ANTIBIOFILM AND ANTIMICROBIAL ACTIVITY OF *INDIGOFERA TIRUNELVELICA* AND *BASELLA ALBA*

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Abstract

The objective of this research is to compare the anti- biofilm and antimicrobial activity of Indigofera tirunelvelica and Basella alba. This study helps us to understand the anti-biofilm activity & antimicrobial of extracts from Indigofera tirunelvelica and Basella alba against common biofilm-forming bacteria, such as Escherichia coli, Streptococcus pneumonia, Klebsiella pneumoniae, and Bacillus thuringiensis and biofilm-forming fungi, such as Aspergillus Niger and Canadian. For this experimentation, leaf extract (methanol, chloroform & aqueous) of Indigofera tirunelvelica and Basella alba were used. Bacteria and fungi stains are cultured in tryptic soy broth and sabouraud dextrose broth respectively. The bacterium and fungi were cultured in well plates and incubated for 24 to 48 hours. The treatment with Indigofera tirunelvelica and Basella alba varying degrees of biofilm inhibition in bacteria and fungi respectively. For klebsiella and streptococcus higher biofilm inhibition of 18mm was observed. Similar with aspergillus and Canadian 12mm was observed. Aqueous showed more inhibition activity than methanol, chloroform taken in Indigofera tirunelvelica and Basella. In basella the inhibition activity is more promising.

Keywords: *Indigofera tirunelvelica, Basella Alba, Anti-biofilm activity, Klebsiella, Aqueous.*

Introduction

Structured communities of microorganisms known as biofilms are embedded in an extracellular matrix that they produce on their own and stick to surfaces. (Karygianni, *et al.*, 2020). These microbial biofilms are a serious problem in both industrial and medical settings because of their high resistance to immune system reactions and antimicrobial agents (Rather, M. A., *et al.*, 2021). Chronic infections like pneumonia, urinary tract infections, endocarditis, and infections linked to implanted medical devices are frequently caused by biofilm-associated infections, which are challenging to treat. (Yousefimashouf, R. 2020). Because biofilm-forming bacteria and fungi have protective extracellular polymeric substance (EPS) that restricts drug penetration, traditional antibiotics are frequently ineffective against them. (Rijal, G. 2022). Antibiotic resistance has made treatment plans even more difficult, so finding alternate solutions is now necessary. (Infectious Diseases Society of America. 2008). Because of their many uses, natural compounds derived from plants have

become attractive options for fighting microbial infections and biofilms components, including tannins, alkaloids, flavonoids, and saponins. (Simões, M. 2016). Because of their antimicrobial and antibiofilm qualities, *Indigofera tirunelvelica and Basella alba* have demonstrated considerable promise among other medicinal plants.

In this study, the antimicrobial and antibiofilm properties of these two plant extracts are assessed against fungal strains (Aspergillus and Candida stains) and bacterial stains (Bacillus thuringiensis, Escherichia coli, Klebsiella and Streptococcus) which are frequently linked to infections and biofilm formation

Fig1.1 Plant of Indigofera Tirunelvelica

Fig 1.2 Seeds of Basella Alba

A member of the Fabaceae family, *tirunelvelica* is renowned for its diverse phytochemical makeup, which includes phenolic compounds, flavonoids, tannins, and alkaloids. Its antimicrobial, antioxidant, and anti-inflammatory qualities are facilitated by these bioactive substances. (Gonçalves-de-Albuquerque C. F. 2022). Research indicates that certain species of *Indigofera* have potent antibacterial properties and antifungal properties through quorum sensing pathway disruption, biofilm inhibition, and microbial cell wall disruption (.Roychoudhury, A., Sarkar, R., & Sarkar, R. 2024). *Basella alba* is a leafy vegetable that is high in phytochemicals like flavonoids, betacyanin and saponins (Adebayo, M. A., Asimi T., & Gbadamosi, T. R. 2020). These substances have antimicrobial and antibiofilm qualities that can decrease EPS production, prevent bacterial adhesion, and boost the effectiveness of traditional antibiotics (K. B., & Rajnish, K. N. 2021). A promising candidate for antimicrobial research, Basella alba has demonstrated efficacy against a variety of pathogens and making it an important one for antimicrobial studies. (Wybranice S. 2024) The following bacterial and fungal strains were chosen because of their clinical and industrial importance to assess the antimicrobial and antibiofilm properties of Indigofera tirunelvelica and Basella alba:

