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## DETECTION OF ANTI-QUORUM SENSING ACTIVITY OF *ROSEMARINUS OFFICINALIS* AND *VALERIANA OFFICINALIS* USING MICROBIAL BIOSENSOR STRAIN

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### Keywords:

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**ABSTRACT:** Quorum-sensing is a phenomenon in which bacteria exhibit several density dependent phenotypes. Quorum-sensing is achieved through production of auto-inducer which on acquiring a threshold concentration, activates the genes, bringing into effect the concerned phenotypes. Ayurvedic medicinal plant extracts are being used to treat a plethora of infectious disease and are known to be rich in phytochemical compounds. This work aims at screening *Rosemarinus officinalis* and *Valeriana officinalis* for their anti-quorum-sensing activity using microbial biosensor strains which includes *Pseudomonas chlororaphis* (aureofaciens) 30-84, *S. aureus* agr P3::blaZ pRN8826 and *C. albicans* 10231. Initially, the medicinal plant extracts are made and minimum inhibitory concentration (MIC) of these extracts is determined. Qualitative phytochemical tests are performed with the extracts in order to find out the phytochemical present in these medicinal plant extracts. This is further followed by reporter assays for the detection of anti-quorum sensing activity involving the microbial biosensor strains and then quantification of the activity. The Rosemary leaves and the Valeriana roots containing alkaloid, catecholic tannin and flavonoid as its active component respectively possess quorum sensing inhibitor. Valeriana roots shows an anti quorum sensing activity against *Pseudomonas chlororaphis* 30-84 and *C. albicans* 10231. On the other hand Rosemary leaves have an anti quorum sensing activity against all the three microbial biosensor strains used. The finding of this study suggests that these plants can be used in combination with conventional antibiotics for therapeutic purposes.

**INTRODUCTION:** Ayurvedic medicines have long been used for curing various ailments and diseases in India. This system of traditional medicine largely makes use of indigenous plants that are used to treat a plethora of infectious diseases as they are rich in phytochemical compounds.

These phytochemicals like alkaloids, glycoside, terpenoids, flavonoids, tannins, etc. possess antibacterial and also at times anti-quorum sensing activity. In today's world antibiotics are the drugs of choice for combating diseases. The excessive and unregulated usage of antibiotics has led to multidrug resistance among pathogens. As the pathogens evolve and acquire resistance to a higher concentration of the drugs, the dosage of the drug may be increased in order to suppress the infection which on the other hand may cause undesirable side effects in the host. Fighting bacteria by interfering with their command language and thereby disrupting virulence expression instead of

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inhibiting growth could serve as an alternative to the conventional ways of combating bacterial infections. Most of the ayurvedic medicine follow this route of therapy and thus by combining ayurvedic and antibiotic chemotherapy, it is possible to eliminate infections whilst decreasing the dosage of antibiotics. Lower antibiotic dosage would mean decreasing the frequency of multidrug resistance as well as overall toxicity in the host. Hence, utilizing medicinal plants is a promising way of combating infectious diseases<sup>1</sup>.

Quorum sensing (QS) is a process of cell-to-cell communication used by many bacterial species which allows them to monitor their environment for the presence of other bacteria. It is mediated by the production, diffusion, and recognition of small signal molecules called auto-inducer which controls a broad range of biological functions like production of virulence factors, pigmentation, enzyme production *etc.* Therefore, as the population, *i.e.* quorum increases so does the concentration of the signal molecules. When the threshold concentration is reached there are enough bacterial cells to activate the transcription of quorum sensing target genes and the different biological functions are exhibited<sup>2-6</sup>. The quorum sensing molecules are different in gram negative, gram positive and yeast. Gram negative produces acyl homoserine lactone (AHL); gram positive produces auto-inducing peptides (AIP) and yeast produces tyrosol and farnesol<sup>2, 6, 9, 10</sup>.

Autoinducer molecules have been found to be subject to biological inactivation by interfering with the QS signal. Quorum sensing inhibitor (QSI) is a low molecular mass molecule whose activity will reduce the expression of QS controlled genes<sup>11-13</sup>. They may either attack the signal generator (lux I homologue) or the signal molecule (auto-inducer) or the signal receptor (LuxR homologue).

