. A blender was used to grind the dried pieces of rhizome into a fine powder. After soaking 10g of powder in 100ml of ethanol for 48 hours, the mixture was filtered. The resulting filtrate is heated to high temperatures in order to evaporate the solvent and produce a thick, crude extract of ginger rhizome.

2.5.3 Extraction from Clove Buds

Using a blender, dry clove buds were ground into a fine powder. 10gm of the powder was then soaked in 100ml of ethanol for 48 hours before being filtered. After filtering, it is heated up to high temperature to evaporate the solvent, leaving behind a thick, raw residue of clove buds' extract.

2.5.4 Extraction from Garlic Bulbs

Garlic bulbs outer, dry covering is removed, cleaned with distilled water, and then blended into a thin paste because drying could harm the beneficial compounds. For 48 hours, 10g of paste was soaked in 100ml of methanol before being filtered. The resulting filtrate is heated to high temperatures in order to evaporate the solvent and produce a thick, crude residue of garlic bulb extract.

2.5.5 Preparation of Stock Solutions of Plant Extracts

The extracted materials were then stored in sealed tubes at 4 °C until future use. Just prior to use, 0.1 g of the corresponding crude extracts were dissolved in 1000µL of dimethyl sulfoxide (DMSO) to create stock solutions with a concentration of 100 mg/ml.

The overall procedure of plant extracts preparation and making stock solutions was clearly mentioned in fig 2.

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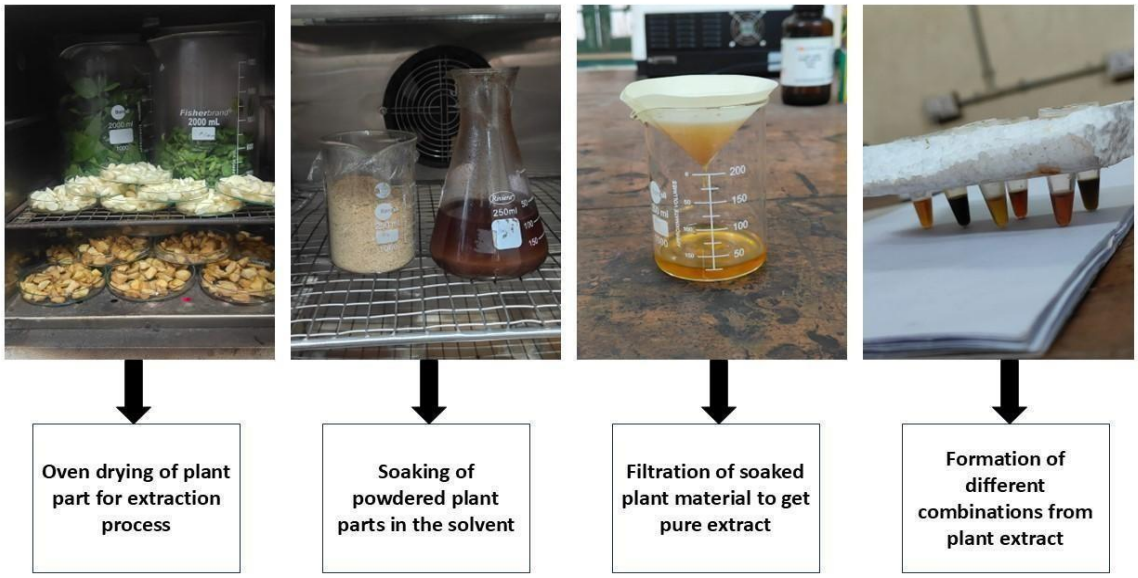

Fig 2: Plant extraction followed by stock solution preparation.

2.6. Analysis of Bioactive Compound Present in Crude Plant Extracts

Gas chromatography and mass spectrometry (GC-MS) was used to analyse the bioactive chemicals found in the crude extract of dried clove buds. Normal-phase and reversed-phase high pressure liquid chromatography were used to analyse the bioactive substances found in the crude extract of garlic bulbs. Thin layer chromatography and high-pressure liquid chromatography were used to analyse the bioactive chemicals found in the crude extract of neem leaves. Gas chromatography and mass spectrometry (GC-MS) was used to analyse the bioactive chemicals found in the crude extract of ginger rhizome.

2.7. Preparation of Bacterial Overnight Culture

A fresh overnight culture of *Pseudomonas aeruginosa* was obtained by inoculating 10µl of purchased pure strain into 10ml LB broth under certain incubation conditions such as 37°C for 24 hours under sterile conditions inside laminar airflow.

