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INVESTIGATION OF ANTI-SWARMING, ANTI-BIOFILM AND ANTI-QUORUM SENSING POTENTIAL OF SOME ORGANIC TEA OF DIFFERENT BRANDS FROM INDIA

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Keywords:

Anti-quorum sensing, Biofilm disruption, Anti-swarming, Tea extract, Bacterial communication

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ABSTRACT: The emergency to find a solution to multidrug resistance of bacteria due to the abuse of antibiotics leads to the search for new antibacterial pathways. Quorum sensing (QS), or bacterial cell to cell communication, is a cell-density-dependent bacterial response. It is mediated by hormone-like compounds called autoinducers (AIs). QS-dependent regulation of gene expression controls various phenotypes, including bioluminescence, biofilm formation, drug resistance, virulence factors expression, and motility. Therefore, the inhibition of QS is considered a new promising target of antimicrobial pathways as anti-virulence compounds that can repress the gene expression of QS. Green tea is one of the most commonly consumed teas in the world. Green tea extract is also a great source of compounds like polyphenols, amino acids, enzymes, pigments, and carbohydrates. Hence, green tea has recently received considerable attention as a new source of safe and effective QS inhibitory substances. In the present study, methanolic and ethanolic various tea extracted were prepared and tested to inhibit quorum sensing mediated bacterial virulence factors such as anti-swarming, and antibiofilm potential against organism P*seudomonas aeruginosa*. Quorum sensing inhibition against *Chromobacterium violaceum MTCC* 2656 has been carried out. The study's outcome shows that most of the methanolic and ethanolic extracts of various tea products show significantly Anti-quorum sensing Potential up to 83% swarming inhibition with P*seudomonas aeruginosa*, inhibition *violaceum pingment* and biofilm disruption were also quite high in most of the Tea extract of ethanol and methanol.

INTRODUCTION: The problem of multidrug-resistant bacteria is becoming a worldwide concern nowadays. This pathogenic organism became resistant to a large range of antibiotics that possess a problem with treating various diseases [1]. Antibacterial drugs are the foremost effective of all medicines. Their success is reflected by their continuous use and thus the decreased morbidity and mortality from bacterial infections over the past 50 years.

However, the continued emergence and spread of multidrug-resistant bacteria have predicted that we are re-entering the predominant era [2]. The event of antibiotic resistance is the key reason for the development of pathogenicity. The microorganism dwelling within the biofilm often develops antibiotic resistance against common disinfectants and antiseptics that are regularly used [3]. So to beat the rapid spread of multidrug resistance, the event

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of recent antimicrobial or antipathogenic agents that influence new microbial targets has become an extremely pressing priority.

Because of the proven fact that quorum sensing is involved in microbial pathogenesis, research efforts have recently focused on developing antipathogenic agents to manage bacterial diseases by inhibiting quorum sensing [4].

Quorum sensing is the ability to detect and reply to cell population density by gene regulation and intracellular communication between bacteria using bacterial products. This population density-dependent mechanism is mediated through small signaling molecules called autoinducers by which bacteria regulate gene expression in gram-negative bacteria; the autoinducers are AHLs that are acyl-homoserine lactone [5].

Quorum sensing enables bacteria to limit the expression of specific genes to high cell densities at which the resulting phenotypes are visiting be most beneficial. QS-dependent regulation of gene expression controls many phenotypes, including bioluminescence, biofilm formation, drug resistance, virulence factors expression, and motility.

Therefore, the inhibition of QS is considered a fresh, promising target of antimicrobial pathways as anti-virulence compounds that could repress the gene expression essential for basic metabolism in vitro, rather than the microorganisms themselves [6].

Quorum sensing might be a good drug target because, unlike antibiotics and biocides that kill bacteria, interfering with signaling system or cell to cell communication relies on the principle that when one bacterium releases autoinducers into the environment, their concentration is just too low to detect, hence when sufficient bacteria present concentration of autoinducers reach threshold level that permits the bacteria to sense critical cell population and to activate target genes [7].