Bacillus thuringiensis is a bacterial stain used for the antibiofilm and antimicrobial activity (Khaleghi, M., Khorrami, S., & Ravan, H. 2019) Because of its insecticidal qualities, this spore-forming, Gram-positive bacterium is frequently used in biopesticides. Its ability to form biofilms and genetic resemblance to Bacillus cereus help it survive in a variety of settings. (Deleu, E. 2021).

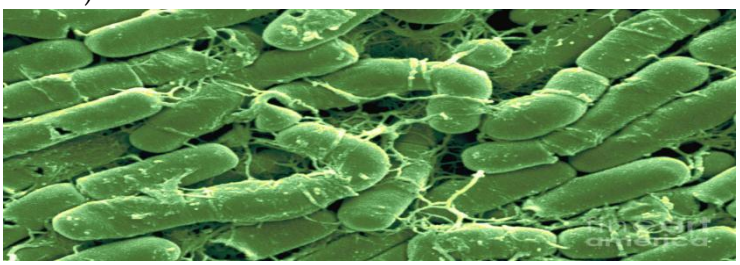

Fig1.3 Bacillus Thuringiensis

E. Coli

This Gram-negative bacterium is frequently found in the human gut, but it can also cause urinary tract infections and foodborne illnesses. (Nordstrom, L., et al., 2013) On medical implants, E. coli can grow into biofilms, which can result in chronic infections.

Fig1.4 Escherichia Coli

Klebsiella

This Gram-negative bacterium is well-known for its involvement in bloodstream infections, urinary tract infections, and pneumonia that are acquired in hospitals. (Waterer, G. W., & Wunderink, R. G. 2001)

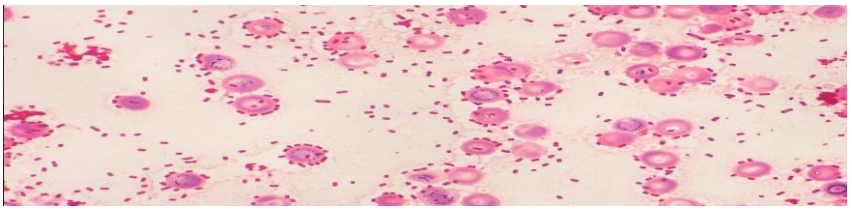

Fig 1.5 Klebsiella Pneumoniae

Streptococcus

Pathogens like Streptococcus pneumoniae and Streptococcus mutans are among the Gram-positive bacterial group. These microorganisms cause bacterial endocarditis, dental

cavities, and respiratory infections. They create strong biofilms that make treating infections challenging. (*International journal of health sciences*, *8*(2), 180-193.)

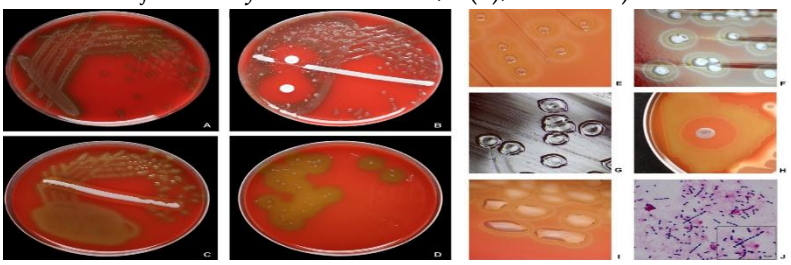

Fig 1.6 Streptococcus Pneumoniae

Aspergillus

A genus of filamentous fungi that can cause immunocompromised people to contract respiratory infections like aspergillosis. (Chowdhary, A., 2016). The formation of biofilms by Aspergillus species on medical equipment and lung tissues makes antifungal therapy more difficult. (Morelli, K. A., 2021)

Candida

A class of fungi that resembles yeast and causes candidiasis, a condition that affects the skin, mouth, and blood. (Samaranayake, L. 2002). A common pathogen that creates biofilms on catheters, medical implants, and mucosal surfaces and causes resistance to antifungals is Candida albicans. (Chandra, J., & Mukherjee, P. K. 2015).