2.8. Experimental Data

To identify the synergistic effects of the selected plant extract combinations, a negative control group and two major treatment groups were included in the experiment. The negative control named CO-1 and CO-2 for neem-ginger combination and garlic clove combination respectively, was a broth and only bacterial culture to provide baseline data for the normal behaviour of the test organism. The treatment groups were set up to study the effect of different ratios of plant extract combinations on bacterial growth. Test tubes 1A, 1B, 1C, 1D and 1E were exposed to the mixture of neem and ginger. The garlic-clove combination was tested in tubes 2A, 2B, 2C, 2D and 2E with different concentrations of extracts in each tube. The tubes marked CO-1 and CO-2 served as combination controls for their respective trial sets, and thus permitted evaluation of the combined effect in the absence of synergistic interaction. This allowed comparisons of each treatment with the negative control and of different extract ratios and individual components within each combination. The treatment groups and the corresponding test tube labels are given in full in Table 2.

Table 2: The treatment groups and their respective test tube names

Treatment Groups	Test Tube Name
Neem + ginger	1A, 1B, 1C, 1D, 1E, CO-1
Garlic + clove	2A, 2B, 2C, 2D, 2E, CO-2

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2.9. Preparation of Synergistic Combinations from Extracts

The stock solutions were made fresh, and two distinct combinations were created, each with five different ratios. The two tested combinations were neem + ginger and garlic + clove. In each combination, the extracts were used in concentrations from 0 to 200 parts. Neem + ginger combination: 1A – 200:0; 1B – 150:50; 1C – 100:100; 1D – 50:150; 1E – 0:200. Similarly, the garlic + clove combination was prepared: 2A – 200:0; 2B – 150:50; 2C – 100:100; 2D – 50:150; 2E – 0:200. 200 µL of neem leaf stock solution was used for 1A, while 150 µL of neem and 50 µL of ginger stock solutions were combined for 1B, 100 µL of neem and ginger solutions were taken for 1C, 50 µL of neem and 150 µL of ginger were combined for 1D, and only 200 µL of ginger were taken for 1E. 200 µL of garlic bulb stock solution was used for 1A, while 150 µL of garlic and 50 µL of clove stock solutions were combined for 1B, 100 µL of garlic and clove solutions were taken for 1C, 50 µL of garlic and 150 µL of clove were combined for 1D, and only 200 µL of clove were taken for 1E. For each series, there were two control treatments (CO-1 and CO-2), which served as negative controls for the respective test series: CO-1 for the neem + ginger series, and CO–2 for the garlic + clove series. In general, all the prepared treatments were tested for antibiofilm activity against *Pseudomonas aeruginosa*, as described below. The detailed list of the treatment groups and test tube labels is given in Table 3.

Table 3: Treatment groups their respective extract ratios and test tube identification.

Treatment Groups	**Combination Ratios**	**Test Tube Names**
Neem + ginger	200:0	1A
Neem + ginger	150:50	1B
Neem + ginger	100:100	1C
Neem + ginger	50:150	1D
Neem + ginger	0:200	1E
Control 1	-	CO-1
Garlic + clove	200:0	2A
Garlic + clove	150:50	2B
Garlic + clove	100:100	2C
Garlic + clove	50:150	2D
Garlic + clove	0:200	2E
Control 2	-	CO-2

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2.10. Biofilm Inhibition Assay