In Gram-negative bacteria, the foremost common autoinducers are N-acyl-homoserine lactones (AHLs), a signalling molecule in higher concentrations that can bind to and activate a transcriptional activator or R protein which successively induces the expression of target genes. Whereas Gram-positive bacteria usually utilize peptides as autoinducers [8].

In recent years, Green tea is becoming increasingly popular worldwide, partly because of many documented evidence about its beneficial effects on health.

Tea contains numerous components, including catechins, caffeine, amino acids, carbohydrates, proteins, chlorophyll, volatile compounds, fluoride, minerals, and other undefined compounds. Several biological properties are associated with tea polyphenols (TP); tea extract can modulate the Quorum sensing [9].

We hypothesize that a variety of its antimicrobial properties is additionally contributed by the QSI phytochemicals present in it.

Therefore, within the current study, we aimed to research the anti-quorum sensing and anti-biofilm potentials of herb polyphenol extract against *Chromobacterium violaceum* CV026 and *Pseudomonas aeruginosa* at sub-inhibitory concentration.

Furthermore, the influence of tea extract on *P. aeruginosa* quorum-sensing- regulated virulence factors production, motility, and biofilm formation were also assayed [10].

MATERIALS AND METHODS:

Chemicals, Bacterial Strains and Culture Medium: All the chemicals and media were used for carrying out this study purchased from High-media Pvt.Ltd and Sigma Aldrich. *Chromobacterium violaceum MTCC* 2656, a pathogenic strain, was procured from MTCC Chandīgarh, India.

Pseudomonas aeruginosa with a trait of swarming motility isolated from soil collected from the campus of Dr. Ambedkar College Deekshabhoomi Nagpur and isolates were maintained on Nutrient Agar medium slant at 2-8°C.

Tea Samples Collection: Organic tea and various brands of tea were purchased from nearby local markets, twelve tea samples were purchased from local market of Nagpur city. India. Which are listed in **Table 1**. The tested tea samples are listed below.

TABLE 1: THE TESTED TEA SAMPLES AND STOCK AND WORKING CONCENTRATION OF TEA EXTRACT

S. no.	Name of extract	Stock Concentration of methanol Extract mg/ml	Stock Concentration of ethanol Extract mg/ml	Concentration of Working methanol & ethanol extract mg/ul
1	Lipton	700	650	1.0
2	Himalaya wellness tea	630	600	1.0
3	Organic India	890	720	1.0
4	24 Mantra Organic Green Tea	820	870	1.0
5	LaPlant Green Tea	670	630	1.0
6	Twinings Green Tea	540	590	1.0
7	Tetley	810	760	1.0
8	Taaza	760	700	1.0
9	Tulsi Green Tea	680	570	1.0
10	TajMahal Green Tea	530	780	1.0
11	Tata tea	520	610	1.0
12	The Indian Tea	660	530	1.0

Preparation of Tea Extract: A modification of previously described procedures (Raaman, 2006), was used to prepare the Methanolic and ethanolic extract of the tea Product. The dried Tea materials (2 gm) were mixed with 20 ml 70% (v/v) ethanol and 20 ml Methanol separately for 8 h in a Soxhlet extractor. The extract was then allowed to evaporate in an oven at 37°C for at least three days. Some of the extracts were dried using IR Concentrator. The dried extract was stored in a refrigerator until used (extracts were tested within 2 weeks of extraction).

Preparation of Stock Solutions: Stock solutions of crude ethanol and methanol extracts were prepared, filter-sterilized (0.25 µm), and stored at 4°C. **Table 1**. Shows the concentration of each stock solutions concentrations working solution.

Swarming Inhibition Assay: Swarming Inhibition assay was conducted with a nutrient medium containing 0.8% agar. Swarm plates were allowed to dry in the incubator before being used. Prepared Swarm plates were then divided into four regions by using a marker and inoculated with the overnight grown bacterial culture of *Pseudomonas aeruginosa* in Nutrient broth. 0.5 µl of culture inoculated over 1µl of tea extract and allowed to absorb sample drop in the medium. These plates were then incubated for 48 h at 37°C. Inhibition in the swarming motility was determined by measuring the diameters of the swarm zones compared to the negative control.