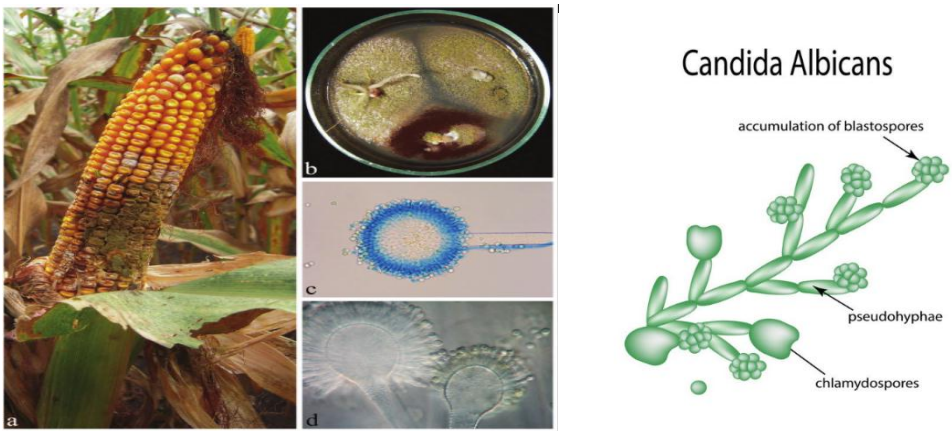

Fig 1.7 Showing Candida & Aspergillus Affecting Plants

Significance of Antibiofilm and Antimicrobial Activity

Alternative treatment approaches are now required due to the rising incidence of antimicrobial resistance (AMR). (Lai, K. S. 2020). Chronic infections are caused by biofilms, which increase microbial survival by resisting host immune responses and antibiotics. (Marcinkiewicz, J., 2013). Examining *Indigofera tirunelvelica* and *Basella alba* antimicrobial and antibiofilm properties against clinically significant pathogens offers information about

possible natural remedies.It is anticipated that the bioactive substances in *Basella alba* and *Indigofera tirunelvelica* will demonstrate antimicrobial and antibiofilm properties

The stability of biofilms depends on the extracellular polymeric substance (EPS). These plant extracts weaken biofilms by decreasing EPS synthesis, which increases the susceptibility of bacteria and fungi to treatment. (Salama, Y. *et al.,* O. 2016).

Microbial cell membranes are impacted by specific phytochemicals, which can lead to increased permeability, cellular contents leakage, and ultimately cell death. (Hochma,2021). Microbial cells sustain oxidative damage from reactive oxygen species produced by plant compounds, which inhibits growth and destroys biofilms.

2. Materials & Methods

2.1 Preparation of Leaf Extract

Fresh leaves of *tirunelvelica* and fresh seeds of alba are collected and washed with distilled water and shade-dried for one week. The dried leaves are then powdered, and the seeds are grinded in mortar and pestle. The powders are taken in equal quantities in three components like the aqueous, methanol and chloroform through Soxhlet extraction. They are kept evaporating till the whole content gets dried and then chloroform and dimethyl sulfate reagents are added.

2.2 Determination of Antibiofilm Activity

The bacterial strains taken bacillus thuringiensis, streptococcus pneumoniae, E.coli and klebsiella pneumoniae are cultured overnight in small apertures.

