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Studies on Quorum Sensing Inhibitory Properties of Floral Extracts of Selected *Musa x paradisiaca* L. Varieties

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***Abstract*- Bacterial cells communicate with each other through chemical signal molecules called autoinducers through the phenomenon known as quorum sensing. This property of bacterial cells enables the regulation of several activities including antibiotic production and resistance, competency, virulence and symbiosis. One of the major challenges faced in the pharmaceutical industry is antibiotic resistance. Hence, investigation of anti-quorum sensing agents from naturally available sources is crucial. The present study focuses on inhibition of quorum sensing in *Pseudomonas aeruginosa* using floral extracts of five *Musa x paradisiaca* L., varieties. This is the first report on quorum sensing inhibitory properties of the above-mentioned plants and envisages the potential for analysis on other bacteria known for quorum sensing.**

***Keywords- Pseudomonas aeruginosa*, quorum sensing, quorum quenching, *Musa x paradisiaca* L., phytochemical analysis, biofilm, pyocyanin, swarming**

I. INTRODUCTION

One of the fundamental phenomena exhibited by bacteria is quorum sensing, a cellular communication process that allows millions of cells to communicate through chemical signal molecules known as autoinducers. This phenomenon allows bacteria to regulate various activities such as antibiotic production and resistance, competency, virulence and symbiosis through regulatory proteins [1].

Traditionally, bacterial infections have been treated using antibiotics [2]. However, their limitations ranging from restricted effectiveness to multidrug resistance, thought to arise from overuse [3], have allowed researchers to focus on antimicrobial agents with higher efficacy and lower side effects, such as plant extracts [4-5]. Secondary metabolites with significant biochemical activity have been observed to be present in most plants [6]. Due to their cytotoxicity towards microbial pathogens, it has been found that plant extracts of medical significance can eliminate the virulent nature of pathogenic bacteria by inhibiting autoinducers.

The present study explores the effects of five varieties of *Musa x paradisiaca* L. (Musaceae), namely puttubale (MPP), bettubale (MPB), karibale (MPK), kattubale (MPKa) and nendrabale (MPN), in blocking quorum sensing. *Musa x paradisiaca* L., is an annual plant known to contain bioactive compounds that have pharmacological effects [7]. Antidiarrheal activity [8], hypoglycaemic activity [9], hypocholesterolaemic activity [10], antioxidant activity [11], wound healing [12], anti-allergic activity [13], antimalarial activity [14] and anti-bacterial activity [15] are exhibited by its flowers.

Formation of biofilm, production of pyocyanin and swarming motility by *P. aeruginosa* are the result of quorum sensing [16], which has been found to be in association with the bacteria's pathogenesis [17]. In this work, *Musa x paradisiaca* L., floral extracts were screened for their quorum quenching properties on *P. aeruginosa*.

II. METHODOLOGY

A. Plant Material and Extraction

Flowers of five cultivars of *Musa x paradisiaca* L., were collected from Kabbinale, India. After separation from the bract, the flowers were surface sterilized by dipping in 70% ethyl alcohol for 30 seconds followed by five to six washes using distilled water. An aqueous extract was prepared by

homogenizing 10g of flowers in 10mL of distilled water using a mortar and pestle. After filtration, the extracts were stored at 4°C until further analysis [18].

B. Bacterial Strain

Pure culture of *Pseudomonas aeruginosa* (NCIM Accession No: 5591) was procured from the National Chemical Laboratory, Pune, India. The culture was maintained on Luria-Bertani agar slants.

C. Phytochemical Analysis of Musa x paradisiaca L., *Floral Extracts*

The screening of *Musa x paradisiaca* L., floral extracts for phytochemical constituents was carried out using methods described by Manjulika et al., [19], with slight modifications. These phytochemical tests are described in Table 1.

D. Estimation of Total Phenolics by FCR method

Total phenolic content was estimated using Folin-Ciocalteau method, described by Cindric et al. with modifications [20]. 0.5mL of extract was taken in different test tubes, to which 0.5mL of Folin-Ciocalteau reagent (FCR) and 5mL of distilled water were added. The contents of the test tubes were mixed well and 1.5mL of Na_2CO_3 solution (w=20%) was added to each tube. The volume was made up to 10mL using distilled water. The blank contained 0.5mL of FCR, 5mL of distilled water and 1.5mL of 20% Na_2CO_3 solution. After one hour of incubation, the absorbance was measured at 765 nm. A standard curve of prepared gallic acid solutions was used to estimate the total phenolic content of the different extracts.