Quorum Sensing Inhibition Assay using *Chromobacterium violacein* CV026: A paper disc diffusion assay was performed to test for Quorum sensing inhibition using. *Chromobacterium violaceum MTCC* 2656. 100 microliter of the. *Chromobacterium violaceum MTCC* 2656 overnight culture grown was spread on agar plates and allowed it to dry. Paper discs were dipped into the extracts and placed on the culture-grown plates. The plates were incubated at 37°C for 24 hrs; then, plates were observed for any pigment inhibition zones and examined for violacein production. Quorum sensing inhibition was detected by a colourless, opaque, but viable halo around the disc. Methanol and ethanol were also used as a negative control [10].

Biofilm Inhibition Assay using Tube Method: In this assay 100 µL of bacterial culture *Pseudomonas aeruginosa* were transferred to glass test tubes containing 5 mL Nutrient Broth and 150 µl of tea extract, and tubes were incubated at 37°C for 24 h. Three controlled tubes were also prepared, one containing culture media only and the other two of ethanol and methanol as a negative control. The media was then removed, and the tubes were washed with distilled water. The tubes are stained with crystal violet and rinsed twice to discharge the extra stain and air-dried. The occurrence of the blue ring above on the wall at the bottom of the tube indicates biofilm production. Two control tubes of ethanol and methanol were also taken for comparison[11].

RESULTS:

Anti-swarming Assay: Ethanol and methanol extract of various Tea extracts were tested for the ability to inhibit swarming in *Pseudomonas aerogenosa*.

The result of this assay was interpreted in a way that is the reduction in the diameter of the swarm zones as compared to control. Data represented in **Table 2** shows no significant variations in ethanolic and methanolic extract, but the Highest Swarming inhibition was found in methanolic and ethanolic extract of Lipton. The size of swarm zones of *Pseudomonas aerogenosa* was significantly reduced in the presence of these extracts as compared to control. In some extracts pattern of motility was found to be different. All the Tea brands significantly effective as an Anti-swarming Agent for the tested organism *Pseudomonas aerogenosa*

TABLE 2: RESULT OF ANTI-SWARMING POTENTIAL OF VARIOUS TEA EXTRACTS

S. no.	Tea Extracts	Diameter of Swarm zone in cm	
		Methanol (% of Swarming inhibition)	Ethanol (% of Swarming inhibition)
	Negative control	1.6	1.8
1	Lipton	0.3 (81.25%)	0.3 (83.33 %)
2	Himalaya wellness tea	0.4 (75%)	0.4(77.77 %)
3	Organic India	0.3 (81.25%)	0.6 (66.66 %)
4	24 Mantra Organic Green Tea	0.5 (68.75)	0.5 (72.22%)
5	LaPlant Green Tea	0.6 (62.5 %)	0.4(77.77%)
6	Twinings Green Tea	0.3 (81.25 %)	0.4(77.77%)
7	Tetley	0.5 (68.75 %)	0.5 (72.22%)
8	Taaza	0.9 (43.75)	1.3 (27.77%)
9	Tulsi Green Tea	0.5 (68.75%)	0.4(77.77%)
10	TajMahal Green Tea	0.5 (68.75%)	0.4(77.77%)
11	Tata tea	0.4 (75%)	0.5 (72.22%)
12	The Indian Tea	0.4 (75%)	0.5 (72.22%)
		The values of swarm zones presented as Mean	

Anti-quorum Sensing Bioassay using *Chromobacterium violaceum MTCC* 2656: The pigment production is one of the phenomena controlled by quorum sensing regulatory mechanism in *Chromobacterium violaceum MTCC* 2656. The paper disc diffusion assay was performed for pigment inhibition in which various Tea extracts tested for only the pigment inhibition and no growth inhibition. The area of colorless turbid zone indicates that the extract is allowing inhibition of the quorum sensing mechanism in the organism. Data of Pigment Production inhibition in *Chromobacterium violaceum MTCC* 2656 presented in **Table 3.**