Medicinal plants like *Valeriana officinalis* (Valerian) and *Rosemarinus officinalis* (Rosemary) are used for therapeutic purpose. Dried roots of Valeriana are rich in phytochemicals like alkaloids, Valerenic acid, Isovaleramide *etc.* and Rosemary leaves are rich in essential oils, flavonoid, rosmarinic acid, terpenoids *etc.*<sup>1, 16-20</sup>. Thus the extracts of these plants can be screened to detect the anti-quorum sensing activity using microbial

biosensor strain of *Pseudomonas chlororaphis*<sup>30-84</sup> which produces hexanoylhomoserine lactone (HHL), *S. aureus* agr P3::blaZ pRN8826 produces auto-inducing peptideII (AIP-II) and *C. albicans* 10231 an over producer of tyrosol<sup>21</sup>. These organisms' exhibit different phenotypes on reaching a particular density. The auto-inducer molecule HHL produced by *Pseudomonas chlororaphis* 30-84 gives a yellow-orange pigment known as the phenazine pigment<sup>22</sup>; *S. aureus* agr P3::blaZ pRN8826 producing AIP-II is responsible for the production of the enzyme beta lactamase 3 and tyrosol produced by *C. albicans* 10231 leads to germ tube formation<sup>23-26, 33-40</sup>.

The aim of the present study is to find out whether the photochemical present in the dried roots of Valeriana and Rosemary leaves contain a quorum sensing inhibitor which targets the auto-inducer molecule of HHL, AIP-II and tyrosol, produced by the microbial biosensor strains. If these medicinal plants possess a quorum sensing inhibitor they can be used for therapeutic purpose in future<sup>41-48</sup>. They can be used to overcome the problem of multidrug resistant organism and thus can be used in combination with conventional antibiotics.

## MATERIALS AND METHOD:

**Preparation of Medicinal Plant Extracts:** The Valeriana roots and Rosemary leaves was purchased from S.R. International shop and Nisarg Nirman Agro Ltd, Mumbai respectively. The aqueous extracts were prepared as suggested by Mojica *et al.*,<sup>15</sup> with slight modification. The plant parts were washed, dried and powdered. Aqueous extracts were prepared by soaking the powders (1gm) in distilled water (10mL). The mixture was then stirred in magnetic stirrer at 23 °C for 90 min followed by filtration through whatmann filter paper No 1. The filtrate was further centrifuged at 5100 rpm for 45 min. The supernatant after centrifugation was dried and the dried aqueous extracts were dissolved in distilled water to known concentration and stored at 4 °C. These extracts were used for phytochemical screening and biological assays.

**Phytochemical Screening of Extracts:** Extracts were qualitatively analyzed for presence of phytochemicals namely, alkaloid, glycosides, phenols, terpenoid and steroid, tannin, flavonoid,

saponin, proteins and amino acid using methods suggested by Ayoola *et al.*, Ghafour *et al.*, and Joshi *et al.*,<sup>27-29</sup>.

### Bacterial Strains:

#### Three Biosensor Strains were used for Anti-QS Activity:

- Pseudomonas chlororaphis* (aureofaciens) 30-84, wild type strain that produces a yellow orange pigment in response to QS (C6 AHL) molecules that it produces. This strain was maintained on *Luria bertanni* (LB) agar.
- S. aureus* agrP3::blaZ pRN8826 that contains agrP3-blaZ fusion plasmid and produces  $\beta$ -lactamase spontaneously due to agr expression, it targets AIP mediated QS. This strain was maintained on LB agar with 10 $\mu$ g/mL chloramphenicol.
- C. albicans* 10231, clinical strain that produces small germ tube in response to QS (tyrosol) molecules that it produces. This strain was maintained on Malt extract Glucose Yeast extract Peptone agar (MGYP)

#### Determination of Minimum Inhibitory Concentration (MIC) of Extracts against Biosensor Strains:

MICs of the extracts were determined essentially to decide sub inhibitory concentrations (SICs) that were used for quantitative anti-QS activity in all the three biosensor strains. MIC was determined using the broth dilution method given by Hammer *et al.*,<sup>18</sup> 964mg/mL aqueous extract of Rosemary and 250mg/mL aqueous extract of Valeriana were used in the broth dilution method. The diluents used for *P. chlororaphis* 30-84, *S. aureus* agrP3::blaZ pRN8826 and *C. albicans* 10231 were LB broth and MGYP broth respectively. Each tube was diluted to a total volume of 1mL and inoculated with 20 $\mu$ L of a bacterial suspension (10<sup>8</sup> CFU/mL) of the *P. chlororaphis* 30-84/ *S. aureus* agrP3::blaZ pRN8826 / *C. albicans* 10231.

