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Studies on Degradation of Biofilm from Plant Extracts

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Abstract: ***Quorum sensing is a process of cell-cell communication that bacteria use to regulate collective behaviors. It depends on the production, detection and group-wise response to extracellular signal molecules called auto inducers. QS- Dependent regulation of gene expression controls wide variety of phenotypes including biofilm formation, drug resistant, virulence factors expression and motility. Administration of flavonoids to Pseudomonas aeruginosa alters transcription of quorum sensing controlled target promoters and suppresses virulence factor production, confirming their potential as anti-infective that do not function by traditional bactericidal or bacteriostatic mechanism. Various sources from plant i.e. Datura, Papaya, Merigold, Tea, Ganja ,Capsicum and onion were collected and flavonoids were extracted from them using methanol extraction method. Biofilm was produced using MRS media and the extract was inoculated with the biofilm for 4-5 days. Dhatura, Papaya, Marigold, Tea, Ganja were able to degrade the biofilm. Effect of antibiotics were seen on degradation of biofilm, Gudchef and zefu have good tendency to degrade the biofilm..Lactobacillus when inoculated along with the biofilm of P.aeruginosa , it inhibited the growth of the biofilm formation in P.aeruginosa***

Keywords: Biofilm, Quorum Sensing, Plant extract, Papaya, Marigold, Tea, Ganja

1. Introduction

Formation of bio-films hassles numerous infection treatment, importantly, those are medical services related [1]. Regularly used antibiotic targeted at hindering the growth of bacteria, Planktonic cells have not potential to cure the illness related to bio-film. Heterogeneous populations with various growth rates exist in bio-films , lodged in matrix of polymeric substance which is extracellular, communication between the cells works as important role[2].This strategy , called quorum sensing (QS), appears to be essential for the development of biofilm, and has arisen as a popular goal for identifying efficient strategies to control biofilm. Both Gram-positive and Gram-negative microbes utilize QS to regulate gene expression in a way dependent on population density. Nevertheless, in terms of signal types, receptors, and signal transduction, the QS systems differ among species [3,4]. N-acyl-l-homoserine lactone (AHL) signalling biomolecules, named autoinducers (AIs), primarily arbitrate QS in Gram-negative bacteria. In general, AHL-type AIs comprise of a moiety couple of homoserine lactone (HSL) with a varying length and oxidation state of fatty acid[5, 6]. The AHL-dependent QS mechanisms are composed of two LuxR and LuxI family proteins, the previous being essential for the activation of the AIs and the latter for their identification and binding[7]. Provided the species sensitivity and broad-spectrum effect, QS is an obvious prospect for discovery of antibiofilm drugs. QS Systems can be inhibited in various ways, notably by hindering the signaling generator, demeaning the signaling molecule, or blocking the signal receptor [10]. Specifically the enzymatic degradation of the signal molecule is called quorum quenching [11]. Even though QS enforces the expression of multiple virulence variables, it is possible to utilize quorum sensing inhibition (QSIs) to reduce virulence of bacteria. In addition, QSIs may create biofilms extra impervious to antibacterial drugs and immune response of host, thus requiring smaller amounts and narrower treatment options for antibiotics [12, 13]. Pertinently, because QS doesn't really influence the bacterial growth, QSIs are predicted to be less likely to develop tolerance than bactericidal compounds [14]. Natural products were a rich source of innovation for the discovery of antimicrobial drugs, not only as antibiotics but also as contributes in drug design [15]. Compared to traditional antibiotics, natural products have complex structures that may provide a wide range of pathways for action. Different types of QSIs were also recognized from natural sources [16]. Among the phytonutrients, a most researched class of QSIs is halogenated furanones from the marine algae Delisea pulchra[18,19,20]. Additionally, various plant-derived compounds and their substituted derivatives and derivatives, such as tannins[21], cinnamaldehyde from cinnamon[22], horseradish iberin[23], garlic ajoene [24] and rosmarinic acid[25] have been observed to show anti-quorum sensing behavior against both Gram-negative and Gram-positive bacteria.

2. Methodology

Following Samples were collected from the respective places, then samples were washed by tap water then by distilled water and dried under the shed area.