TABLE 3: PIGMENT PRODUCTION INHIBITION IN *CHROMOBACTERIUM VIOLACEUM* MTCC 2656

S. no.	Sample	Methanol Extract	Ethanol extract
1	Lipton	+++	++
2	Himalaya wellness tea	-ve	+++
3	Organic India	+++	+
4	24 Mantra Organic Green Tea	+++	+++
5	LaPlant Green Tea	+	++
6	Twinings Green Tea	++	+
7	Tetley	-ve	-ve
8	Taaza	-ve	-ve
9	Tulsi Green Tea	+++	+++
10	TajMahal Green Tea	+	+
11	Tata tea	-ve	+++
12	The Indian Tea	++	+++

+++ High, ++ Moderate, + Least

Biofilm Disruption Assay by Tube Method: *P. aeruginosa* shows strong biofilm formation; the different Tea extracts were screened for their biofilm disruption Potential against *P. aeruginosa*. The crystal violates staining method is easily and widely used to measure biofilms' formation and inhibition in a tube. The results of this assay are presented in **Table 4**. All most all the methanol and ethanol extract were showed significant Biofilm reduction as compared to negative control except for ethanol and methanol extract of Taza

TABLE 4: RESULT OF BIOFILM DISRUPTION ASSAY BY TUBE METHOD

S. no.	Sample	Methanol Extract	Ethanol extract
1	Lipton	+++	++
2	Himalaya wellness tea	++	+++
3	Organic India	+++	++
4	24 Mantra Organic Green Tea	+++	++
5	LaPlant Green Tea	+++	++
6	Twinings Green Tea	++	+
7	Tetley	+	+
8	Taaza	-ve	-ve
9	Tulsi Green Tea	++	+++
10	TajMahal Green Tea	+++	+++
11	Tata tea	+	+
12	The Indian Tea	++	+++