All experiments were performed in triplicate and the tubes were incubated at RT (*P. chlororaphis* 30-84 / *C. albicans* 10231) or 37 °C (*S. aureus* agrP3::blaZ pRN8826) for 24h. 20 $\mu$ L of 1mg/mL aqueous solution of TTC (2,3,5-Triphenyl-2H-tetrazolium chloride) (Hi media) was added as a

redox indicator. Reduction of TTC to its formazan product was a clear indication of growth/no growth. Change in colour to pink was noted as growth. Positive and negative controls were used. Concentrations lower than the cidal concentrations were then used as SICs.

#### Anti-QS Activity of Plant Extracts Using Gram Negative Biosensor Strain:

**Bioassay for Phenazine Pigment Detection:** The agar well diffusion assay was adopted to detect the anti-QS activity using a modified method of Zahin Maryam *et al.*,<sup>1</sup> and was performed by *P. chlororaphis* 30-84 for determining pigment inhibition activity by plant extracts. Luria agar plates were overlaid with LB agar butt containing 0.6mL of appropriately diluted (10<sup>8</sup>CFU/mL) freshly grown culture of *P. chlororaphis* 30-84. Wells of 8mm diameter were made on the LB agar plate and hundred microlitres (100 $\mu$ L) of diluted and neat solution of the plant extracts were loaded in each agar well. Sterile distilled water was used as a negative control. Plates were incubated for 48-72h at RT.

**Quantification of Phenazine Pigment:** For quantitative studies of the anti-QS activity, a method of Maddula *et al.*,<sup>30</sup> was used. Phenazine (PZ) was extracted from strain *P. chlororaphis* 30-84 and the amount of pigment produced was estimated. Briefly, the 30-84 strain was washed with saline and cell density adjusted (optical density at 540nm = 0.4). 10% of the inoculum was added to LB broth containing the medicinal plant extracts and grown to late exponential phase (optical density at 620nm = 1.8). Concentrations of the plant extracts that were selected were below the MIC and equaled 50MIC (N/2), 25MIC (N/4), 12.5MIC (N/8) for Rosemary and 25MIC (N/2), 12.5MIC (N/4), 6.25MIC (N/8) for Valerian.

Medium without the extracts served as a positive control and LB broth with no culture served as the negative control. Cell-free supernatants were prepared by centrifugation (2500  $\times$  g) for 30 min. PZs from cell-free supernatants were extracted with an equal volume of acidified benzene, and the benzene phase was separated and evaporated under air. Dried PZs were dissolved in 0.1N NaOH and quantified by UV-visible spectroscopy using 0.1N NaOH as the blank. The absorption maxima for

Phenazine 1 carboxylic acid (PCA) and 2-hydroxy phenazine 1 carboxylic acid (2-OH-PCA) were measured at 367nm and 484nm, respectively. The relative amounts of PCA and 2-OH-PCA were calculated by multiplying their absorption maxima by their standard extinction coefficients.

### Anti-QS Activity of Plant Extracts Using Gram Positive Biosensor Strain:

**Beta Lactamase Assay:** Quantitative measurement of agr activity was done using the *S. aureus* agrP3::blaZ pRN8826 reporter assay, originally described by Ji *et al.*,<sup>31</sup>. This assay measured RNAPIII production in the form of  $\beta$ -lactamase activity using the chromogenic cephalosporin, nitrocefin, as a substrate. *S. aureus* agrP3::blaZ pRN8826 was grown overnight in LB broth containing 10 $\mu$ g/ml of chloramphenicol at 37 °C. The overnight broth culture was adjusted to a logarithmic phase (optical density at 600nm = 0.4). In a microtitre plate, 10 $\mu$ L of the aqueous plant extract was added to 90 $\mu$ L of log phase culture such that it reached a sub inhibitory concentration equivalent to 4MIC (N/2), 2MIC (N/4), 1MIC (N/8) for Rosemary and 10MIC (N/2), 5MIC (N/4), 2.5MIC (N/8) for Valerian. 90 $\mu$ L of culture was added to 10 $\mu$ L of LB broth in positive control and 10 $\mu$ L of the plant extracts in extract control.