E. Estimation of Antioxidant Activity by DPPH Assay

The antioxidant activity of the extracts was found using DPPH assay as described by Rajesh and Natvar [21]. 50ml of all extracts were taken in different test tubes, to which 100µL of methanol was added to make the final volume up to 150µL. For the blank, only 100µL of methanol was taken. To all of the tubes, 3 mL of methanol was added, followed by 150µL of DPPH (1, 1-Diphenyl –2-picrylhydrazyl). For the control, 150µL of methanol and 150µL of DPPH were taken. After incubating at room temperature for 15 minutes, the absorbance was measured at 517 nm. A standard curve of prepared ascorbic acid solutions was used to estimate the total antioxidant capacities of the different extracts. The percentage of scavenging was calculated using Eq. (1).

$$\% \text{ scavenging activity} = \left[1 - \frac{(\text{absorbance of test sample})}{(\text{absorbance of control})} \right] \times 100 \quad (1)$$

F. Estimation of Glucose by Dinitro Salicylic Acid Method

Total glucose content was found by DNS method [22]. 1mL of the extracts were taken, to which 1mL of distilled water and 2mL of DNS reagent were added. The blank contained 2mL of distilled water and 2mL of DNS reagent. All of the tubes were incubated in a boiling water bath for 10 minutes. The absorbance was measured at 540 nm after 6mL of distilled water was added to each tube.

TABLE I

Preliminary Phytochemical Tests for *Musa x paradisiaca* L., Extracts

Phytoconstituents	**Test**	**Observation**
Alkaloids	1mL of extract + few drops of Wagner's reagent	Reddish brown precipitate
Glycosides	1mL of extract + 2mL of CH_3COOH + 2mL of $CHCl_3$ + few drops of H_2SO_4 (conc.)	Blue to green colouration
Flavonoids	1mL of extract + few drops of $Pb(OAc)_4$	Yellow precipitate
Tannins	1mL of extract + 3-4 drops of $FeCl_3$	Blue to black colouration
Diterpenes	1mL of extract + 3-4 drops of $Cu(OAc)_2$	Emerald green colouration
Triterpenes	1mL of extract + $CHCl_3$, filter filtrate + few drops of H_2SO_4 (conc.) + shake	Golden yellow colouration
Saponins	1mL of extract + 1 ml of distilled water + shake	Foam persisting after 10 minutes

A standard curve of prepared glucose solutions was used to estimate the total glucose content of the different extracts and the results were given in terms of µg glucose equivalents / mL of extracted compound.

G. Estimation of Biofilm Formation

Biofilm formation by *P. aeruginosa* was estimated using tube assay method [23]. Sterilized test tubes containing 5mL of fresh Luria-Bertani broth with and without 500µL of extract were inoculated with 500µL of culture grown overnight and incubated for 18-20 hours at 37^0C. Free unbound cells were discarded and tubes were washed with distilled water five to six times. The adherent biofilm was stained with crystal violet dye (65%) for 10 minutes and washed with distilled water. The test tubes were air dried and biofilm was observed. Subsequently, the crystal violet stain was solubilized in ethanol (95%) and biofilm formation was estimated at 620 nm.

H. Estimation of Pyocyanin Production

To estimate pyocyanin production, sterilized test tubes containing 5mL of fresh King A broth with and without 500 µL of extract were inoculated with 500µL of *P. aeruginosa* culture grown overnight and incubated for 18-20 hours at 37^0C [24]. Pyocyanin was extracted using 3mL of chloroform. To the chloroform extract, 1mL of 0.2N HCl was added to get a pink colour and the production of pyocyanin was estimated at 520nm. (OD*17.072) [25].

I. Assay for Swarming Motility

Swarm plates were prepared using 0.65% agar, 0.5% peptone, 0.2% yeast extract and 1% glucose. *P. aeruginosa* culture was inoculated in triplicate on swarming media with and without 500 µL of extract and incubated for 18-20 hours at 37^0C [26]. Swarming movement was observed and diameter was measured.