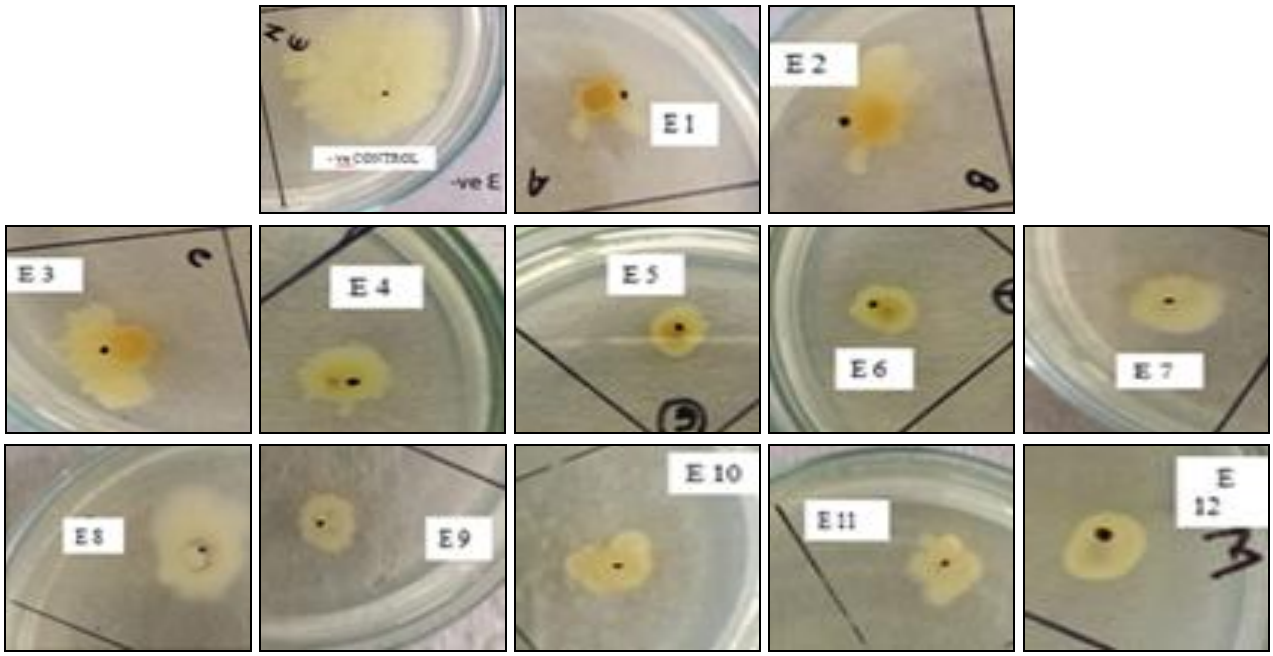

FIG. 1: THE PHOTOGRAPH OF SWARMING INHIBITION ASSAY BY ETHANOLIC TEA EXTRACTS USING *PSEUDOMONAS AERUGINOSA*

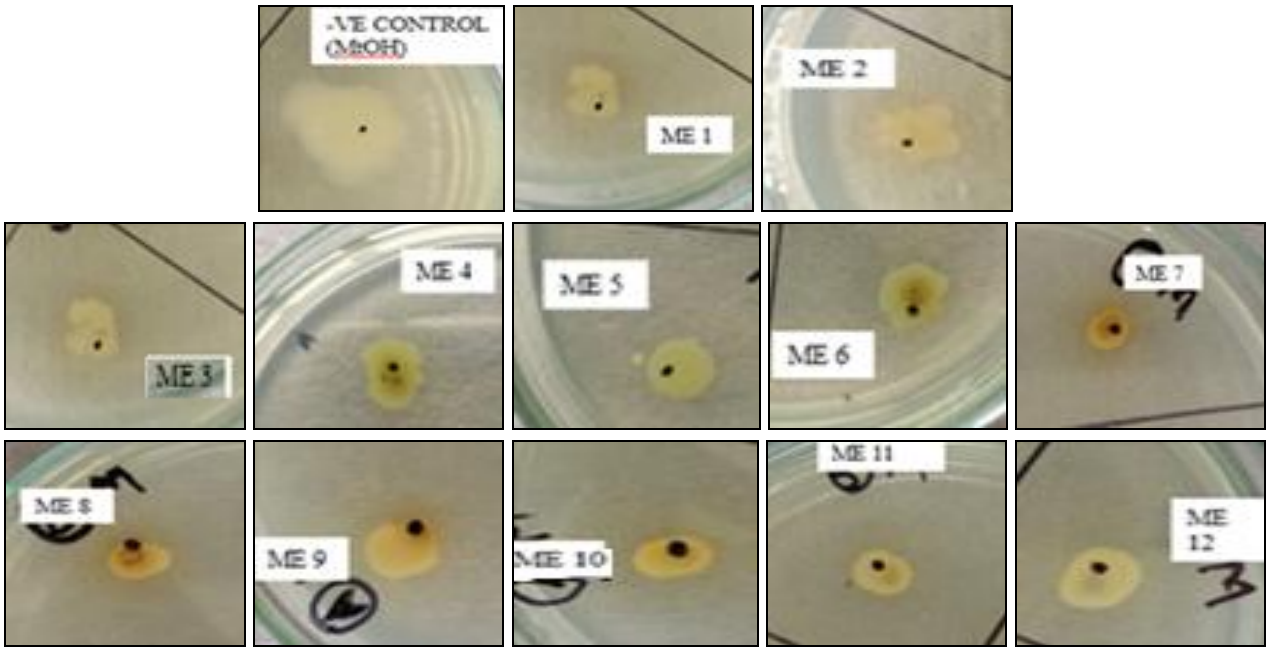

FIG. 2: THE PHOTOGRAPH OF SWARMING INHIBITION ASSAY BY MTHANOLIC TEA EXTRACTS USING *PSEUDOMONAS AERUGINOSA*

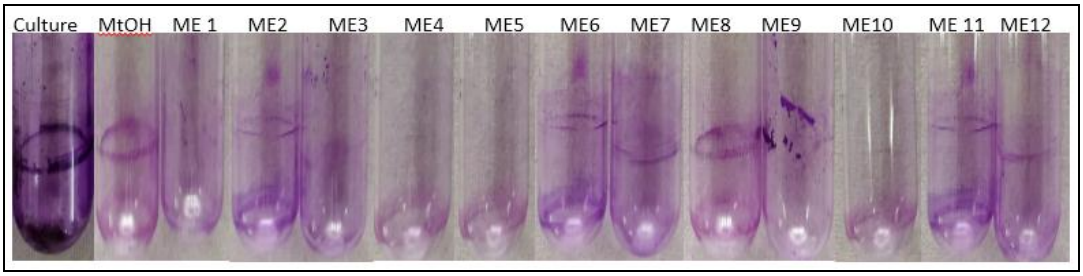