The plates were incubated for 2.5h at 37 °C. After incubation 66 $\mu$ L of nitrocefin prepared by dissolving 16 nitrocefin discs (BD diagnostics) in 4mL of 0.1M sodium phosphate buffer (pH 5.8) was added. In negative and extract control 66 $\mu$ L of

sodium phosphate buffer was added instead of nitrocefin.  $\beta$ -lactamase activity was measured in Biorad imark microplate reader using kinetic mode at  $\epsilon$ 490- $\epsilon$ 650nm. One unit of  $\beta$ -lactamase was defined as an increase in 0.001 OD units per min. All assays were performed in duplicates and average percent enzyme activity was calculated with respect to the positive control.

### Anti-QS Activity of Plant Extracts Using *C. albicans* ATCC 10231 Biosensor Strain:

**Germ Tube Assay:** A germ tube assay was used to detect whether the plant extracts possess an anti-QS activity against tyrosol, based on a modified method of Chen H *et al.*,<sup>25</sup>. *C. albicans* ATCC 10231 was adjusted to give a final concentration of 10<sup>4</sup> yeast cells/mL. The aqueous plant extract prepared in MGYB broth was added to the *C. albicans* culture such that it reached a sub inhibitory concentration equivalent to 25MIC (N/2), 12.5MIC (N/4), 6.25MIC (N/8) for Rosemary and 0.5MIC (N/2), 0.25MIC (N/4), 0.125MIC (N/8) for Valeriana and incubated for 2h at RT. The positive control contained the culture (CFU 10<sup>4</sup>cells/mL) in CGYP broth. After 2 hrs of incubation the no of cells showing germ tube was counted on a haemocytometer slide and the morphology of Candida cells was assessed directly by microscopy analysis (Motic Microscope Phase Contrast). All assays were performed in duplicates and the average percentage of germ tube induction was calculated with respect to the positive control.

**TABLE 1: MEAN MINIMUM INHIBITORY CONCENTRATION OF VALERIANA AND ROSEMARY AQUEOUS EXTRACTS AGAINST BIOSENSOR STRAINS**

Plant Extract	Biosensor Strains	MIC			Average (mg/ml)
		I	II	III	
Valerian Stock Concentration 250mg/mL	<i>S. aureus</i> agrP3::blaZ pRN8826	20	20	20	20
	<i>Pseudomonas chlororaphis</i> 30-84	50	50	50	50
	<i>C. albicans</i> 10231	1	1	1	1
Rosemary Stock Concentration 964mg/mL	<i>S. aureus</i> agrP3::blaZ pRN8826	8	8	8	8
	<i>Pseudomonas chlororaphis</i> 30-84	100	100	100	100
	<i>C. albicans</i> 10231	50	50	50	50

## RESULTS AND DISCUSSION:

**Phytochemical Screening:** The aqueous extract of Valeriana when screened for phytochemical constituents showed strong reactions with FeCl<sub>3</sub>

giving a greenish black color, thus confirming the presence of catecholic tannins. The alkaline reagent test for flavonoids showed yellow coloration of the extract, thus confirming that the Valeriana plant

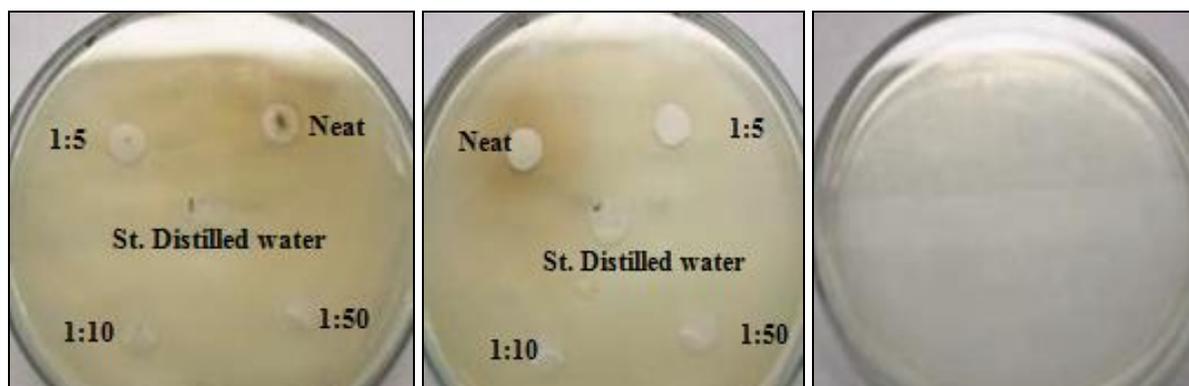
was rich in flavonoids. On the other hand Rosemary aqueous extract showed strong reaction with Mayer's reagent showing a cream color precipitate and thus confirmed that the Rosemary plant was rich in alkaloids<sup>27-29</sup>.