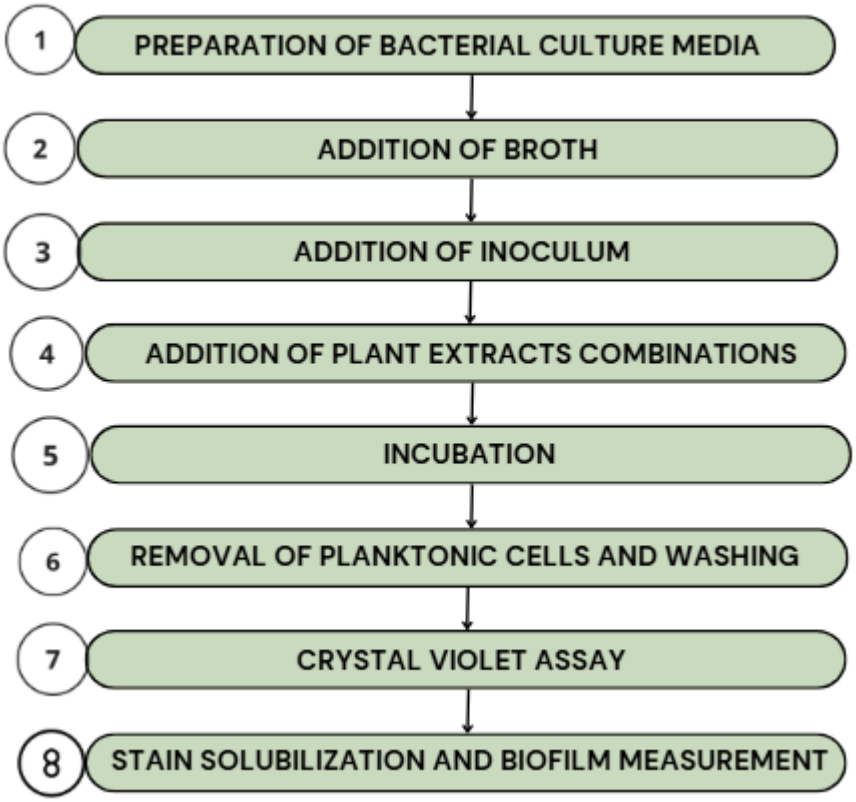

Fig 3: Schematic Representation of the Experimental Procedure of biofilm inhibition assay

Step 1: Preparation of Bacterial Culture Broth

2.4 g of LB broth powder was weighed and dissolved in 120ml distilled water to prepare culture broth. The media, along with 12 test tubes and a small measuring cylinder is autoclaved at 121°C temperature and 15psi pressure for 15 min.

Step 2: Addition of Broth

This step was carried out completely in sterile laminar airflow conditions. Test tubes were labelled with their respective names, and 4.7 ml of broth was measured using a measuring cylinder and poured into each test tube that was supposed to be treated, and 4.9 ml of media was added into the control test tube.

Step 3: Addition of Inoculum

Add 100µl of overnight bacterial culture into all the test tubes using a micropipette.

Step 4: Addition of Plant Extracts Combinations

Add the prepared combination solutions made from stock solutions into their respective test tubes as mentioned in the table.

Step 5: Incubation

These test tubes were incubated at 37°C for 24 hours to determine the efficiency of these synergistic combinations in inhibiting the early biofilm formation of *Pseudomonas aeruginosa*.

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Step 6: Removal of Planktonic Cells and Washing

After 24 hours, the broth from the test tubes was gently poured into a beaker and then washed with distilled water.

Step 7: Crystal Violet Assay

These test tubes were now stained with 1% crystal violet solution and left for 15min so that the bacteria would take up the stain. The crystal violet solution from the test tubes was discarded and washed with distilled water to remove excess stain, followed by drying of the test tubes.

Step 8: Solubilization of Stain and Measurement of Biofilm OD

Ethanol was added to all the test tubes to solubilise the stain, which is indicative of the amount of biofilm formed and the ethanol in the test tube was not disturbed for 15 min to completely dissolve the stain. The ethanol present in the test tubes was used to determine the biofilm OD at 545nm using a spectrophotometer.

The whole process of biofilm inhibition assay was best explained in fig 3 and visually present in fig 4.

Fig 4: The procedures for inoculation, the incorporation of plant extracts, and the test tubes used for conducting the crystal violet assay.

2.11. Calculation of Biofilm Inhibition

Biofilm inhibition OD = (Control OD – Biofilm OD) * 100 / Control OD Equation 1

In the equation 1, OD means optical density at 545nm.

The formula in equation 1 is used for calculating the percentage of biofilm inhibition. A higher percentage indicates greater inhibition, and a lower percentage indicates lower inhibition.

3. RESULTS AND DISCUSSION

In this preliminary investigation, neem, ginger, garlic, and clove were used singly and in combination to assess the suppression *Pseudomonas aeruginosa* early biofilms using the crystal violet assay. These chemicals' synergistic action

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was identified and contrasted with the suppression of individual substances. They were combined in various ratios to create the synergistic combinations. Inhibition values vary depending on the ratio.