FIG. 3: RESULT OF BIOFILM DISRUPTION ASSAY USING *PSEUDOMONAS AERUGINOSA* BY METHANOLIC EXTRACT OF DIFFERENT TEA

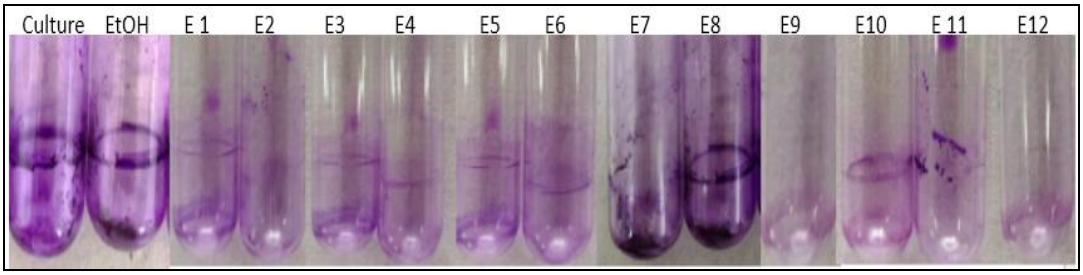

FIG. 4: RESULT OF BIOFILM DISRUPTION ASSAY USING *PSEUDOMONAS AERUGINOSA* BY ETHANOLIC EXTRACT OF DIFFERENT TEA

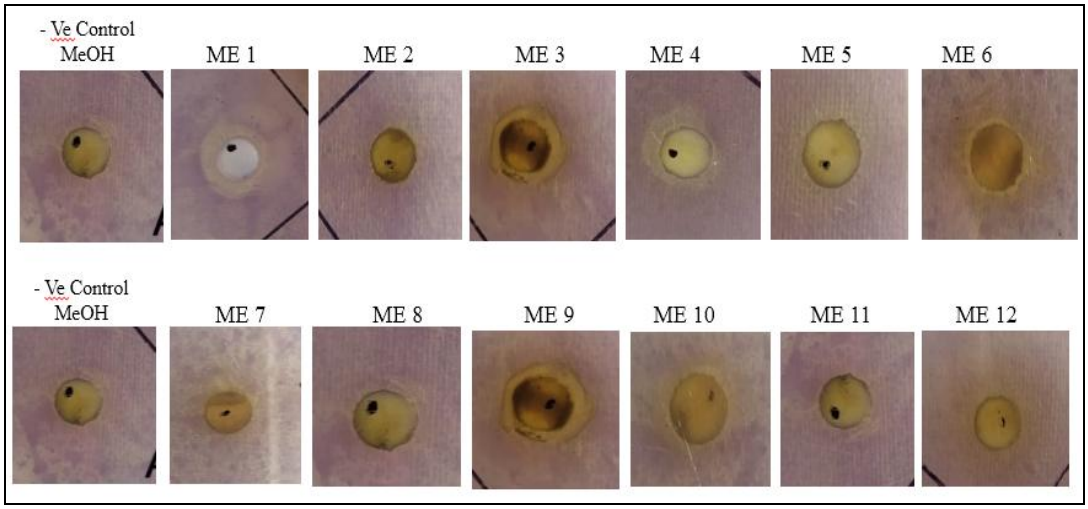

FIG. 5: RESULT OF QUORUM SENSING INHIBITION ASSAY USING *CHROMOBACTERIUM VIOLACEUM* MTCC 2656. BY METHANOLIC EXTRACT OF DIFFERENT TEA PRODUCT