**Determination of Minimum Inhibitory Concentration (MIC) of Extracts against Biosensor Strains:** The MIC of the aqueous plant extracts were carried out in triplicates (**Table 1**) using TTC as the redox indicator.

**Anti-QS Activity of Plant Extracts Using Gram Negative Biosensor Strain:**

**Bioassay for Phenazine Pigment Detection:** The *Pseudomonas chlororaphis* 30-84 strain produced yellowish to faint orange phenazine pigment on

exposure to diluted samples of the Rosemary and Valeriana extracts (**Fig. 1**). There was no zone of inhibition observed around the wells containing the plant extracts. This indicates that the plant extracts does not show any anti-quorum sensing activity against hexanoylhomoserine lactone, an auto-inducer molecule produced by *Pseudomonas chlororaphis* 30-84 strain. The phenazine pigment produced by the strain was however less in amount and faint in colour. Thus further quantification of the pigment needs to be done in order to ensure the anti-quorum sensing activity of the extracts. As reported by Vattem *et al.*,<sup>32</sup> *Rosemary officinalis* is known to inhibit the purple pigment production in *Chromobacterium violaceum* thus having an anti quorum sensing activity.



**FIG 1: BIOASSAY FOR PHENAZINE PIGMENT DETECTION: NO ZONE OF INHIBITION AROUND THE WELLS CONTAINING DIFFERENT DILUTION OF PLANT EXTRACTS; (A) LURIA AGAR PLATE CONTAINING ROSEMARY EXTRACT IN THE WELLS (B) LURIA AGAR PLATE CONTAINING VALERIANA EXTRACT IN THE WELLS (C) LURIA AGAR CONTROL PLATE**

**TABLE 2: QUANTITATIVE STUDIES OF ANTI-QUORUM SENSING ACTIVITY DETECTED AS PIGMENTATION IN *P. CHLORORAPHIS* 30-84 IN THE PRESENCE OF SUB-INHIBITORY CONCENTRATIONS OF PLANT EXTRACTS**

Plant Extract	Sub-inhibitory Concentration (mg/ml)	Concentration of PCA (moles)	Concentration of 2-OH PCA (moles)	Total phenazine pigment (moles)	Percentage of Phenazine
Valeriana	N/2	0.0000149	0.014	$1.66 \times 10^{-5}$	7.41%
	25				
	N/4	0.0000119	0.012	$1.34 \times 10^{-5}$	5.98%
	12.5				
	N/8	0.0000159	0.007	$1.67 \times 10^{-5}$	7.45%
Rosemary	6.25				
	N/2	0.00000781	0.062	$6.04 \times 10^{-5}$	26.9%
	50				
	N/4	0.00000969	0.077	$15.8 \times 10^{-5}$	70.5%
	25				
	N/8	0.0000164	0.131	$14.8 \times 10^{-5}$	66%
	12.5				
Positive control	-	0.576	0.277	$22.4 \times 10^{-5}$	100%
Negative control	-	0.000	0.000	0.000	0%

Note: For Valeriana N = 50mg/ml, Rosemary N = 100mg/ml (N=MIC in mg/ml)

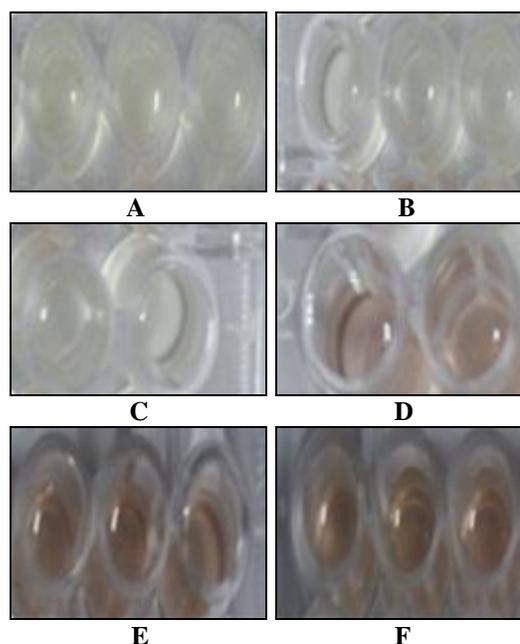
PCA is Phenazine 1 carboxylic acid, 2-OH PCA is 2-hydroxy Phenazine 1 carboxylic acid