III. RESULTS AND DISCUSSION

A. Phytochemical Analysis

Phytochemical screening of the *Musa x paradisiaca* L., floral extracts revealed the presence of a wide range of secondary metabolites. Flavonoids, alkaloids, glycosides and triterpenes were found to be present in all five varieties of flowers, with the latter three exhibiting copious presence. Saponins and diterpenes were present in all varieties besides MPB, whereas tannins were present in all varieties besides MPB and MPN.

B. Estimation of Total Phenicol Content

The total phenolic content in the samples determined by Folin-Ciocalteau method are presented

TABLE II

Results of Phytochemical Analysis of *Musa x paradisiaca* L., Extracts

+++ = Copiously present; ++ = moderately present; + = slightly present; - = absent

Phytoconstituents	**MPP**	**MPB**	**MPK**	**MPKa**	**MPN**
Alkaloids	+++	+++	+++	+++	+++
Glycosides	+++	+++	+++	+++	+++
Flavonoids	+	+++	+	+	+
Tannins	+	-	++	+	-
Diterpenes	++	-	+++	+++	+++
Triterpenes	+++	+++	+++	+++	+++
Saponins	++	-	++	+	+

in Table III. Phenolic content normally correlates to the physiological function of the compounds as antioxidants [27]. MPP was found to have the highest phenolic content among the *Musa x paradisiaca* L., extracts studied.

TABLE III
Total Phenolic Content in Samples

Sample	Yield (µg/mL)
MPP	49.50
MPB	34.50
MPK	39.50
MPKa	17.83
MPN	36.72

C. Estimation of Antioxidant Activity

The percentage of scavenging activity determined by DPPH assay indicated the presence of antioxidants in the *Musa x paradisiaca* L., extracts, and hence their ability to be used as effective medicines against bacterial infections. However, the levels were observed to be relatively low.

TABLE IV
Antioxidant Activity of Samples

Sample	Scavenging Activity (%)
MPP	28.89
MPB	11.87
MPK	22.77
MPKa	15.92
MPN	22.53

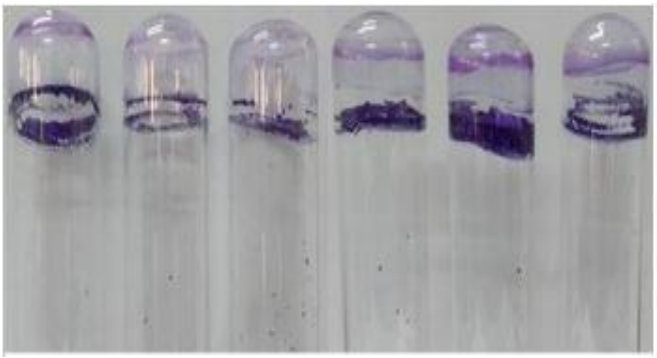
D. Estimation of Glucose Content

Total glucose content in the samples estimated using DNS method is shown in Table 5. Carbohydrates act as a direct energy source for bacteria and hence promote microbial growth. The low concentration of glucose in the *Musa x paradisiaca* L., extracts may indicate high potential in restricting bacterial growth.

TABLE V
Glucose Content in Samples

Samples	Amount of Glucose (mg/mL)
MPP	3.49
MPB	1.56
MPK	1.04
MPKa	1.15
MPN	1.74

E. Effect of Musa x paradisiaca L., Extracts on Biofilm Formation



Positive Control MPP MPB MPK MPKa MPN

Fig. 1 Biofilm formation in the presence of *Musa x paradisiaca* L., extracts

Formation of biofilm by *P. aeruginosa* in the presence of MPB extract was observed to be lower in comparison to that in the presence of the other *Musa x paradisiaca* L., floral extracts. The absorbance values measured in each case have been depicted in Fig.2. All data represent mean ± standard deviation (error bars) for three separate trials.