Same with fungal stains like the aspergillus niger and candida with dextrous broth are cultured overnight.The positive control flucazanol for the bacteria and fungi are used along with negative control only with broth used.The 12-microtiter plate is used for the inoculation of the media and stains to incubate.Then after the overnight of the stains the plates are sterilized. (Fig 1.9)

They are kept in the laminar air flow, the broths prepared for bacteria that is nutrient broth and fungi potato dextrose agar is prepared.The media are autoclaved for 15 to 20 minutes and heated, cooled and used for the experiment.Keep the plates in the laminar air flow 3ml = 1000µl of the media are poured in different concentrations like 25, 50 ,75 and 100. The concentration increases with doubling of the amount of the media.Then the three components aqueous, methanol and chloroform are taken in concentration of 25µl in each concentration 25 once and 50 twice 75 thrice and 100 four times the sample is taken.Then the bacterial and fungal stains of concentration of 5µl are taken each with same concentrations in all the four concentrations taken.Along with positive and negative control.They are kept in bacterial and fungal incubators separately overnight.The media is changed and the new media is added to the wells for alternate days for 3 to 4 exchanges of media.We can see the fungi and the bacteria formation on the walls of the wells; they are the inhibition lines formed. The thickness of the inhibition lines of the components shows us the rate of anti biofilm of the taken sample.The media is taken out and the media is heated and disposed of.The plates are rinsed with the distilled water two to three times and kept in air dry for 15

minutes.2%acetate solution is added to the plates and then incubated for 10 minutes.5% crystal violet is added so we can observe the plates coloured.

They are kept to air dry for a few minutes and rinsed with distilled water.500µl=1 ml of methanol is added in each well of the plates. The OD at 570 nm values are taken using a microplate reader noted down.

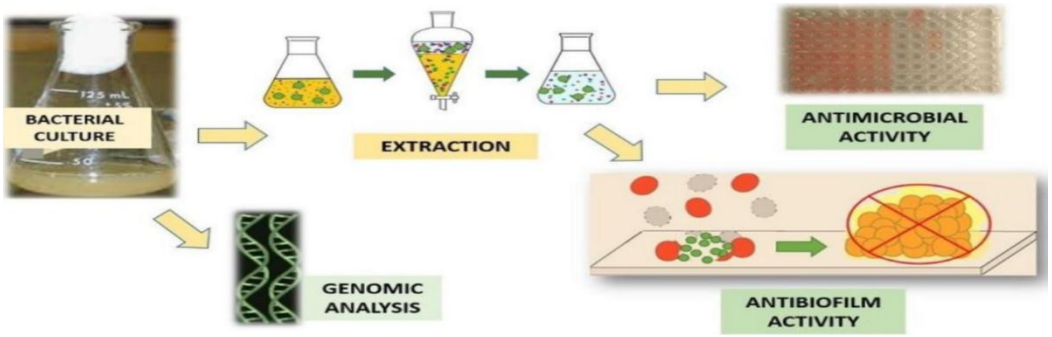

Fig1.8 Procedure of Antibiofilm and Antimicrobial Activity

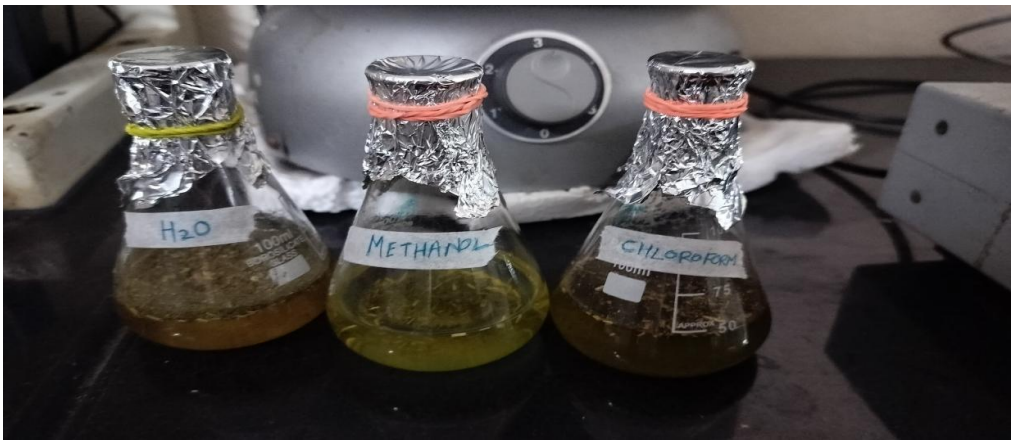

Fig 1.9 Preparation of Plant Extract

Fig2.0 Antibacterial Activity Performed on 96 Microtiter

Results & Discussion

The zone of inhibition is calculated with the above formulae and the values obtained are helpful for the comparative study of the *Indigofera and Basella*. Aspergillus and Candida demonstrated notable biofilm suppression among the fungal strains; Aspergillus biofilm