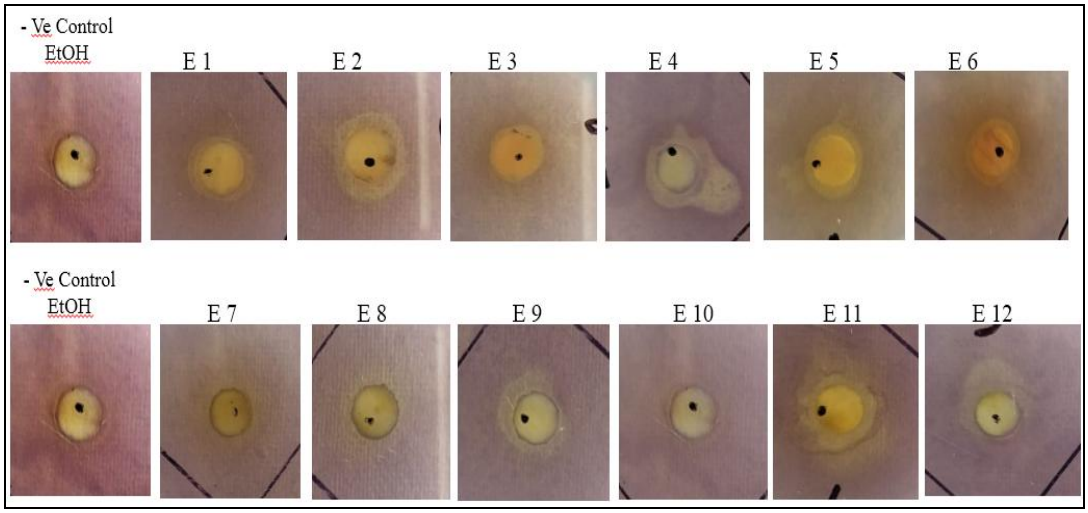

FIG. 6: RESULT OF QUORUM SENSING INHIBITION ASSAY USING *CHROMOBACTERIUM VIOLACEUM* MTCC 2656. BY ETHANOLIC EXTRACT OF DIFFERENT TEA PRODUCT

DISCUSSION: Green tea is one of the most popular beverages worldwide due to its health-promoting properties, which reflect the presence of antioxidants and also antimicrobial substances that are active against various Gram-positive and Gram-negative bacteria [12-16]. Both activities are mainly due to polyphenols, the most abundant of which is the catechins, particularly epigallocatechin gallate (EGCG), representing 50–80% of the total catechin content [17]. A deeper understanding of Bacterial cell-cell communication promises to shed light on the complexities of the host-microbe relationship and may lead to novel therapeutic applications. Many species of bacteria use quorum sensing to coordinate gene expression according to the density of the local population. Hence in the present study anti-quorum sensing activities of the twelve organic tea of various brands has been studied for their inhibition of Quorum sensing, controlled phenotype Swarming and biofilm formation of *Pseudomonas aeruginosa* and pigment production *Chromobacterium violaceum MTCC* 2656. The tea extract effectively inhibited Quorum sensing and controlled phenotypes of both tested organisms. This study's findings support other investigations that demonstrated anti-QS activity in certain Tea extracts. Further works are required to find active QSI ingredients of tea extracts and to clarify the mechanism of their action as a Quorum sensing inhibitor. A few tea extract showed negative result may be due to insufficient active ingredient concentration in that extract for inhibition of respective Quorum sensing controlled Phenotypes.

CONCLUSION: It is concluded that anti-QS is as important as antibacterial activity as it will unlikely cause resistance problems as it does not pose selection pressure. Anti-swarming, Biofilm, and QS are major virulence factors of most pathogenic microorganisms. The bioassay of the 12-studied tea extracts revealed significant Quorum sensing controlled bacterial phenotype inhibition activities. Further research is needed to investigate the molecular mechanism and active ingredient in each tea extract. Biofilm-associated bacterial infections frequently caused by *P. aeruginosa* are found in most diseases. The effectiveness of many antibacterial drugs has been lost due to the evolution of pathogenic resistance. Therefore alternative ways of reducing biofilms are essential. The anti-QS compounds present in green tea products help enhance the susceptibility of the bacteria and thus eradicate biofilms. Green tea may therefore offer a new source of antimicrobial compounds that can be used to reduce our reliance on antibiotics by inhibiting bacterial virulence without selecting for resistant bacterial strains [18-20].

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