Table 1: Summarization of sample collection from different locations

S. No.	Sample	Scientific name	location
1	Papaya leaves	*Carica papaya*	Vikas nagar Area, Lucknow
2	Datura leaves	*Datura metel*	Shadab Colony, Lucknow
3	Merigold flower	*Tagetes erecta*	Lekhraj panna, Lucknow
4	Ganja leaves	*Cannabis sativa*	Vibhuti khand ,Gomtinagar,Lucknow
5	Red bell pepper	*Capsicum annuum*	Vikas nagar, Lucknow
6	Onion bulb	*Allium cepa*	Aliganj, Lucknow
7	Tea	*Camellia sinensis*	Vikas nagar, Lucknow

Marketed drugs

Table 2: Marketed drugs sample that is utilized to tes antibiofilm activity against given pathogen

S.No.	Antibiotics	*Location*
1.	Oflomac	Local market , Lucknow
2.	Mauxykind	Local market , Lucknow
3.	Zefu	Local market , Lucknow
4.	Goodchef	Local market , Lucknow
5.	Ceptax	Local market , Lucknow
6.	Clavaun	Local market , Lucknow
7.	Oflox	Local market , Lucknow
8.	Lcin500	Local market , Lucknow
9.	Ciplaux	Local market , Lucknow
10.	Merigold oil	MRDlifesciences, Lucknow

Sample preparation from marketed medicine:
All the drugs were crushed and mixed with distilled water and prepared with concentration i.e., 1000mg/ml and collected for their various test.

Pathogen used to form biofilm:
P. aeruginosa and *L. Rhamnosus* these are the pathogens which is provided by MRD Lifesciences, Lucknow, India

Flavonoids extraction:
1gm of Dried papaya leaves, Dhatura leaves, Capsicum, Onion bulb, ganja and marigold petals were collected and individually dipped in 4 different solvent i.e. acetone, methanol, propanol, petroleum ether for overnight. The filtrate part was collected after 24hrs and stored at 40ºC in hot air oven. The extract was dissolved in DMSO and collected in micro centrifuge tubes for antibiotic sensitivity test.

Antibiotic sensitivity test
The idea of the agar's well spreading is similar to that of the agar plate's diffusion test. Standardized inoculum concentration of the given volume is distributed evenly around the plate surface of the gelled agar. A hole with such a sterile cork borer which ranges from 6-8 mm in diameter is drilled aseptically in the middle. A fixed volume of plant extract is then well injected into the bored agar and incubated at optimal temperature and period, depending on the test micro-organism **.**In this study, 20 µL pathogen was distributed on the agar media of nutrients, well prepared, 45 µl extract sample was well fitted and incubated in incubator at 37^0c for 24 hours. Hydrolysis region after incubation was calculated.

BIOFILM FORMATION
MRS media was designed by using Beef extract 10g/l, Peptone 10g/l, Dextrose 20g/l, Yeast extract 5g/l, Na2HPO4 2g/l, Tween 1ml/l, Tri Ammonium Citrate 2g/l, Sodium Acetate 5g/l, MgSO4 0.2g/l, MnSO4 0.2g/l, having pH 6.2-6.6 and transferred 3ml each it into Test Tube and autoclaved it. 20µl of pathogens (*P. Aeruginosa and L. rhamnosus*) was inoculated under sterile condition and incubated at 37°C for 1 week.

Biofilm staining
Biofilm formed was initially washed with phosphate buffer saline (PBS). After that crystal violate is added to the biofilm and kept for 1min. then it was washed with PBS, and kept for drying.

Biofilm treatment:
With acetonic extract: - Extract was prepared by dipping 2g of dried sample and added to 5ml of solvents and added in equal volume and incubated at 37°C. With various medicines Antibiotics, antipyretics and analgesics used are ciprofloxacin, amoxicillin, azithromycin, antipyretics, ibuprofen, paracetamol, naproxen, aspirin, diclofenac and aceclofenac, The medicinal extract was prepared after crushing the tablets and made a fine powder of it and mixed with 1ml distilled water. And added to equal volume to the biofilm and incubated at 37°Cat shaker incubator.

Treatment of biofilm with *Lactobacillus* strain
The bacterial strain was isolated and identified using various biochemical tests i.e., gram staining, catalase test, endospore staining, mannitol test, using Bergey's Manual which was showing antimicrobial activity against and L.rhamnosus. Which was further inoculated in equal proportion to the biofilm and incubated at 37°C in shaker incubator for 12 hrs. Biofilm was prepared, stained and washed with PBS buffer. Samples were prepared in a Test tube,Poured it into the Test tube containing biofilm so that the biofilm gets dipped. Incubated it at 37°C
For 24hrs and check the activity.