3.1. Synergic Action of Neem Ginger Combination

Table 4: Percentage inhibition and biofilm OD for neem-ginger combinations

Test tube name	Biofilm OD at 545nm	Percentage inhibition
1A	0.301	No inhibition
1B	0.977	No inhibition
1C	0.102	35.8%
1D	0.109	31.4%
1E	0.089	44.0%
CO-1	0.159	-

The OD for Neem alone (200:0) was 0.301 with no inhibition, and the 150:50 combination had an OD of 0.977 and showed no inhibition either. The combination of 100:100 gave an OD of 0.102 with inhibition of 35.8%, and the combination of 50:150 gave an OD of 0.109 with inhibition of 31.4%. Ginger alone (0:200) showed the maximum inhibition of 44.0% with an OD of 0.089. Thus, ginger alone had better antibiofilm activity than the tested neem–ginger combinations as shown in fig 5 and table 4. In general, the combinations did not show a clear increase in inhibition, suggesting that the activity differed depending on the ratio of extracts. In the cases of 1C, 1D, and 1E, the biofilm OD achieved is lower than the control OD.

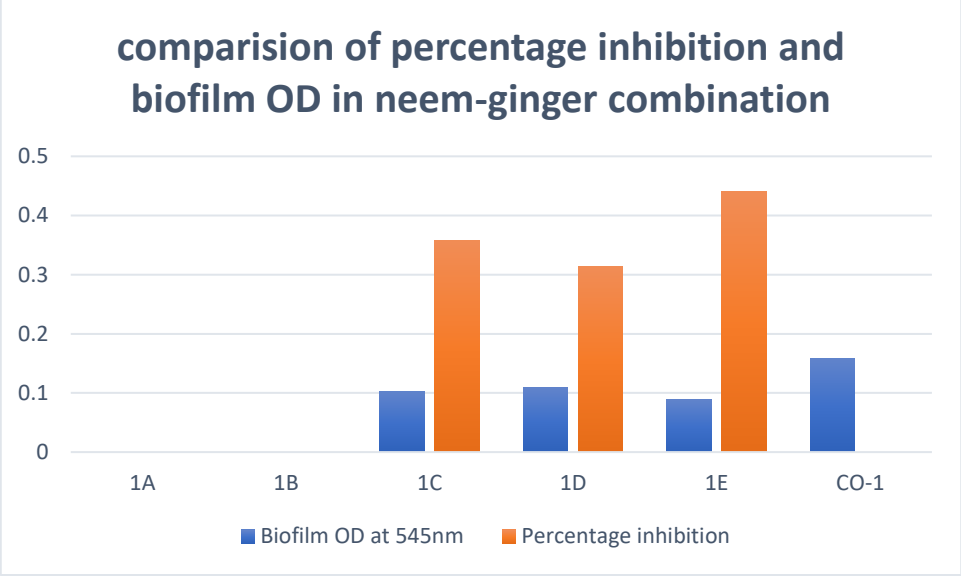

Fig 5: The various inhibitory percentages depending on the ratios of neem and ginger combinations and the OD values relative to the control OD.

3.2. Synergic Action of Garlic and Clove Combination

Table 5: Percentage inhibition of garlic-clove combinations

Test tube name	Biofilm OD at 545nm	Percentage inhibition
2A	0.069	56.6%
2B	0.089	44.0%
2C	0.102	35.8%

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2D	0.108	32.0%
2E	0.094	40.8%
CO-2	0.159	-

Alone, garlic (200:0) had an OD of 0.069 and the highest inhibition, 56.6%. The combination 150:50 had an OD value of 0.089 with 44.0% inhibition, and the combination 100:100 had an OD value of 0.102 with 35.8% inhibition. 50:150 combination yielded an OD of 0.108 and 32.0% inhibition. Only clove showed an OD of 0.094 (0:200) with inhibition of 40.8%. Overall, garlic alone was more effective in preventing biofilm formation than the combinations tested. In general, a trend to less inhibition was seen with decreasing ratio of garlic to clove. However, clove alone also showed significant inhibition, indicating that the two extracts possess antibiofilm activity as seen in fig 6 and table 5.