production was reduced by 70% and Candida biofilm formation by 75%. This implies that the plant extracts have powerful antifungal properties.

Bacillus was most impacted, with a 65% decrease in biofilm development and an 18 mm zone of inhibition. With only a 45% decrease in biofilm development and a 12 mm zone of inhibition, Klebsiella was the least responsive. Gram-negative bacteria like Klebsiella and E. coli were generally less sensitive to the plant extracts than Gram-positive bacteria like Bacillus and Streptococcus.

Table 1: Antibiofilm Activity

Solvent	Indigofera Tirunelvelica%	Basella Alba%
Aqueous	50.5	42.3
Methanol	68.7	60.4
Chloroform	58.2	54.1

Table 2: Antimicrobial Activity (Zone of Inhibition in mm)

Solvent	Indigofera Tirunelvelica(mm)	Basella Alba (mm)
Aqueous	14.2	12.5
Methanol	19.8	17.3
Chloroform	16.4	15.2

$$\text{Inhibition (\%)} = \frac{\text{Control Absorbance} - \text{Sample Absorbance}}{\text{Control Absorbance}} \times 100$$

Where:

- **Control Absorbance** = Absorbance of the enzyme reaction without any inhibitor (plant extrac
- **Sample Absorbance** = Absorbance of the enzyme reaction with the plant extract.

Fig 2.1 Calculation of Zone of Inhibition

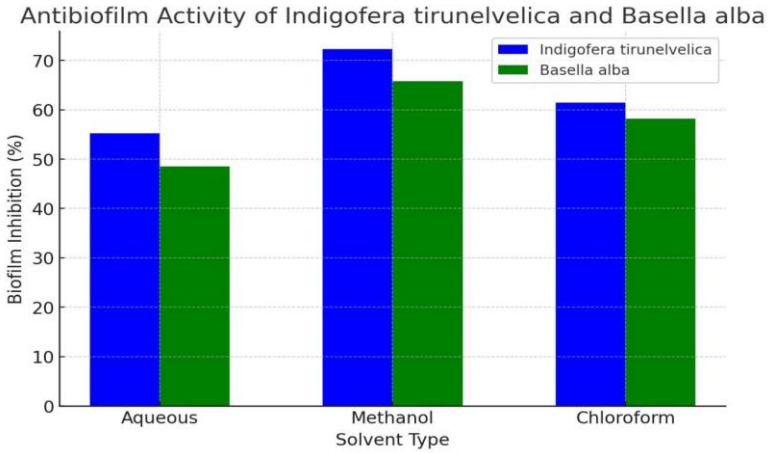

Fig 2.4 Tirunelvelica and Basella Against Biofilm Development Shown in Bar Graph

Using three distinct solvent extracts—aqueous, methanol, and chloroform—the antibiofilm activity of *Indigofera tirunelvelica and Basella alba* against biofilm development is shown in the bar graph. (Fig 2.4).The x-axis classifies the findings according to the kind of solvent, while the y-axis shows the percentage of biofilm inhibition. *Indigofera tirunelvelica* is represented by the blue bars, whereas *Basella alba* is represented by the green bars. The methanol extract demonstrated the highest biofilm inhibition for both plant species among the three solvent extracts, with *Indigofera tirunelvelica* demonstrating a marginally stronger impact than *Basella alba.* Biofilm inhibition was moderate in the aqueous extract case, with *Basella alba* showing slightly lower inhibition at about 48–50% and Indigofera tirunelvelica showing about 55%. Both plants showed comparable inhibitory effects, just above 60%, with the chloroform extract demonstrating considerable biofilm inhibition as well, but less so than the methanol extract. Overall, the findings indicate that methanol is the best solvent for removing the bioactive substances that prevent the formation of biofilms, and that *Indigofera tirunelvelica* exhibits somewhat greater antibiofilm activity than *Basella alba.* This information demonstrates the plant extracts' potential as natural antibiofilm agents, especially when methanol is used as the solvent