**Quantification of Phenazine Pigment:** Amount of Pigment produced by *Pseudomonas chlororaphis* 30-84 on exposure to sub-inhibitory concentrations of the extracts showed a decrease as compared to the positive control; which contains only the phenazine pigment extracted from the *Pseudomonas chlororaphis* 30-84 strain. Rosemary and Valeriana extract showed anti-QS activity at subinhibitory concentrations (Table 2). The Valeriana extract showed more inhibition of the pigment thus showing a better anti-QS activity as compared to the Rosemary extract.

This might be due to the reason that there are certain active components present in the Rosemary extract whose concentration is less. Since the extract is a crude preparation these active components showing anti-QS activity needs to be concentrated by further purifying the extract. These components when concentrated and used might then give a better anti-QS activity.

**Anti-QS Activity of Plant Extracts Using Gram Positive Biosensor Strain (Anti-agr Activity):**  $\beta$ -lactamase produced as a result of QS in biomonitor strain *S. aureus* agrP3::blaZ pRN8826 was estimated using chromogenic cephalosporin that on breakdown by  $\beta$ -lactamase gave a red colored product (Fig. 2). Estimation of the enzyme units of  $\beta$ -lactamase was used as a measure of agr activity and hence, QS in the *S. aureus* agrP3::blaZ strain. The  $\beta$ -lactamase enzyme activity did not decrease in case of Valeriana plant extract whereas on exposure to the subinhibitory concentration of the Rosemary extract the  $\beta$ -lactamase enzyme activity decreased as that compared to the positive control (Table 3). Thus the phytochemical alkaloid present in the Rosemary extract gives an anti QS activity against the gram positive biosensor strain. However, the anti-agr activity is not drastic as compared to the positive control.

This indicates that apart from alkaloid there might be some other active components present in the extract which has an anti-QS activity and thus gives a decrease in the  $\beta$ -lactamase enzyme activity, but these active components are present in less concentration and therefore the decrease is not drastic. Since Rosemary extract is a crude preparation, it needs to be further purified so that these active components can be concentrated. Such a purified extract if used might give a better anti-QS activity against the *S. aureus* agrP3::blaZ pRN8826 strain producing AIP II.



**FIG 2:  $\beta$ -LACTAMASE QUANTITATIVE ASSAY. (A) VALERIANA EXTRACT CONTROL: MICROTITER PLATE CONTAINING VALERIANA EXTRACT, YELLOW IN COLOUR DUE TO THE ABSENCE OF NITROCEFEN (B) ROSEMARY EXTRACT CONTROL: MICROTITER PLATE CONTAINING ROSEMARY EXTRACT, YELLOW IN COLOUR DUE TO THE ABSENCE OF NITROCEFEN (C) NEGATIVE CONTROL (D) POSITIVE CONTROL (E) VALERIANA EXTRACT TEST: PINK IN COLOUR DUE TO THE BETA LACTAMASE ACTIVITY ON ADDITION OF NITROCEFEN (F) ROSEMARY EXTRACT TEST**

**TABLE 3: QUANTITATIVE STUDIES OF ANTI-QUORUM SENSING ACTIVITY DETECTED AS  $\beta$ -LACTAMASE ACTIVITY IN BIOSENSOR STRAIN *S. AUREUS* agrP3::blaZ IN THE PRESENCE OF SUB-INHIBITORY CONCENTRATIONS OF PLANT EXTRACTS**

Plant Extract	Set	Sub-inhibitory Concentration (mg/ml)	Enzyme activity (%)	Mean enzyme activity (%)
Valeriana	I	N/2	81.63	79.76
	II	10	77.9	
	I	N/4	115.3	107.06
	II	5	98.83	
	I	N/8	82.65	109.92
	II	2.5	137.2	

Rosemary	I	N/2	51.02	42.95
	II	4	34.88	
	I	N/4	104.08	92.73
	II	2	81.39	
	I	N/8	68.36	62.08
	II	1	55.81	
Positive control	I	-	100	100
	II	-	100	
Negative control	I	-	0	0
	II	-	0	

Note: The  $\beta$ -lactamase assay is carried out in duplicates.