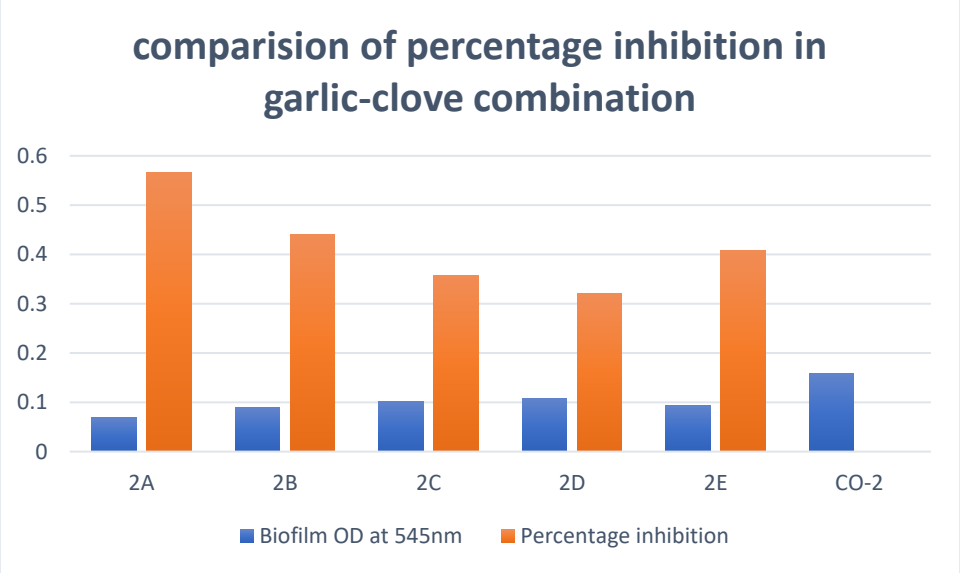

Fig 6: The various inhibitory percentages according to the ratios of garlic and clove combinations. Additionally, how the OD values compare to the control OD.

3.3. Comparison of Neem-Ginger and Garlic-Clove Treatments

Both treatment groups were compared in fig 7, and it was found that the garlic–clove treatments showed higher biofilm inhibition than the neem–ginger treatments in general. The highest inhibition was observed with only garlic at 56.6%, followed by only ginger and the 150:50 garlic–clove combination, both showing 44.0% inhibition. The combinations of neem and ginger showed less inhibition, 35.8% for 100:100 and 31.4% for 50:150. Generally, garlic was the most effective extract among the four plant extracts tested under the experimental conditions. The results also indicate that individual extracts showed higher inhibition than most of the combinations. Thus, the current findings did not show any clear synergistic effect between neem-ginger or garlic-clove combinations, as activity was dependent on the extract and combination ratio used.

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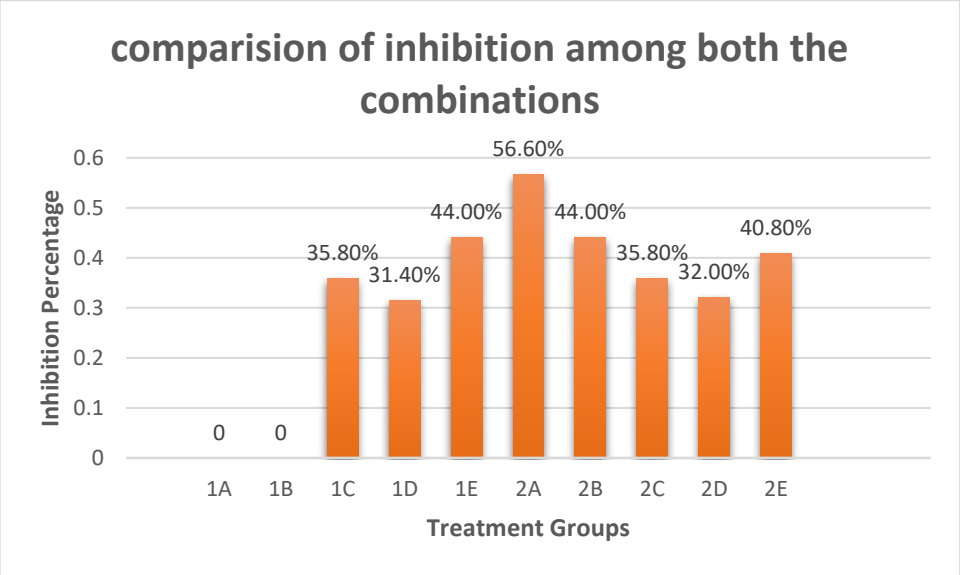

Fig 7: comparison of inhibitory activity of all treatment groups, including the garlic-clove and neem-ginger combinations.