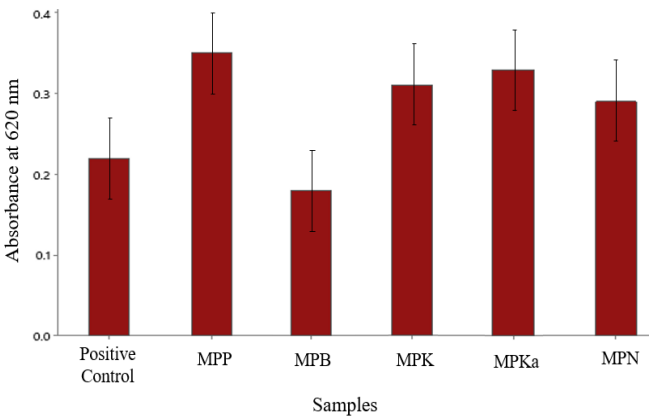


Fig. 2 Quantification of biofilm formation in the presence of *Musa x paradisiaca* L., extracts

F. Effect of Musa x paradisiaca L., Extracts on Pyocyanin Production

The disappearance of the green colouration of pyocyanin, typically produced after 24 hours of growth of *P. aeruginosa*, indicates low levels or absence of pyocyanin. With the exception of MPK and MPN, pyocyanin production was observed to have reduced in the presence of *Musa x paradisiaca* L., extracts. However, only MPKa exhibited a significant reduction in pyocyanin production. The absorbance values measured in each case have been depicted in Fig.3. All data represent mean ± standard deviation (error bars) for three separate trials.

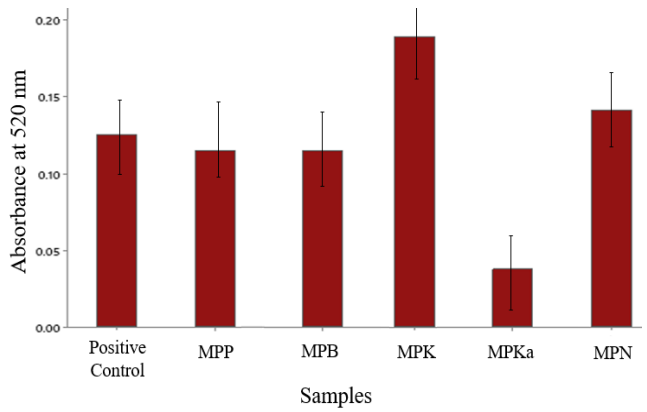


Fig. 3 Pyocyanin production in the presence of *Musa x paradisiaca* L., extracts

G. Effect of Musa x paradisiaca L., Extracts on Swarming Motility

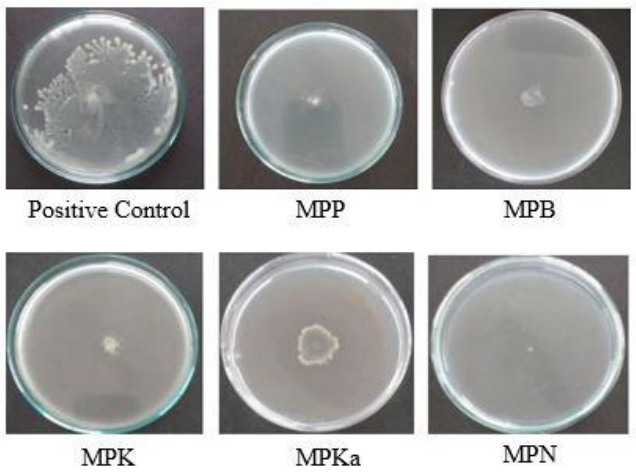


Fig.4 Swarming motility of *P. aeruginosa* in the presence of *Musa x paradisiaca* L., extracts

Swarming motility was observed to be highly regulated in the presence of the *Musa x paradisiaca* L., extracts, with a significant decrease in diameter observable in the presence of each of the five varieties considered.

TABLE VI
Swarming Diameter

Source	Swarming Diameter (cm)
Positive Control	8.5
MPP	0.9
MPB	0.3
MPK	1.1
MPKa	1.5
MPN	0.5

IV. CONCLUSION

The present study assessed the quorum quenching properties of *Musa x paradisiaca* L., in *Pseudomonas aeruginosa*. Floral extracts from five varieties of the medicinal plant were prepared and screened for phytochemical constituents. The extracts studied exhibited restriction in swarming motility, pyocyanin production and biofilm formation. This investigation advances the idea of the use of *Musa x paradisiaca* L., floral extracts in tackling the bacterial infections caused by *P. aeruginosa* and indicates promise in the future use of medicinal plants against other bacteria known for quorum sensing.

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