For Valeriana N = 20mg/ml, Rosemary N = 8mg/ml (N = MIC in mg/ml)

**Anti-QS Activity of Plant Extracts Using *C. albicans* ATCC 10231 Biosensor Strain (Germ Tube Assay):** *C. albicans* 10231 produces very less farnesol (<0.005mg/g dry weight) and more of tyrosol, thus accelerating short germ tube formation (Fig. 3). The germ tube induction in presence of the subinhibitory concentration of the extracts was determined. The percentage of germ tube induction was calculated and compared to that of the positive control (Table 4). Valeriana and Rosemary extracts showed a decrease in the germ tube induction as that compared to the positive control (Fig. 4). The active phytochemicals present in the extract blocks the production of tyrosol and hence there is a drastic decrease in the germ tube induction. Thus the alkaloid, tannin and flavonoid present in Rosemary and Valeriana extracts respectively has an anti-QS activity against *C. albicans* ATCC 10231.

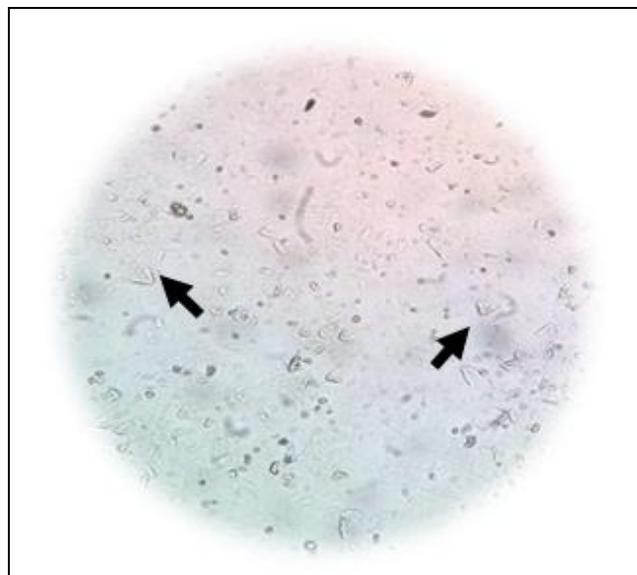


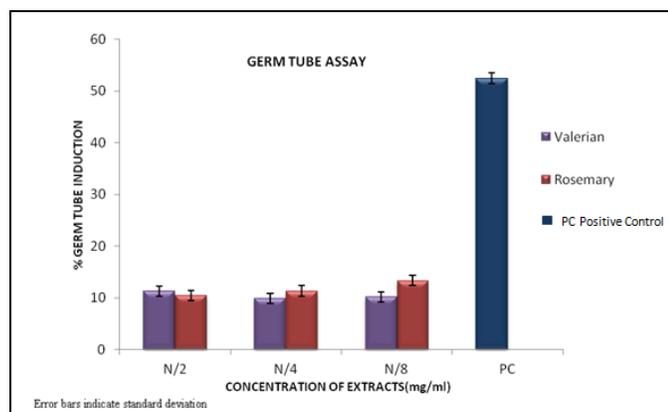
FIG 3: MICROSCOPIC VIEW OF SHORT GERM TUBE PRODUCED BY *C. ALBICANS* 10231 OBSERVED UNDER PHASE CONTRAST MICROSCOPE (45X)

TABLE 4: QUANTITATIVE STUDIES OF ANTI-QUORUM SENSING ACTIVITY DETECTED AS GERM TUBE INDUCTION IN *C. ALBICANS* 10231 IN THE PRESENCE OF SUB-INHIBITORY CONCENTRATIONS OF PLANT EXTRACTS