Treatment of Biofilm with the Flavonoids Extracted
Biofilm was made using MRS media, inoculated with *Pseudomonas aeruginosa* and Incubated for 3 to 4 days. The biofilm formed was washed with phosphate buffer saline and stained with crystalviolet.The flavonoids extracted were poured into the stained biofilm test tubes and kept forincubation in dark for about 3-4 days.

To Check Flavonoids Effect on Quorum Sensing Inhibition
Biofilm was made using MRS media, inoculated with *Pseudomonas aeruginosa*. Equal volume of the extract was mixed with it and incubated at 37^0C in rotary shaker Incubator for 3-4 days.

Treatment of Biofilm With *Lactobacillus* strain
Lactobacillus was isolated from raw milk of cow by spreading and pure culture was obtained by streaking .Nutrient broth was prepared and inoculated with *lactobacillus* and incubated for 24 hrs. *Lactobacillus* broth was collected and poured into biofilm at regular intervals to checkits effect on biofilm formation and degradation.

Phytochemical Analysis of Extracts
Different-different activities possessed by plants in due presence of certain bioactive. Components on the secondary metabolites. Trease and Evans *et al*., 1989 gave a standard procedure for identification these Secondary metabolites.

3. Result

Antibiogram of different plant extracts against *A uriginousa.*

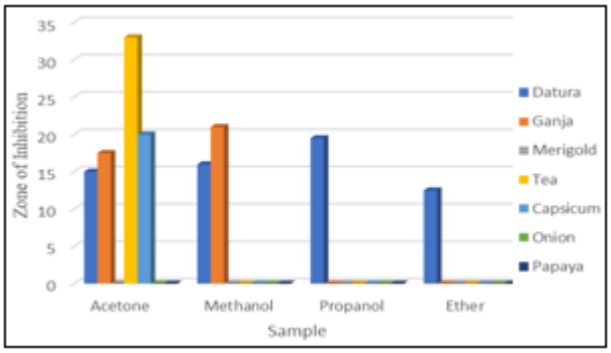

Figure 1: Antibacterial analysis of various plant samples against Pseudomonas aeruginosa. Tea extract from acetone showed maximum zone of hydrolysis

Production of biofilm

A prominent layer of Biofilm was formed after one week of incubation. White colour ring indicates biofilm formed.

Figure 2: Biofilm formation

Staining of Biofilm:

Biofilm was stained using crystal violet and kept for drying. Purple color ring indicates stained biofilm.

Figure 3: Biofilm Staining

Treatment of Biofilm with the flavonoids extracted

Extracts of Ganja, Datura , Merigold and Tea were able to successfully degrade biofilm. A comparative study shows that the biofilm on the left of the image was being degraded by the following sample which is shown on the right of the image.

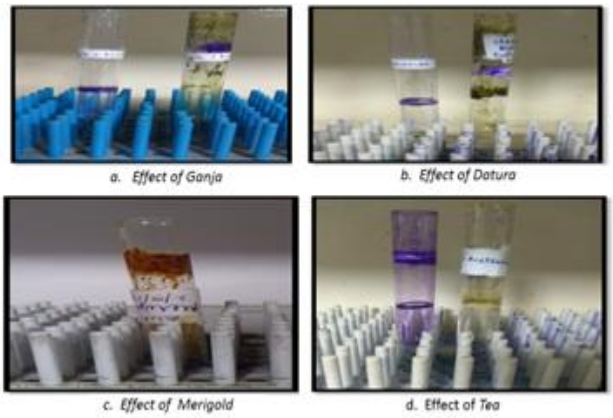

Figure 4: Effects of selected Plant's extract were seen on degradation of biofilm. Extract of Ganja and tea showed high potential for biofilm degradation

Treatment of Biofilm with Various Antibiotics

Gudchef and zefu were able to completely degrade the biofilm. The image below depicts a control on right and effect of the antibiotic on the biofilm on left. Antibiotics Gudchef and zefu were successfully able to degrade the biofilm, hence no purple colour stained ring is seen on the left.