3.4. Analysis of Bioactive Compound Present in Crude Plant Extracts

Among the 45 phenolic compounds found in the crude extract of clove, the GC-MS analysis revealed eugenol, eugenol acetate, and caryophyllene as the main constituents. Among the 27 bioactive chemicals found, the GC-MS analysis of ginger crude extract, indicated Zingerone, α-zingiberene, (6)-shogaol, α-farnesene, β-funebrene, 6-gingerol, and α-curcumene as significant compounds. Alliin, isoalliin, methiin, cycloalliin, γ-L-glutamyl-S-methyl-L-cysteine, γ-L-glutamyl-S-(2-propenyl)-L-cysteine, and γ-L-glutamyl-S-(trans-1-propenyl)-L-cysteine were identified as the main components in the garlic extract using normal-phase and reversed-phase high pressure liquid chromatography. Astragalin, quercitrin, isoquercitrin, nicotiflorin, and rutin were identified as flavonoids in the neem leaves extract using TLC and HPLC.

The commonly recognized harmful bacterium Pseudomonas aeruginosa uses quorum sensing (QS) to manufacture virulence factors and create biofilms. One emerging method for reducing its pathogenicity is to disrupt the regular QS connections between signal molecules and their corresponding receptors (Kim et al.,2015). 6-gingerol can lessen *Pseudomonas aeruginosa* virulence and biofilm development, which is essential for boosting antibiotic efficacy and lowering *Pseudomonas aeruginosa* pathogenicity. These findings showed that 6-gingerol inhibited biofilm formation just as well as furanone C-30, a synthetic anti-QS molecule (Kim et al.,2015). 6-shogaol inhibits the overall structural development of the biofilm while concurrently limiting the attachment and survival of bacteria and fungus within, which effectively suppresses the formation of EPS, a major component of biofilms (Jung et al.,2025). When a garlic clove is crushed, alliinase converts its precursor, alliin, into allicin in a matter of seconds. Allicin has a special bactericidal impact on bacteria that are embedded in biofilms. The viability of biofilm bacteria was lost along with the decrease in biofilm mass. Allicin decreased the bulk of Pseudomonas aeruginosa biofilms by lowering the adhesion ratio, extracellular matrix synthesis, and virulence factor expression. Allicin primarily functioned as an inhibitor of quorum sensing. (Wu et al.,2015). Jakobsen's bioassay-guided fractionation of garlic extracts revealed that ajoene, a sulphur-containing molecule with potential as an antipathogenic medication, was the main QS inhibitor found in garlic (Jakobsen et al.,2012). Ajoene has the ability to block genes related to pathogenicity that are regulated by quorum sensing (Yang et al.,2016). Rutin present in crude neem extract acts as a principal anti-biofilm compound. Rutin affects biosynthesis of capsular polysaccharide (cps) which is essential in getting contact with the other bacterial cells (bacterial adhesion) leading to microcolonies which is responsible for biofilm formation (Wang et al.,2017).

CONCLUSION

Application of extracts from neem, ginger, garlic, and cloves to stop *Pseudomonas aeruginosa* from developing biofilms used both individual extracts and combinations of those four extracts in different ratios and analysed by using crystal violet assay. Neem ginger combinations also exhibited approximately same inhibition percentages as that of the garlic and clove combination whereas in in neem ginger combination the ginger alone exhibited the highest percentage of

DOI: 10.17148/IARJSET.2026.13909

inhibition when compared to its synergistic combination. Of all the treatment groups employed in this investigation, garlic extract alone showed the highest percentage of inhibition. As the percentage of cloves rose and the percentage of garlic decreased, the inhibition rate decreased. Clove and garlic alone inhibited the early biofilms to greater extent when compared to their synergistic combinations. The early biofilms were somewhat hindered by the extracts and these investigations are critical for developing formulations that prevent the formation of biofilms.

FUTURE PERSPECTIVES

Future research can concentrate on testing various plant extract concentrations and combinations, assessing how well they work against mature biofilms and other bacterial strains, and looking into how they might be used in conjunction with antibiotics. To ascertain their suitability for upcoming applications, more safety and toxicity research is also required. Our study shows that plant-derived extracts are efficient against *Pseudomonas aeruginosa*. Future studies should focus on characterising and identifying the most effective inhibitory plants and extracts, as well as on different bacteria, as our work only looks at early biofilm inhibition.

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IARJSET ISSN (O) 2393-8021, ISSN (P) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Impact Factor 8.066 ⁜ Peer-reviewed & Refereed journal ⁜ Vol. 13, Issue 9, September 2026

DOI: 10.17148/IARJSET.2026.13909

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