Plant Extracts	Sub-inhibitory Concentration (mg/ml)	Total no. of organism/ml of suspension		Mean	Total No. of organism with germ tube/ml of suspension		Mean	Germ tube induction (%)
		Set I	Set II		Set I	Set II		
Valeriana	N/2	Set I	Set II		Set I	Set II		
	0.5	190×10 <sup>4</sup>	113.25×10 <sup>4</sup>	151.62×10 <sup>4</sup>	17×10 <sup>4</sup>	17.25×10 <sup>4</sup>	17.12×10 <sup>4</sup>	11.29
	N/4	230×10 <sup>4</sup>	166.75×10 <sup>4</sup>	198.37×10 <sup>4</sup>	21.25×10 <sup>4</sup>	18×10 <sup>4</sup>	19.62×10 <sup>4</sup>	9.89
	0.25							
	N/8	184.75×10 <sup>4</sup>	269.5×10 <sup>4</sup>	227.12×10 <sup>4</sup>	19.75×10 <sup>4</sup>	26.5×10 <sup>4</sup>	23.12×10 <sup>4</sup>	10.17
Rosemary	0.125							
	N/2	164.25×10 <sup>4</sup>	113.5×10 <sup>4</sup>	138.87×10 <sup>4</sup>	18.5×10 <sup>4</sup>	10.5×10 <sup>4</sup>	14.5×10 <sup>4</sup>	10.44
	25							
	N/4	139.75×10 <sup>4</sup>	128.75×10 <sup>4</sup>	134.25×10 <sup>4</sup>	14.5×10 <sup>4</sup>	16×10 <sup>4</sup>	15.25×10 <sup>4</sup>	11.35
	12.5							
Positive Control	N/8	104.5×10 <sup>4</sup>	131.25×10 <sup>4</sup>	117.87×10 <sup>4</sup>	14.25×10 <sup>4</sup>	17.25×10 <sup>4</sup>	15.75×10 <sup>4</sup>	13.36
	6.25							
Positive Control	-	203.5×10 <sup>4</sup>	221×10 <sup>4</sup>	212.25×10 <sup>4</sup>	110×10 <sup>4</sup>	112.75×10 <sup>4</sup>	111.3×10 <sup>4</sup>	52.43

Note: The assay is carried out in duplicates

For Valeriana N = 1mg/ml, Rosemary N = 50mg/ml (N = MIC in mg/ml)



**FIG 4: COMPARISON OF THE PERCENTAGE GERM TUBE INDUCTION IN PRESENCE OF THE SUB-INHIBITORY CONCENTRATIONS OF ROSEMARY AND VALERIANA EXTRACTS WITH THE POSITIVE CONTROL FOR ANTI-QUORUM SENSING ACTIVITY OF PLANT EXTRACTS USING BIOSENSOR STRAIN *C. ALBICANS* 10231**

**CONCLUSION:** The discovery of antibiotics has been a boon to human civilization but the current traditional antimicrobials need to be reviewed for their efficacy due to the increasing occurrence of multidrug resistant strains. One novel therapeutic approach to overcome the problem of resistance is the use of antipathogenic drugs that target key regulatory bacterial systems responsible for the expression of virulence factors. Since quorum sensing leads to expression of virulence genes in several known pathogens, agents that can interrupt bacterial communication can be used as anti-pathogenic drugs.

The study on Rosemary leaves and Valeriana roots aqueous crude extracts containing alkaloid; catecholic tannin and flavonoid as its active component respectively are shown to have a quorum sensing inhibitor thus giving an anti-quorum sensing (QS) activity. However, the anti-QS activity of both the extracts is different with respect to the different microbial biosensor strains used. Valeriana roots containing tannin and flavonoid shows anti-quorum sensing activity against *Pseudomonas chlororaphis* 30-84, a gram negative organism that produce hexanoyl-homoserine lactone as the auto-inducer molecule and *C. albicans* 10231 which produces tyrosol as the quorum sensing molecule. Rosemary leaves containing alkaloid as its active component shows an anti QS activity against all the three types of organism, though the activity is a little less in case of *S. aureus* agrP3::blaZ pRN8826, a gram positive

strain producing AIP II as the autoinducer molecule and *Pseudomonas chlororaphis* 30-84. Thus, from the above inferences it can be stated that Rosemary and Valerina gives a better anti-QS activity against *C. albicans* 10231 microbial strain, a eukaryotic model.

However Valeriana and Rosemary can further be used to treat infections caused by *Pseudomonas* for e.g. pneumonia, bacteremia, etc, infections caused by *C. albicans* for e.g. oral thrush, vaginal candidiasis etc. Rosemary to a certain extent can be used for treating infections caused by *S. aureus* which are capable of colonizing on human catheters and medical tubing giving rise to urinary tract infection, and also infections caused due to biofilm formation. These extracts might also be combined with antibiotics and used, so that the dosage of the antibiotics can be lowered to a certain extent and the performance of the antibiotic is improved.

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