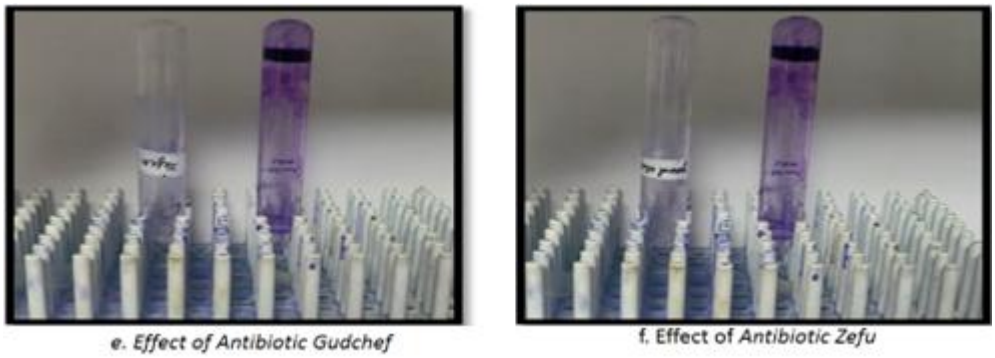

Figure 5: Effect of antibiotics were seen on degradation of biofilm, figure illustrated that Gudchef and zefu have good tendency to degrade the biofilm.

Treatment of Biofilm with *Lactobacillus* strain

Lactobacillus inoculated with already formed biofilm Formation of double layer of purple colour indicates that *Lactobacillus instead* of degrading the biofilm formed by *P.aeruginosa*, contributed in the formationof another layer of biofilm which can be depicted in the image given below.

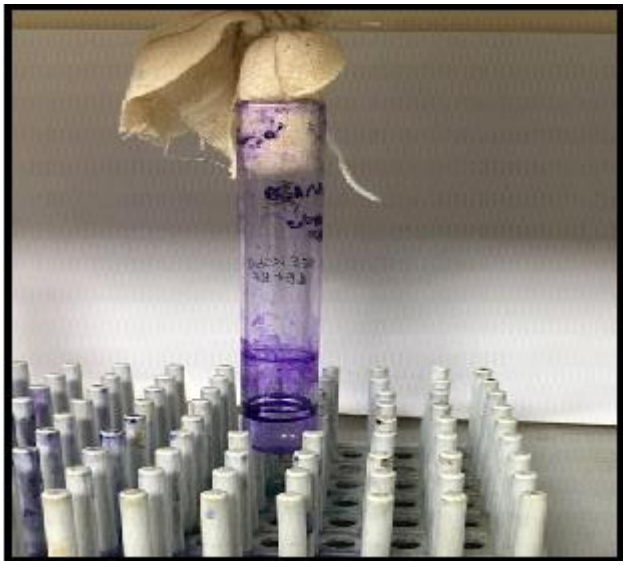

Figure 6: Lactobacillus stained, upper ring is formed by biofilm of lactobacillus.

Lactobacillus inoculated along with biofilm .Lactobacillus when inoculated along with the biofilm of *P.aeruginosa* , it inhibited the growth of the biofilm formation in *P.aeruginosa.*

Figure 7: No biofilm formation was seen

Phytochemical Analysis of Extracts

Table 3: Summary of Phytochemical analysis of various samples

S.No.	Sample	*Phtochemical analysis*							
		Terpenoid	flavonoid	Phlobatanin	Tannin	Leucoanthocyanin	coumarin	steroid	Fatty acid
1.	**Datura**	**+ve**	**+ve**	**+ve**	**-ve**	**-ve**	**+ve**	**-ve**	**-ve**
2.	**Ganja**	**+ve**	**+ve**	**+ve**	**-ve**	**-ve**	**-ve**	**+ve**	**-ve**
3.	**Merigold**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**	**-ve**
4.	**Tea**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**	**+ve**	**-ve**

4. Conclusion

Dhatura, Papaya, Marigold, Tea, Ganja are a well-known medicinal plant due to its high content of phytochemicals and secondary metabolites. It is broadly employed as alternative drug for human health. The present basic analysis has reported the biofilm inhibition potential of Dhatura, Papaya, Marigold, Tea, and Ganja against the biofilm forming *P. aeruginosa* and *L. Rhamnosus*at minimum concentrations. Further analysis are required to detect the mechanism indulged in the ant biofilm property of the Dhatura, Papaya, Marigold, Tea, Ganja extracts at the molecular level and evaluate their biological functions for their safety in developing an herbal product.

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