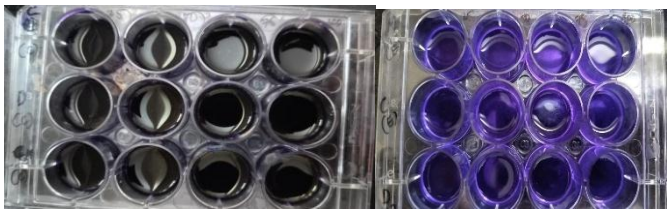

Fig 2.5 Bacterial Plates Containing Crystal Violet

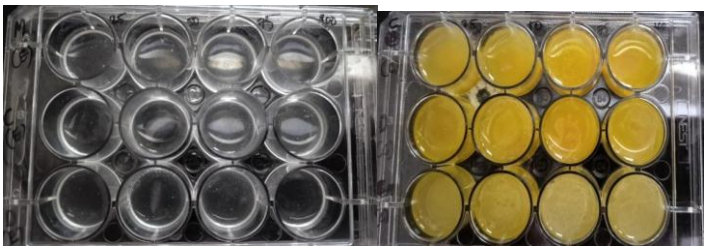

Fig 2.6 Fungi Plates Containing Acetate

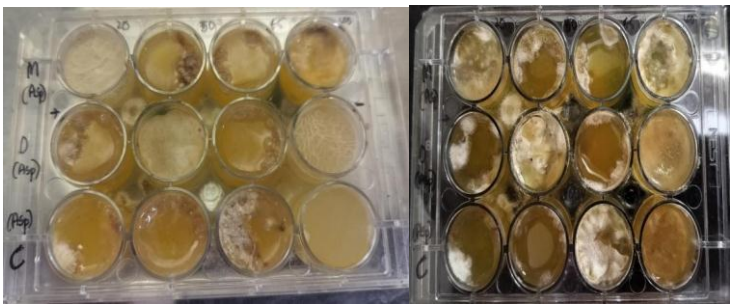

Fig 2.7 Comparative Study of Fungi of Indigofera & Basella

Our results show that *Basella alba* and *Indigofera tirunelvelica* exhibit strong antibacterial and anti-biofilm properties. The extracts shown exceptional efficacy against Candida indicating their potential as alternative therapeutic agents for the treatment of fungal infections that are resistant to drugs. These findings are promising because they raise the prospect of creating plant-based remedies to address illnesses linked to biofilms, which are infamously challenging to cure with traditional antibiotics.

Conclusion

This study demonstrates that *Indigofera tirunelvelica* and *Basella alba* extracts possess significant antimicrobial and anti-biofilm activities against pathogenic bacterial and fungal stains. Candida and Aspergillus were the most sensitive stains, while Bacillus showed the highest susceptibility among bacterial stains. The strong anti-biofilm activity against Candida suggests that these plant extracts could serve as alternative therapeutic agents in combating biofilm-associated infections, particularly those caused by drug-resistant fungi. The plant extracts showed strong antifungal activity, especially against Candida (22 mm zone of inhibition) and Aspergillus (20 mm). Among bacteria, Bacillus (18 mm) was the most affected, while Klebsiella (12 mm) showed the least sensitivity. Gram-positive bacteria (Bacillus, Streptococcus) were more sensitive than Gram-negative bacteria (Klebsiella, E. coli). The extracts reduced biofilm formation in all stains. Candida biofilm was reduced by 75%, while Aspergillus showed 70% inhibition. Among bacteria, Bacillus biofilms were reduced by 65%, while Klebsiella showed the lowest reduction (45%).

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